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Chapter 2

4-Hydroxyquinolin-2-ones and their Close Structural Analogues as a New Source of Highly Effective Pain-Killers

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Additional information is available at the end of the chapter

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1. Introduction

Despite the most unflattering epithets and fear, pain was and still remains the normal response of any living organism on strong physical, chemical or mechanical stimuli. It has the most important protection function in nature – at just the right time pain immediately signals about appearance of exogenic or endogenic destructive effects on a certain organ [1-6], and it is simply necessary for the organism's survival as a biological unit. Unfortunately, it presents not only by disagreeable sensation. Being rather complex psychophysiological phenomenon pain (especially strong and continued) is often accompanied by very powerful emotional stresses [7-9], which can rapidly exhaust the body's adaptation resources and cause the serious disorders of its vital functions. Obviously it is for this reason that International Association for the Study of Pain considers pain as a global factor causing problems in modern society not only of medical, but also of socio-economic character [10-13].

Pains of various origin and pain syndromes occur so often as it is difficult to find a person among the world population that does not know this feeling. Hence it is not surprising that pain-killers are among the most popular and often used drugs. The drug arsenal of this pharmacological group that is available in modern medicine is exceedingly wide [14]. However, even under such conditions the appropriate pain relief is not always successful. The cause of it can be side effects and, as a consequence, numerous contraindications and restric-

tions in using drugs [15]. That is why the vital task of pharmaceutical and medical chemistry is the search of new, highly effective and, most notably, safe pain-killers.

Quinoline as a basic structure of such investigations is of special interest. The precondition of it is the natural origin, practically unlimitted synthetic potential and, of course, the analgetic action, which is inherent to many of its derivatives. For example, quinine (**1**, Figure 1) – the main alkaloid of cinchona tree bark – does not only inhibit malaria parasites actively, but reveals nonspecific analgesic properties. It potentiates the action of narcotic and nonnarcotic analgesics, thanks to which it has been widely used in the composition of finished drug combinations for headache. Lysergic acid diethylamide (**2**, more known under abbreviation LSD) created semisynthetically as a vigorous psychodelic is currently prohibited to therapeutic use by the laws in most countries. Nevertheless, in spite of its illegal status, researchers continue to be interested in LSD because of its unique medicinal properties. In particular, it has been found that as an analgesic this substance acts more effectively and sustained than opiates in low doses that do not cause any psychologic effects. And as for inhibition of cluster headaches – a rare syndrome causing particularly intensive pain, it has no equal at all for the present [16].

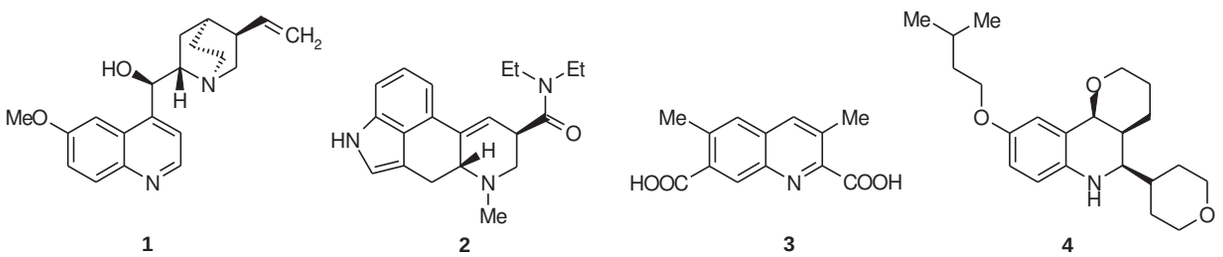

Figure 1. Natural (**1**), semisynthetic (**2**) and synthetic (**3** and **4**) quinoline analgesics

Natural resources are limited and not always reproducible. Besides, isolation of biologically active substances from the plant or animal raw material, their subsequent purification and standardization is, as a rule, difficult and time-consuming. That is why it is quite natural that the search of new analgesics of the quinoline Internet resources reveals a lot of publications concerning the given topics [17-23]. Thus, promising substances are created based on various derivatives both quinoline (**3**) itself and its hydrogenized analogs (**4**).

2. Synthesis and analgesic activity of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid *N*-R-amides

Until recently 4-hydroxyquinolin-2-ones have not even mentioned as analgesics in scientific literature. Only some years ago the situation turned over when based on preliminary virtual screening we obtained hydrochlorides of [(alkylamino)alkyl]amides of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid as potential opioid receptor antagonists [24]. Further pharmacological research has confirmed the presence of "calculated"

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biological properties of the compounds synthesized. At the same time it has been noted that some substances do not block the pain-killing action of narcotic analgesics, but vice versa, prolong it greatly. It is this obsevation that has become the first step for conducting extensive studies in purposeful research of substances with a new type of the pharmacological effect on a living organism for this class of compounds, i.e. potential pain-killers, in the range of 4-hydroxyquinolin-2-ones derivatives.

The beginning of this big and complex work was the synthesis of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid alkyl-, hydroxyalkyl-, *cyclo*-alkyl-, arylalkyl- and hetarylalkylamides (**6**, Figure 2) carried out by the reaction of methyl 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylate (**5**) with the corresponding primary amines in boiling methanol [25, 26].

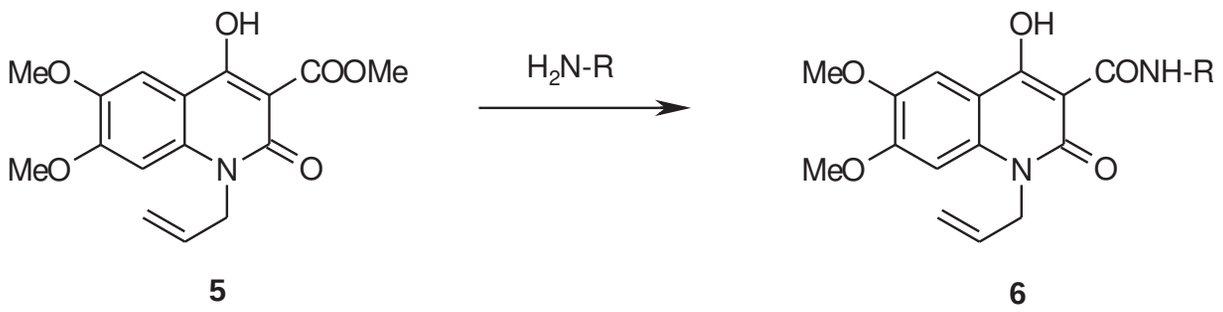

Figure 2. Synthesis of 1-allyl-6,7-dimethoxy-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**6**)

The screening test of analgesic properties of 1-*N*-allylsubstituted amides **6** convinced us in correctness of the chosen direction – each and all compounds revealed the analgesic effect to a greater or lesser degree in oral introduction to white rats in the dose of 0.00005 Mol/kg (on the average it is approximately 20 mg/kg) [25, 26]. While carrying out the biological experiments the animals were treated in accordance with the European Convention for Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Of 50 samples studied with the general formula **6** approximately half of them do not yield Diclofenac in activity, and three of them (**6**, R = $-(CH_2)_2OH$, $-CH_2C_6H_4$-Cl-4 or furfuryl) even exceed one of the most powerful nonnarcotic analgesics Ketorolac. In the experiments of the given series the standard model of rectal mucosa irritation by electric current was used. Therefore, the central component influencing on the nociceptive system is present in the mechanism of the analgesic action of amides **6**. One regularity that can be interesting for future research has come to our attention. It is clearly traceable in all groups of compounds with the same aromatic ring in the arylalkylamide fragment, e.g., benzyl- → 2-phenylethyl- → 3-phenylpropylamide; 4-chlorobenzyl- → 2-(4-chlorophenyl)ethylamide, etc. It appeared that the farther the aromatic substituent from the amide nitrogen atom is, the less are the analgesic properties of the corresponding 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid arylalkylamides.

3. Chemical modification of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides

After revealing a new biological activity in compounds of any chemical class usually the complex of works directed to improvement of their pharmacological and(or) pharmaceutical properties follows. This methodology being generally recognized and traditional for medical chemistry consists in gradual introduction of various structural modifications into a basic molecule allowing to have changes in its characteristics in the right direction. We tried to implement such approach in practice in our further research; by its result the theoretically important regularities of the "structure – activity" relationship at least can be determined. And if one is fortunate (the element of chance is always present in such works), it is realistic to reveal the promising lead compounds with a practical significance concerning the solution of the problem dealt with.

3.1. Halocyclization in 2-bromomethyl-7,8-dimethoxy-5-oxo-1,2-dihydro-5*H*-oxazolo[3,2-*a*]quinoline-4-carboxamides

The ability of 1-*N*-allylsubstituted 4-hydroxyquinolin-2-ones to cyclize readily in oxazoloquinolines [27] while interacting with the molecular bromine in acetic acid was used by us for transformation of amides **6** described above into their tricyclic derivatives **7** (Figure 3). This interesting reaction occurs instantly and quantitatively, but its direction is insensitive to the structure of substituents and it always primarily occurs as bromocyclization [28, 29]. Nevertheless it should be remembered that carrying out such reactions requires the strict observance of the equimolar ratio of reagents. It is clear that the lack of bromine will lead to partial transformation of allyl derivatives **6** into oxazoloquinolines **7**. However, the excess of bromine is also inadmissible since in this case formation of complexes of di(2-bromomethyl-5-hydroxy-7,8-dimethoxy-4-R-carbamoyl-1,2-dihydrooxazolo[3,2-*a*]quinolinium) ditribromides with bromine [25] or (if there is structural background for it) bromination of the molecule's amide fragment are possible [26].

According to the data of the biological research transfer from bicyclic 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides **6** to their tricyclic oxazoloquinoline derivatives **7** as such does not have a significant effect on analgesic properties and, therefore, it is not likely to be considered practical. Nevertheless, rather high reactivity of 2-bromomethyl oxazol fragment of these compounds in relation to various nucleophiles allows conducting more profound transformation into 2-aminomethyl- (**8**) or 2-methylene- (**9**) oxazoloquinolines that have not studied yet pharmacologically and even into 1-acetonyl derivatives (**10**) [27]. Taking into account immensely wide synthetic possibilities the probability of success in future studies concerning these directions remains at the very high level.

3.2. 1-*N*-Allyl group removal

The next variant of the chemical modification of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**6**) was obvious and simple removal of 1-*N*-allyl

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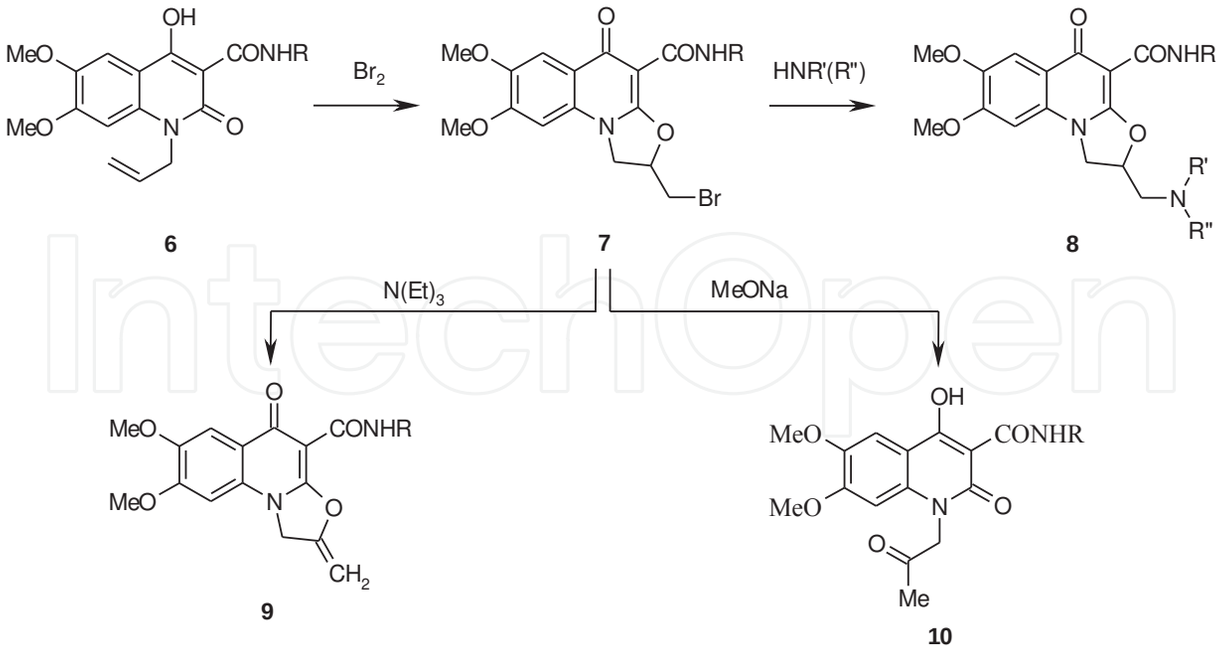


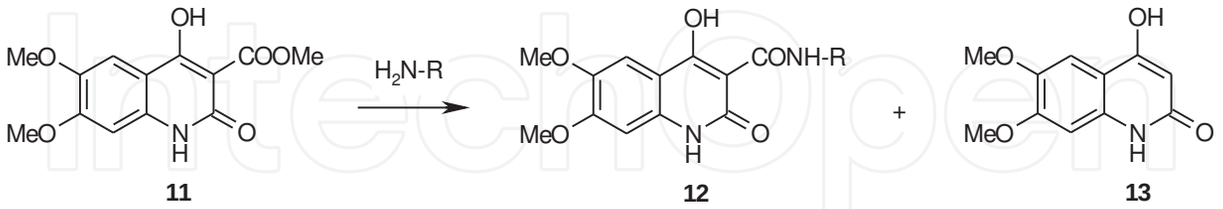
Figure 3. Synthesis and chemical transformations of 2-bromomethyloxazolo[3,2-*a*]quinoline-4-carboxamides (**7**)

substituent from the basic molecule. More specifically, obtaining of the target products only externally looks like removal of 1-*N*-allyl fragment. In reality the first stage of alkylation for initial methyl 4,5-dimethoxyanthranilate is simply excluded from the synthetic scheme of amides **6** obtaining [30].

Lower esters of 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids have a high reactivity [31-34]; due to it their transformation into various *N*-R-amides usually causes no complications. That is why problems arosen in amidation of dimethoxy substituted ester **11** by primary alkylamines appeared to be unexpected to a great extent. For example, after the synthesis in boiling DMF used because of the low solubility of ester **11** in other organic solvents, along with target alkylamides **12** formation of a noticeable amount of 4-hydroxy-6,7-dimethoxy-1*H*-quinoline-2-one was observed (**13**). The cause of appearance of this admixture proved to be water present in the reaction mixture; its effect could be eliminated by amidation at the temperature of about 80 °C. As a result a great number of target alkyl-, hydroxyalkyl-, *cyclo*-alkyl- and arylalkylamides of 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**12**, Figure 4) have been obtained with high yields and purity [35, 36]. Anilides and hetarylamides **12** do not form in these conditions. The more rigid conditions are necessary for their synthesis such as the temperature of approximately 120 °C and quite little amount of DMF (1-2 ml per 0.01 mol) [37, 38]. It is of interest that in a greater volume of the solvent amidation of alkyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates by anilines and hetarylamines takes place incredibly slow.

The analgesic activity of seventy 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides in empty position 1 with the general formula **12** has been studied in white mice. In the experiments the classical model of ”acetic acid induced writhing” [39] allowing to

estimate the peripheral component of the pain relieving effect of the tested samples has been used. We immediately note that simplification of the structure of the objects under research initiated by us did not affect their biology cardinally – in this set of experiments there were no examples of complete or substantial loss of analgesic properties.



11 **12** **13**

Figure 4. Synthesis of 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**12**)

Most alkylamides **12** demonstrate a moderate and statistically significant ($p \leq 0.05$) analgesic effect comparable with Piroxicam at the same dose (20 mg/kg, orally) [35]. Hydroxyl or alkoxyl groups at the terminal carbon atom of amide fragments only decrease the activity. And transfer from alkylamides with the normal structure to their cyclic analogs is not so unambiguous. For example, in the case of propyl derivatives the transformation mentioned is accompanied with almost complete loss of analgesic properties. However, with prolongation of alkyl chains the effect changes to the opposite one – *cyclo*-pentyl - and *cyclo*-hexylamides **12** are more active than their acyclic analogs. Of all the group of alkyl-, hydroxyalkyl- and *cyclo*-alkylamides only propylamide (**12**, R = Pr) is worthy. It has demonstrated the better results on the "acetic acid induced writhing" model than Piroxicam, and even than more effective drugs Nabumetone and Diclofenac.

Arylalkylamides **12** are of much greater interest. Many of them do not yield, and some of them even exceed generally accepted analgesics used in tests by their analgesic action in much lower doses [36]. Thus, the structural biological regularity found while studying 1-*N*-allylsubstituted amides **6** has been confirmed once more, namely, with introduction of the aryl ring into the alkylamide fragment the activity increases, but with its moving from the nitrogen amide atom it gradually decreases.

Involvement of new classes of compounds, in particular anilides (**12**, R = Ph or substituted Ar) [38], in the range of the objects under research has supplemented this regularity with one more observation that is important for future investigations – the total absence of any methylene bridge between nitrogen amide atom and aryl substituent reflects negatively on analgesic properties.

In the group of hetarylamides only pyridine derivatives (**12**, R = Py or 2-Py-Me) [37] synthesized as structurally related carbonyl analogs of Piroxicam have been studied. The biological testing of these compounds has shown that the majority of them are approximately equal to Piroxicam by the level of their analgesic activity. In the range of isomeric unsubstituted pyridylamides a distinct dependence of their analgesic action on the position of the nitrogen atom in the pyridine fragment: 3 < 4 < 2 is observed. In the next group of isomers – monomethyl substituted pyridyl-2 amides – there are somewhat different regularities: although C-methyl-

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ation of the pyridine ring promotes intensification of analgesic properties, however, in general, the effect appears to be insignificant and, furthermore, with low sensitivity to the methyl group position.

Hetarylalkylamides of 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**12**, R = picolyl-2, 3 or 4; furfuryl or tetrahydrofurfuryl) should be particularly mentioned. The bioisosteric replacements methodology [40-43] used in medical chemistry fruitfully and for a long period of time was the theoretical background for the synthesis of these compounds. In classic case implementation of this approach is replacement of an atom or a group of atoms with another ones having approximately the same size, shape and similar electronic configuration [44]. It is expected that after such modification a substance will possess the biological effect, which is close to the initial structure, and, probably, the more expressed one [45]. Based on these particular considerations we substituted the phenyl ring in the most active compound studied – *N*-benzyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**12**, R = CH_2Ph) – by pyridinic or furan nuclei being isosteric to it. According to the results of pharmacological studies transfer to tetrahydrofuran and especially furan derivatives has been recognized as unsuccessful as it led to the marked loss of the analgesic activity. But Ph → Py replacement appeared to be really bioisosteric. Moreover, in this case the strength of the effect is determined by the position of a heteroatom in the pyridinic cycle: 4-Py ≤ Ph = 2-Py < 3-Py. A significant enhancement of analgesic properties of *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**12**, R = CH_2Py-3) was a solid argument for choosing it as a lead compound at the given stage of our research.

3.3. Modification of the benzene moiety of quinolone ring

The next fragment of our research is devoted to making modifications into benzene and (or) other moieties of the quinolone ring only exclusively by the lead compound (Figure 5).

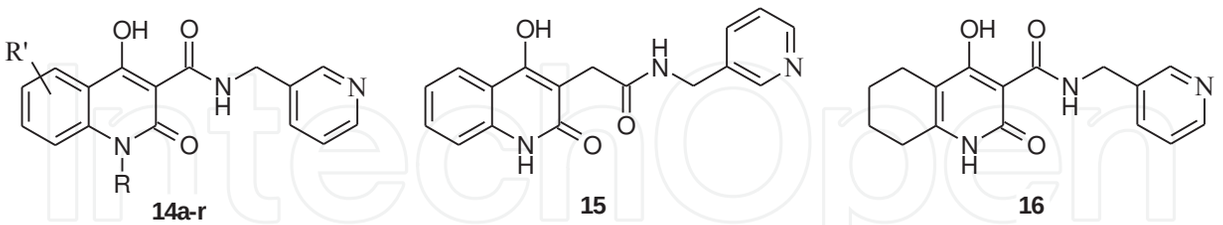

Figure 5. Structural analogs of the lead compound modified in benzene and other moieties of the quinolone ring

Pharmacological testing of this group of substances (Table 1) has demonstrated that on the model of "acetic acid induced writhing" with oral administration in the dose of 20 mg/kg they all are highly active analgesics, which do not yield or exceed the known drugs taken in the doses that correspond to their ED_{50} [46]. Therefore, there is every reason to believe that generally substituents in the quinolone ring affect weakly the analgesic properties of 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides. Nevertheless, *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide has still retained its

leading positions. In particular, it has been found that removal of one methoxy group from its molecule (amides **14a,b**), as well as 1-*N*-ethylation (amide **14j**) result in some decline of analgesic properties. Halogens in the benzene moiety of the molecule (amides **14c-i**) cause the similar effect; therefore, their presence should be also admitted as undesirable. The exception is only 6-bromine derivative **14g** appeared to be even somewhat more active than the lead compound. But in the whole, increase of the activity is quite negligible (see Table 1). In addition, in this case it is necessary to consider the possible increase of toxicity due to the presence of a halogen atom in the molecule.

Compound	R	R'	Analgesic activity (decrease in the amount of "acetic acid writhing", %)	Compound	R	R'	Analgesic activity (decrease in the amount of "acetic acid writhing", %)
14a	H	6-OMe	64.3	**14k**	H	H	70.6
14b	H	7-OMe	60.2	**14l**	Me	H	61.4
14c	H	6-F	51.2	**14m**	Et	H	50.2
14d	H	6,7-F_2	48.6	**14n**	All	H	75.9
14e	H	6-Cl	54.6	**14o**	Pr	H	74.3
14f	H	7-Cl	67.9	**14p**	Bu	H	63.1
14g	H	6-Br	78.3	**14q**	*i*-Bu	H	59.0
14h	H	6,8-Br_2	54.2	**14r**	Am	H	57.8
14i	H	6-I	69.7	**15**	–	–	45.0
14j	Et	6,7-$(OMe)_2$	63.4	**16**	–	–	80.7
Lead compound (20 mg/kg)			75.3	**Metamizole sodium** (55 mg/kg)			35.1
Piroxicam (20 mg/kg)			34.5	**Diclofenac** (5 mg/kg)			51.6
Piroxicam (92 mg/kg)			50.0	**Nabumetone** (50 mg/kg)			50.7

Table 1. The analgesic properties of picolyl-3-amides **14-16** on the the "acetic acid induced writhing" model ($p \leq 0.05$)

Cheap and more available synthetically picolyl-3-amides without substituents in the benzene moiety of the molecule (**14**, R' = H), especially 1-*N*-allyl (**14n**) and 1-*N*-propyl (**14o**) derivatives, demonstrated excellent indices. However, separation of 3-carboxamide and quinolone fragments by the methylene bridge, i.e. transfer to *N*-(3-pyridylmethyl)-2-(4-hydroxy-2-oxo-1,2-dihydro-3-quinolinyl)acetamide (**15**), influences on the analgesic activity negatively. According to the results of the primary screening it should be recognised that the most successful chemical modification of the lead compound is removal of both methoxy groups with the simultaneous reduction of the benzene moiety of the quinolone ring – *N*-(3-pyridyl-methyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**16**) appeared to be the most powerful analgesic of the given group. Unfortunately, after thorough analysis of all

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the pros and cons we had to refuse the further study of some highly reactive compounds for various reasons. Amides **14k-r**, for instance, were published earlier as objects for searching antituberculous drugs [47]. Therefore, their proper patent protection as analgesics is already impossible in principle. In case of hexahydroderivative **16** we faced another problems that were more serious.

3.4. Polymorphism of *N*-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide

A high analgesic activity of *N*-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**16**) found during the primary pharmacological screening caused, of course, an intense interest in it as a potentially new lead compound. However, the second sample of amide **16** sent to the biological laboratory unexpectedly demonstrated the result approximately two times lower than the first one. And they both were the products of the same synthesis! Multiple repeated experiment under the similar conditions for both samples simultaneously confirmed finally the significant differences in their analgesic properties. At first there were even doubts that we dealt namely with amide **16** in both cases. But NMR spectroscopy and combined gas chromatography mass-spectrometry assuaged these doubts rapidly and confirmed the absolute identity of the first and second samples.

Amide **16** is insoluble in water and it was introduced orally to the experimental animals as a fine aqueous suspension stabilized by Tween-80. Since the tested substance entered the organism as a solid, then the crystalline structure became one of the most probable factors influencing considerably on its biological properties [48]. The tendency of many substances to form various crystalline modifications (polymorphism) has attracted the attention of scientists for a long time. In particular, drug polymorphism is capable to change their characteristics so cardinally that currently all serious pharmaceutical manufacturers can not ignore this problem. And the government regulatory authorities also pay attention to the issues of obtaining, determination, description, purity and properties of crystalline forms of products used in pharmacy. As a result – today registration of a new drug in many countries of the world has become impossible without such information. It should, however, be recognised that although polymorphism has turned into an individual science, but it still remains an unsolved phenomenon of nature to a large extent. Until the present researchers only state the fact of formation of one or another polymorphic modifications of a substance. For the present one fails to predict theoretically or calculate this process and, particularly, predetermine conditions providing formation only the necessary polymorph.

Taking into account the given data we consider expedient to conduct the study of the phase composition of highly and lower active samples of *N*-(3-pyridylmethyl)-4-hydroxy-2-oxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (**16**) by the methods of X-ray powder and single-crystal X-ray structural analysis. Tailing of most peaks on the X-ray powder diffraction patterns complicated their analysis greatly and allowed to state with certainty only the fact that each sample consisted of several phases in various ratios. A thorough microscopic analysis led to similar results, but at the same separate shiny triclinic crystals suitable for conducting

the single-crystal X-ray structural research were observed in the total powder mass of the active sample (Figure 6).

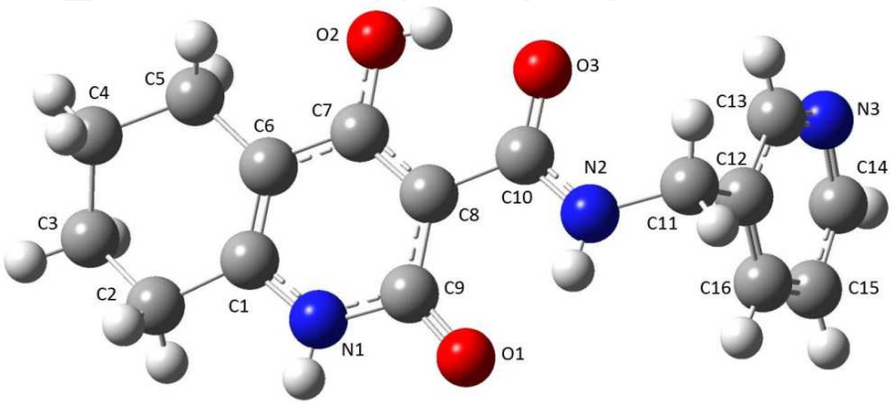

Figure 6. The structure of 1,2,5,6,7,8-hexahydroquinoline-3-carboxamide **16** molecule with numbering of the atoms

In the independent part of the elementary cell of this crystalline phase of amide **16** two molecules – **A** and **B** differing in some geometric parameter were found. The cyclohexone fragment in each of these both molecules is disordered by two *half-chair* conformations – **A1** and **A2**, **B1** and **B2** (folding parameters [49]: $S = 0.69$, $\Theta = 35.4°$, $\Psi = 29.9°$ 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 $O_{(1)}$–$C_{(9)}$, $O_{(3)}$–$C_{(10)}$ and $C_{(7)}$–$C_{(8)}$ are extended, and bonds of $O_{(2)}$–$C_{(7)}$ and $C_{(8)}$–$C_{(9)}$ are shortened comparing 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).

In the low active sample of amide **16** such crystalline phase has not found and it is probably the cause of decrease of its biological activity. This conclusion is not final, of course, since any

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polymorphic modification of amide **16** in the pure form has not been obtained and studied (as, for example, it was successful in the case of 6-hydroxy-*N*-(4-methoxyphenyl)-4-oxo-1,2-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinoline-5-carboxamide [50] passing clinical trials as a new quinolone diuretic). The external factors caused the changes of the phase composition of the second sample 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 (**16**) highly prone to polymorphism. And what's the main – it is not likely reasonable its further study as a potential pain-killer until at least the conditions, which would allow obtaining polymorphic modifications of this substance that are entirely highly active in regard to pharmacology and, not least importantly, with the guarantee of their stability while storing, are found.

4. Structure, physicochemical and analgesic properties of 4-R-2-oxo-1,2-dihydroquinoline-3-carboxylic acids

Even skimming of the scientific literature devoted to 4-hydroxyquinoline-2-ones reveals an extremely wide spectrum of biological properties that are common to these compounds. At the same time in the range of derivatives of 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids the overwhelming majority of publications is devoted to *N*-R-amides and products of their further chemical transformations. Esters are investigated much more rarely and the data concerning acids are practically absent at all. Meanwhile, being the basis of many *N*-R-amides possessing a high analgesic activity, 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids themselves also are of a certain interest as possible pain-killers.

4.1. 4-Hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids and their close analogues

There are few methods for obtaining 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acids known at present; moreover, all of them are similar and based on transformation of the corresponding esters [51]. It is our opinion that the most successful of them is hydrolysis in the AcOH–HCl–H_2O system, which allows to obtain target products with good yields and purity, as well as to avoid decarboxylation. It is this method that has been used in the synthesis of 4-OH-derivatives **17-19** (Figure 7, Table 2). Acids **20a**,**b** unsubstituted in position 4, their 4-chloro- (**20c**) and methyl (**20g-j**) derivatives are much more stable to decarboxylation and can be obtained by the common alkaline hydrolysis of lower alkyl esters of the corresponding quinoline-3-carboxylic acids. Only in the case of 4-alkyl- and 4-arylamino derivatives (**20e**,**f**) another synthetic scheme was used – interaction of alkylamines or anilines with 2-oxo-4-chloro-1,2-dihydroquinoline-3-carboxylic acids [51]

The ionization constants of the compounds synthesized determined by potentiometric titration show that they all are relatively weak acids. At the same time their dissociation constants (p*K*a) by the carboxy group consistently correlate with the influence of substituents present in the quinolone ring (Table 2). Of special note are 4-amino derivatives: 4-amono group (acid **20d**) possessing electron-donor properties decreases acidity of COOH-group so greatly that it

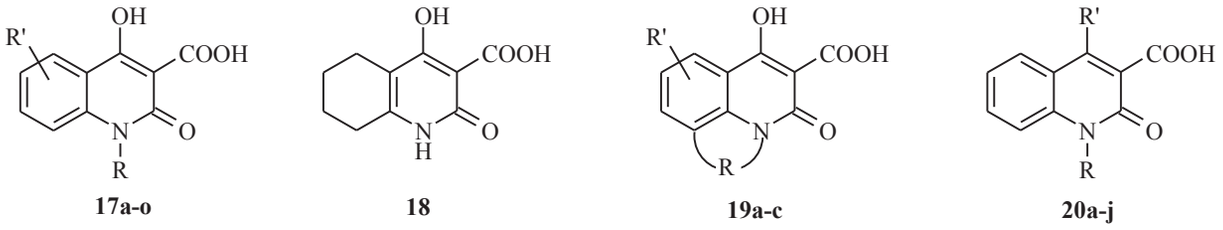

Figure 7. 2-Oxo-1,2-dihydroquinoline-3-carboxylic acids

could not be determined by potentiometric titration (the measurement rang is p*K*a ~ 14). The benzyl substituent in 4-amino group (acid **20e**) does not change the situation, and only aryl fragments (for example, 4-chlorophenyl in acid **20f**) promote some enhancement of acid dissociation of carboxyl. By comparison it is notable that many known drugs of nonnarcotic analgesics group (for example, Diclofenac or Ketorolac, Table 2) are so strong acids, from the chemical standpoint, that even being as salts they exert the ulcerogenic action and, therefore, have a lot of contraindications [52].

The study of the analgesic activity of acids **17-20** has been carried out by the method used when testing 1-*N*-allylsubstituted amides **6** described above. Thus, the experimental data obtained testify that in an hour after introduction of the tested compounds the pain threshold increases in all experimental animals by 7.2-77.3% comparing to the initial level (Table 2). In other words, in spite of significant differences in the potency of the effect exerted all acids **17-20** without any exception reveal analgesic properties. Thus, if the first representative of 4-hydroxy derivatives group – acid **17a** – does not yield Diclofenac in its activity, then introduction of N-alkyl, benzyl or phenyl substituents (acids **17b-g**) leads to the marked decrease of the analgesic action. At the same time carbamoylethyl derivative **17h** exceeds all the reference drugs used, including the narcotic analgesic Tramadol, by its analgesic effect.

In most cases modification of the benzene moiety of 4-hydroxy-2-oxo-1,2-dihydroquinoline ring (acids **17i-o**, **18**, **19**) negatively reflects on biological properties. Nevertheless, highly active compounds have been also found in this range. For example, 6-bromo-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**17l**) appeared to be a more powerful pain-killer than Tramadol. It is interesting that 6-bromo derivative appeared to be also the most active in the case of picolyl-3-amides **14** (see Table 1). However, additional bromine atom in position 8 (acid **17m**) almost completely deprives the molecule of analgesic properties. 4-Hydroxy-2-oxo-1,2,5,6,7,8-hexahydro- and 1-allyl-4-methyl-2-oxo-1,2-dihydroquinoline-3-carboxylic acids (**18** and **20i**, respectively) exceeding nonnarcotic analgesics Diclofenac and Ketorolac by specific activity and yielding Tramadol a little are also worthy.

However, of all 4-R-2-oxo-1,2-dihydroquinoline-3-carboxylic acids **17-20** considered we think 4-benzylamino derivative **20e** attracts the most interest. With its high analgesic activity this compound is surprisingly a very weak acid. That is why unlike Diclofenac and Ketorolac there should not be any serious gastrointestinal disorders with its possible medical application (at least in such pronounced form).

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Compound	R	R'	pKa^{COOH}	Analgesic activity (increase of the pain threshold, %)
17a	H	H	7.16	34.1
17b	Me	H	7.49	28.6
17c	Et	H	7.53	13.9
17d	All	H	7.30	14.4
17e	Pr	H	7.61	7.8
17f	Bn	H	7.15	17.2
17g	Ph	H	6.91	17.0
17h	$CH_2CH_2CONH_2$	H	7.06	77.3
17i	H	6-F	6.87	10.4
17j	H	6-Cl	6.76	7.2
17k	H	7-Cl	Insoluble	13.8
17l	H	6-Br	6.69	69.1
17m	H	6,8-Br_2	5.69	8.7
17n	H	6-I	6.63	34.6
17o	H	6,7-$(OMe)_2$	7.68	10.4
18	–	–	8.25	54.9
19a	$(CH_2)_2$	H	7.20	17.1
19b	$(CH_2)_3$	H	7.61	8.7
19c	$(CH_2)_2CH(Me)$	9-F	7.32	15.9
20a	H	H	8.74	30.5
20b	Pr	H	8.99	21.2
20c	Et	Cl	6.29	8.7
20d	H	NH_2	> 14	52.4
20e	H	NH-Bn	> 14	75.4
20f	H	NH-C_6H_4-Cl(4)	10.48	19.6
20g	H	Me	7.15	36.7
20h	Et	Me	7.10	33.4
20i	All	Me	6.95	51.5
20j	Pr	Me	7.17	15.6
	Diclofenac (10 mg/kg)		4.15	34.1
	Ketorolac (10 mg/kg)		3.49	46.4
	Tramadol (25 mg/kg)		-	57.2

Table 2. Acidic and analgesic properties of 2-oxo-1,2-dihydroquinoline-3-carboxylic acids **17-20**

4.2. 4-*N*-R-Substituted 2-oxo-1,2-dihydroquinoline-3-carboxylic acids and functional derivatives thereof

Naturally the combination of characteristics of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**20e**) that are important for a possible future drug have not gone unnoticed. This compound is of a real interest as an intermediate leading structure in the search of potential pain-killers with improved properties. With the purpose of revealing the structural fragments affecting the most actively manifestation of analgesic properties the synthesis of series of the closest analogs of this compound and their pharmacological screening have been carried out.

The first representative of modified derivatives was 4-benzylaminoquinoline-2-one (**21**, Figure 8) obtained readily by decarboxylation of the parent structure **20e** or by the reaction of 4-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylic acid with benzylamine in high-boiling solvents [53]. As it turned out, removal of the carboxy group from the molecule results in substantial reduction of the analgesic activity – at the same time the ability to increase the pain threshold three times decreases comparing to the initial acid **20e** (see Table 3). Esterification of the carboxy group (ethyl ester **22a**), 1-*N*-ethylation of the quinolone ring (acid **23**), as well as esterification with the simultaneous 1-*N*-alkylation (1-*N*-propylsubstituted ester **22b**) lead to the similar consequences. The result obtained is a convincing proof of the essential role of the carboxy group in exhibiting the biological effect, first of all. Introduction of 1-N-alkyl substituents, as judged by the examples described, is undesirable; though in general their impact is not so definite and it can be the subject of further study in principle.

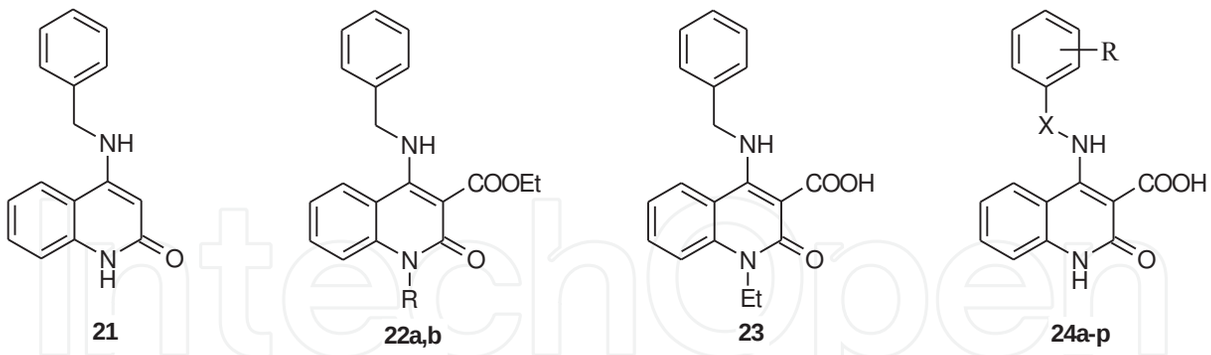

Figure 8. Modified analogs of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**20e**)

Taking into account the abovementioned facts all our further efforts concerning the chemical modification of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**20e**) were directed to make changes entirely to the benzyl moiety of its molecule. The synthesis of 4-N-R-substituted quinoline-3-carboxylic acids **24a-p** was carried out according to the scheme of the same type by interaction of the corresponding primary amines with 4-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylic acid in boiling ethanol (i.e. under conditions intentionally excluding the possibility of decarboxylation).

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The chemical modification of the benzyl moiety of acid **20e** conducted can be conditionally divided into three separate directions. The first two deal with separately the methylene unit or phenyl ring, respectively, the third involves both groupings simultaneously. The pharmacological testing has demonstrated that removal of the methylene bridge separating the secondary amino group and the aromatic ring (4-N-phenylsubstituted acid **24a**) is equal to decarboxylation described above by its effect on the analgesic properties, i.e. it also results in about three times decrease of the activity (Table 3). The replacement of the methylene unit by ethylene and, especially, propylene chains should be also considered unsuccessful. If with trasfer to 2-phenylethyl derivative **24b** the analgesic effect though twice decreases, but still remains at the level of Diclofenac, then in the case of 3-phenylpropylsubstituted acid **24c** it is practically lost at all.

Methylation of the methylene unit of acid **20e** has brought the unexpected results. As a result of such transformation one asymmetrical carbon atom appears in the molecule, hence, the final product can be racemic mixture **24d** or one of the enantiomers with *S*- or *R*-configuration of the chiral center (**24e** or **24f**, respectively). In the synthesis of these compounds racemic and optically pure 1-phenylethylamines are used; that is why the structure of aminoquinolines **24d-f** obtained on their basis is without any doubt. Depending on the spatial structure of the biological target and a number of other factors optical antipodes can exert both the same pharmacological properties and the properties varying so widely. Thus, preservation of the activity by a racemate usually observed in practice at the same level in the first case and its essential decrease or even its complete loss in the second case are quite logical. In this connection a rather high analgesic activity of racemic 1-phenylethylsubstituted quinoline-3-carboxylic acid **24d** on the background of optically pure enantiomers **24e** and **24f** that are absolutely inert in biological respect appears to be somewhat unexpected. The test substances introduced to the experimental animals as aqueous suspensions are insoluble in water and that is why it is not improbable that the cause of the effect found is in differences of crystalline forms. But the final conclusion on this point can be made only after special additional research.

The second direction of the chemical modification of lead compound **20e** is represented by 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acids **24g-n** containing substituents in the aromatic ring of the benzyl fragment. Unfortunately, in all the examples considered a stable tendency to decrease analgesic properties is observed irrespective to the nature of the substituents introduced and their position in the ring (Table 3).

And finally, the third way of modification of 4-N-benzyl substituent of acid **20e** intending introduction of changes into the methylene unit and the aromatic ring simultaneously is presented only by two compounds – optically active 4-[1-(4-methoxyphenyl)-ethylamino]-2-oxo-1,2-dihydroquinoline-3-carboxylic acids **24o** and **24p**. Here, the influence of the spatial configuration of asymmetric carbon on the strength of the analgesic effect is clearly visible: *S*-enantiomer **24o** is noticeably more active than its *R*-antipode **24p**. It is also interesting to note the fact that the methyl group introduced separately into the methylene unit (acid **24e**) or 4-methoxy introduced into the aromatic ring (acid **24l**) lead to the complete loss of the analgesic properties by basic structure **20e**. However, the effect of the same substituents introduced

Compound	R	X	Analgesic activity (increase of the pain threshold, %)
21	–	–	24.8
22a	H	–	18.0
22b	Pr	–	28.8
23	–	–	7.6
24a	H	none	26.0
24b	H	$(CH_2)_2$	35.2
24c	H	$(CH_2)_3$	7.5
24d	H	(±) CH(Me)	40.1
24e	H	*S*(+) CH(Me)	2.2
24f	H	*R*(–) CH(Me)	2.0
24g	4-F	CH_2	35.0
24h	2-Cl	CH_2	42.7
24i	4-Cl	CH_2	18.1
24j	4-Me	CH_2	20.5
24k	2-OMe	CH_2	5.8
24l	4-OMe	CH_2	11.9
24m	3,4-$(OMe)_2$	CH_2	28.3
24n	3-O-CH_2-O-4	CH_2	32.2
24o	4-OMe	*S*(+) CH(Me)	46.1
24p	4-OMe	*R*(–) CH(Me)	31.6

Table 3. Analgesic properties of 4-amino-2-oxo-1,2-dihydroquinolines **21-24** on the model of rectal mucosa irritation by electric current ($p \leq 0.05$)

simultaneously is not any more categorical. In particular, the activity of acid **24o** remains unchanged at the level of one of the most powerful nonnarcotic analgesics Ketorolac.

Thus, according to the results of the research performed the conclusion can be made that in the structure of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acids the carboxy group plays a key role in the process of binding with receptors beyond any doubt. The benzyl group is one more important structural fragment providing a large interaction with a biological target. At the same time the role of 1-*N*-alkyl substituents is not so simple and requires more profound study. The significance of the benzene moiety of the quinolone ring, as well as the secondary amino group in position 4 remains completely unclear.

4.3. The crystalline structure of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids as the factor that their analgesic activity

When studying biological properties of substances containing asymmetric carbon various situations are possible such as: enantiomers show the same clinical picture [54]; only one isomer stipulates the desirable effect, whereas the second one is low active or inactive at all [55]; enantiomers reveal quite different (sometimes directly opposite) physiological properties [56]; one of the isomers is unambiguously harmful [57]. It is clear that only in the first case drugs prepared on the basis of optically active compounds can be racemic mixtures. In all other situations it is expedient to use one of the enantiomers. However, it should be remembered that actually sometimes even under the most favorable pharmacological indications in favor of one of the optical isomers a drug racemate enters the market after all since obtaining the required optically pure enantiomer presents various difficulties [58].

One more varient of manifestation of biological properties by chiral compounds is theoretically possible, and it can occur in practice (although quite rarely), when a racemate appears to be much more active than enantiomers [59]. Frequently this phenomenon is explained by synergy of effects that are inherent to each of the optical isomers individually (see, for example, a detailed study of the mechanism of the analgesic action of Tramadol [60]). We came up against a similar situation while investigating 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids **24d-f**. However, there are some differences, which can not be explained only by synergy – racemate **24d** (**R**) appeared to reveal the marked analgesic activity on the background of enantiomers **24e,f** (**E**) being practically inert in the biological respect. We tried to find out the cause of this effect in this section.

Acids **24d-f** are insoluble in water and introduced orally as aqueous suspensions to the experimental animals. That is why previously we made an assumption about possible dependence of the pharmacological action on the crystalline structure of the substances under study, the more especially as there are many examples of interactions of such kind [48].

The X-ray diffraction analysis has demonstrated that optically pure enantiomers *S*- and *R*-configuration **24e,f** obtained independently have the same crystalline structure and, as we might expect for chiral compounds, they are crystallized in the noncentrosymmetric space group $P2_1$ [53]. On the contrary, racemate **24d** crystallizes in the centrosymmetric space group $P2_1/n$.

The comparative analysis of the structure of the racemic and enantiomeric molecules (on the example of an isomer with *R*-configuration of the chiral center) of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids has show that it is generally rather similar. In both cases the heterocycle, nitrogen atom $N_{(2)}$, carboxide and carboxy group are in the same plane with accuracy to 0.02 Å (Figure 9), it is conditioned by formation of two strong intramolecular hydrogen bonds: $N_{(2)}$–$H_{(2N)}$…$O_{(2)}$ [H…O 1.81 Å, N–H…O 146° in the enantiomer structure and H…O 1.74 Å, N–H…O 150° in the racemate] and $O_{(3)}$–$H_{(3O)}$…$O_{(1)}$ [H…O 1.43 Å, O–H…O 148° in **E**, H…O 1.59 Å O–H…O 154° in **R**]. As a result of formation of hydrogen bonds a marked electron density redistribution also occurs in the quinolone fragment as evidenced by bond lengthening of $O_{(1)}$–$C_{(9)}$ to 1.273(1) Å in **E** and to 1.268(2) Å in **R**, $O_{(2)}$–$C_{(10)}$,

to 1.234(2) Å in **E** and to 1.225(2) Å in **R** comparing to their mean value of 1.210 Å, as well as the bond of $C_{(7)}$–$C_{(8)}$ to 1.410(2) Å in **E** and to 1.418(2) Å in **R** (the mean value is 1.326 Å). At the same time some bonds are shortened on the contrary: $O_{(3)}$–$C_{(10)}$ to 1.316(1) Å in **E** and to 1.327(2) Å in **R** (1.362 Å), $C_{(8)}$–$C_{(9)}$ to 1.420(2) Å in **E** and to 1.433(2) Å in **R** (1.455 Å).

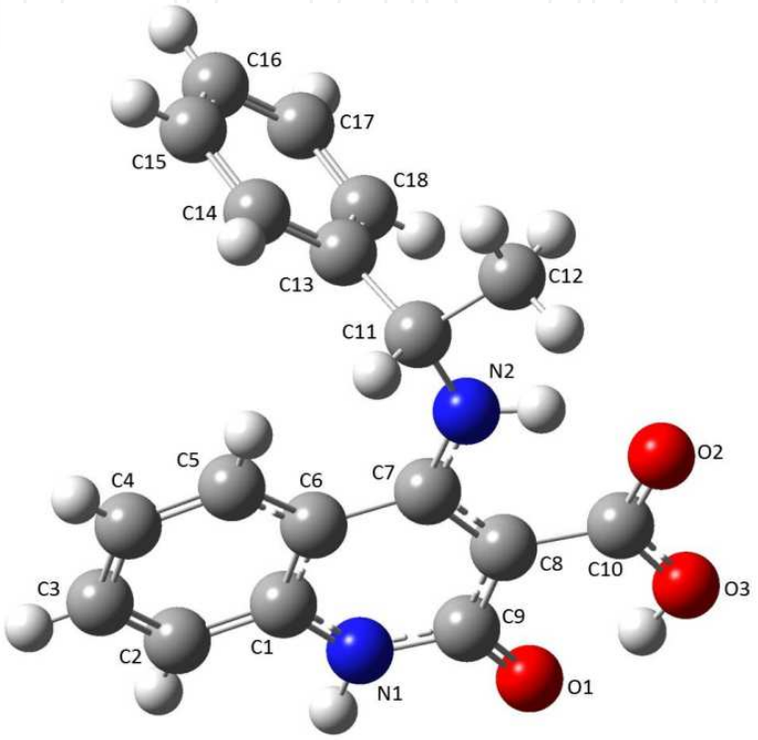

Figure 9. The structure of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids **24d-f**

The substituent at the amino group is in *syn*-periplanar conformation in relation to $C_{(6)}$–$C_{(7)}$ bond [the torsional angle is $C_{(11)}$–$N_{(2)}$–$C_{(7)}$–$C_{(6)}$ -19.7(2)° in **E** and -1.6(2)° in **R**] and turn in such way that the methyl group is in –*ac*-orientation in relation to $C_{(7)}$–$N_{(2)}$ bond in the structure **E** and in *ap*-orientation in **R** [the torsional angle is $C_{(7)}$–$N_{(2)}$–$C_{(11)}$–$C_{(12)}$ -143.0(2)° in **E** and 171.3(1)° in **R**]. The phenyl substituent is practically perpendicular to $C_{(7)}$–$N_{(2)}$ bond and somehow turn to $N_{(2)}$–$C_{(11)}$ bond in the enantiomer structure [torsional angles are $C_{(7)}$–$N_{(2)}$–$C_{(11)}$–$C_{(13)}$ 94.6(2)° and $N_{(2)}$–$C_{(11)}$–$C_{(13)}$–$C_{(14)}$ 10.7(2)°]. In the racemate the phenyl substituent is in –*sc*-conformation in relation to $C_{(7)}$–$N_{(2)}$ bond and noticeably turn to $N_{(2)}$–$C_{(11)}$ bond [torsional angles are $C_{(7)}$–$N_{(2)}$–$C_{(11)}$–$C_{(13)}$ -67.3(2)° and $N_{(2)}$–$C_{(11)}$–$C_{(13)}$–$C_{(18)}$ -36.3(2)°]. Such position of the substituent at the amino group leads to appearance of a strong repulsion between it and atoms of the aromatic cycle of the quinolone fragment [shortened contacts are $H_{(5)}$…$C_{(11)}$ 2.46 Å in **E** and 2.44 Å in **R** (the sum of van der Waal radii is 2.87 Å), $H_{(5)}$…$H_{(11)}$ 2.07 Å in **E** and 1.98 Å in **R** (2.34 Å), $H_{(5)}$ …$C_{(13)}$ 2.42 Å in **E** and 2.57 Å in **R** (2.87 Å), $H_{(11)}$…$C_{(5)}$ 2.65 Å in **E** and 2.75 Å in **R** (2.87 Å), $H_{(5)}$

…$C_{(14)}$ 2.77 Å in **R** (2.87 Å), $C_{(11)}$…$C_{(5)}$ 3.09 Å in **E** and 3.10 Å in **R** (3.42 Å), $C_{(13)}$…$C_{(5)}$ 3.30 Å in **E** and 3.22 Å in **R** (3.42 Å)]. As is known [61], the benzene ring is conformationally flexible and under the influence of the environment can be rather deformable. From these considerations we have sugested that the steric strain in the enantiomer structure is partially compensated by disflattening of the aromatic cycle of the quinolone fragment, distortion in some torsional angle, as well as some pyramidalization of nitrogen atom of the amino group [53]. In the racemate structure the steric strain is compensated only by the substituent's deviation at atom $C_{(7)}$ from the quinolone fragment plane [the torsional angle is $C_{(5)}$–$C_{(6)}$–$C_{(7)}$–$N_{(2)}$ -7.6(2)°]. The shortened intramolecular contacts of $H_{(2)}$…$H_{(1N)}$ 2.23 Å in **E** and 2.29 Å in **R** (2.34 Å), $H_{(12a)}$…$H_{(2N)}$ 2.24 Å in **E** (2.34 Å), $H_{(12b)}$…$C_{(18)}$ 2.78 Å in **E** (2.87 Å) and $H_{(14)}$…$N_{(2)}$ 2.51 Å in **E** (2.67 Å) have been also found in the molecule.

Packing of molecules in crystals of chiral and racemic 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids is much more different. For example, molecules of pure enantiomers form endless zigzag chains in the crystal along the crystallographic line [0 1 0] owing to intermolecular H-bonding of $N_{(1)}$-$H_{(1N)}$…$O_{(2)'}$ (- x, 0.5 + y, 1 - z) H…O 2.15 Å, N–H…O 148° (Figure 10). In turn, these chains form stacks along the crystallographic line [1 0 0], in which the distance between the aromatic cycle of the bicyclic fragment and π-system of carbonyl and carboxy groups of adjacent molecules is 3.37 Å; it allows to suggest the existence of stacking interaction between them. The intermolecular C–H…π hydrogen bond of $C_{(16)}$–$H_{(16)}$…$C_{(9)'}$ (x, y, 1 + z) (H…π 2.84 Å, C–H…π 152°) has been also found in the enantiomer crystal.

In the crystal of racemate the molecules of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acid form centrosymmetric dimers owing to intermolecular H-bonding of $N_{(1)}$-$H_{(1N)}$…$O_{(1)'}$ (1 - x, 1 - y, -z) H…O 1.79 Å, N–H…O 175° (Figure 11). The distance between π-systems of adjacent dimers (3.49 Å), as well as degree of their overlapping allow to assume the existence of stacking interaction. Adjacent dimers are bound with each other by weak intermolecular hydrogen bonds of C–H…π: $C_{(12)}$–$H_{(12b)}$…$C_{(10)'}$ (x, 1 + y, z) (H…π 2.81 Å, C–H…π 130°) and $C_{(11)}$–$H_{(11)}$…$C_{(9)'}$ (x, 1 + y, z) (H…π 2.85 Å, C–H…π 145°).

Thus, the research conducted shows the essential distinctions in the crystalline structure of enantiomeric and racemic 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids. It is known [48] that it is this factor that often determines the most important pharmacokinetic determinants of the drug biological action such as bioavailability, distribution in tissues, metabolic rate, etc. Therefore, it can serve as a prime cause of differences in analgesic properties of the substances studied. It is evident that specific packing of the racemate molecules in the crystal promotes their easy bioavailability – hence it is its higher activity. The satisfactory evidence of this conclusion is the fact that the mechanical racemate of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acids obtained by a simple mixing of equimolar amounts of optically pure enantiomers **24e** and **24f** without subsequent crystallization (it is its fundamental difference from the true single-crystal racemate **24d** described above) is no different by the biological properties from the chiral products composing it. In other words, bioavailability of enantiomers of 2-oxo-4-(1-phenylethylamino)-1,2-dihydroquinoline-3-carboxylic acid **24e**,**f** remains low irrespective of how they are introduced into the organism of an experimental animal – individually or as a simple mechanical mixture.

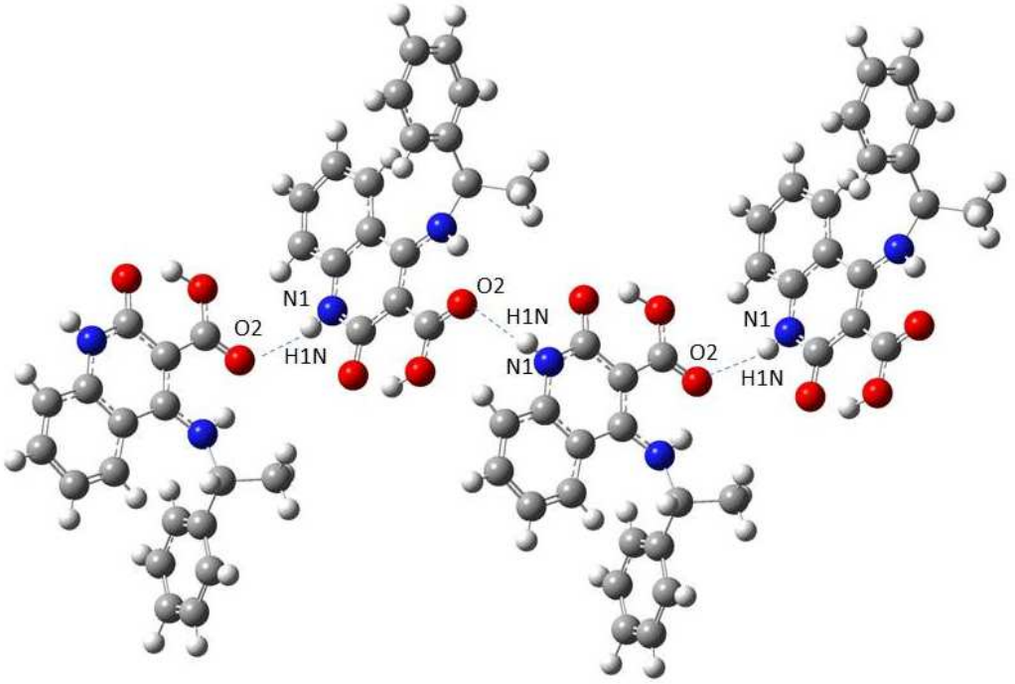

Figure 10. Zigzag chains formed in the crystal by the molecules of enantiomers **24e,f**. The dotted lines indicate the intermolecular hydrogen bonds

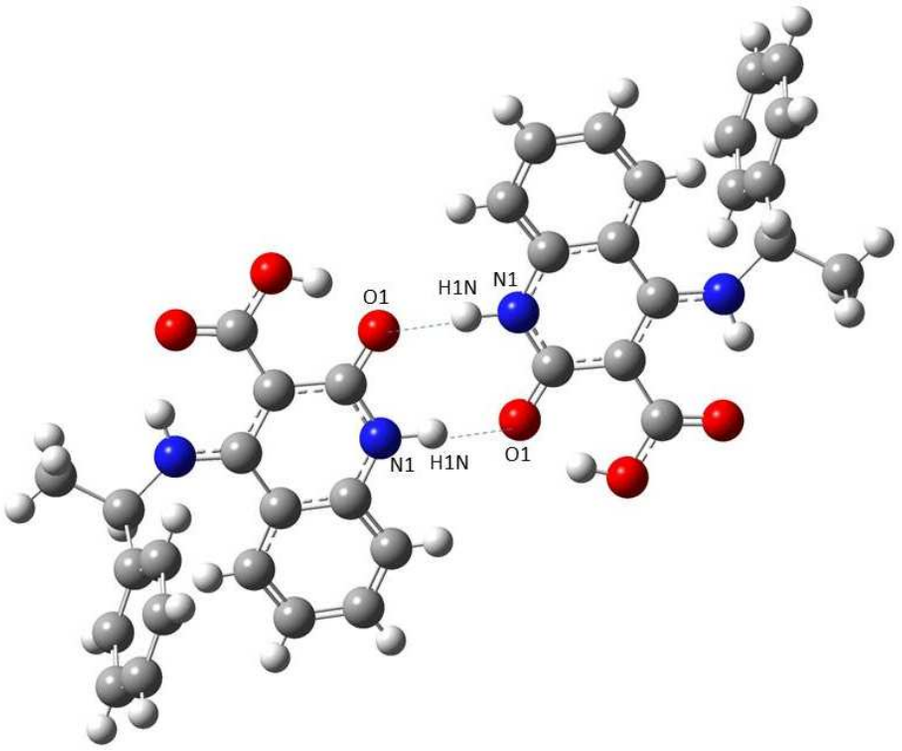

Figure 11. Centrosymmetric dimers formed in the crystal by the molecules of racemate **24d**. The dotted lines indicate the intermolecular hydrogen bonds

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Taking this circumstance into account the research of the phase composition revealing the biological activity of a single-crystal racemate is of interest. The X-ray phase analysis [62] performed has demonstrated that the sample is single-phased and fully corresponds to the racemate's structure determined for a single crystal. Impurity lines, including those that could refer to the structure of one of the enantiomers crystallized in group $P2_1$ on the powder diffraction pattern have not been found. Since the parameters of a primitive unit cell of the crystals of enantiomer (**E**) and racemate (**R**) differ markedly, one may state that the X-ray phase analysis was carried out with considerably fine precision.

4.4. 4-(Hetarylmethyl)amino-2-oxo-1,2-dihydroquinoline-3-carboxylic acids

The conception of bioisosteric replacements suggested at the beginning of the last century [44] currently remains one of the most powerful means for creating effective and safe medicines [40-43]. Its application allows not only to optimize biologically active substances already known, but to reveal new structures with the similar or related properties and so to enhance the patent protection of a future drug.

This methodology has proven its value completely while working with 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**12**). That is why we attempted once more to use it in our research – now for modification of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**20e**), which we made by the classical isosteric replacement of the benzene ring with the heterocycle being similar in many physical and chemical characteristics. As is known [44], they are pyridine, thiophene and in some way furan.

The target 4-(hetarylmethyl)amino-2-oxo-1,2-dihydroquinoline-3-carboxylic acids **25a-f** (Figure 12) have been synthesized by the interaction of the corresponding primary amines with 4-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylic acid [63]. In parallel with hetarylmethyl amino substituted acids **25a-f** cyclohexyl derivative **25g** has been obtained. Of course, this compound cannot be classed to heteroanalogs of acid **20e**, however, the possibility of finding additional information concerning the key functional groups owing to it became a solid ground to its synthesis. By these reasons the reaction of 4-chloro-2-oxo-1,2-dihydroquinoline-3-carboxylic acid with some secondary amines has been studied. Unfortunately, the corresponding quinoline-3-carboxylic acids with tertiary amino groups in position 4 appeared to be extremely unstable substances readily decarboxylized in boiling ethanol immediately after their formation. As a result, we succeded only in isolating 4-N-R,R'-aminoquinolin-2-ones **26** and **27**. Nevertheless, there is a benefit from the experiments carried out. Firstly, they allow to clarify that 4-amino-2-oxo-1,2-dihydroquinoline-3-carboxylic acids are relatively stable only with the presence of even one proton in the amino group. Secondly, the substances obtained are themselves of interest for pharmacological research as a particular type of new structural analogs of the basic molecule. And still, the initially entirely specific task set of this experiment – explain the role of 4-NH-proton in the process of binding of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid with a biological target – has not be solved yet.

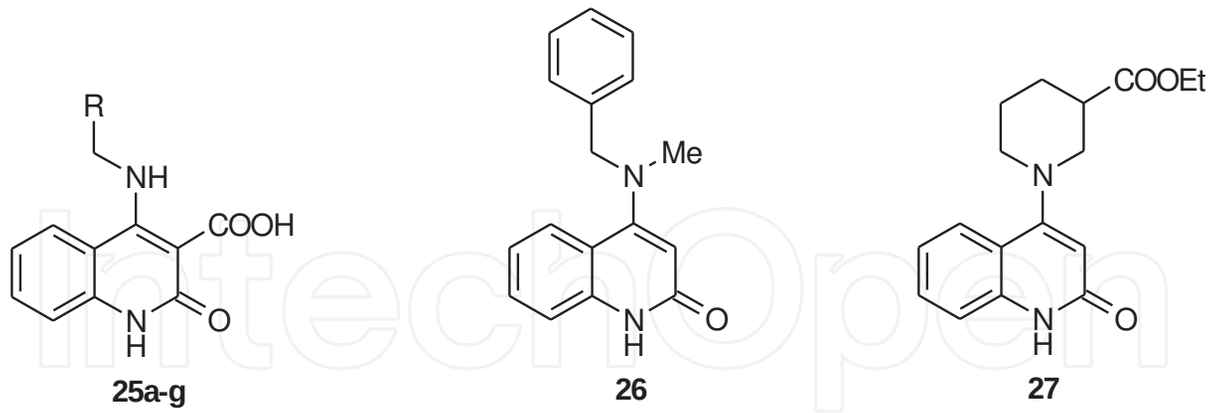

Figure 12. Heteroanalogues of 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**20e**)

The analgesic activity of aminoquinolines **25**-**27** has been studied under conditions being similar to those in testing quinoline-3-carboxamides **12**. Analysis of the data presented in Table 4 shows that our replacement of the aromatic ring of the benzyl fragment in 4-benzylamino-2-oxo-1,2-dihydroquinoline-3-carboxylic acid by isosteric heterocycle is mainly accompanied with some decrease in the analgesic properties. In the case of pyridine derivatives the dependence of the potency of the effect exerted on the position of the nitrogen atom is distinctly visible. Thus, pyridine-3-ylmethylamine substituted acid **25b** does not practically differ from benzyl analog **20e** by its activity, whereas *ortho*-isomer **25a** yields it more than three times.

Benzene has much more similar physical and chemical characteristics with thiophene then with furan [44]. Therefore, it is quite regular that thiophenemethyl derivative **25f**, but not furfuryl analogs **25d,e**, is closer to benzyl prototype **20e** by its biological properties.

Compound	R	Analgesic activity (decrease in the amount of "acetic acid writhing", %)	Compound	R	Analgesic activity (decrease in the amount of "acetic acid writhing", %)
25a	2-Py	21.8	**25f**	Thiophen-2-yl	49.1
25b	3-Py	65.4	**25g**	*cyclo*-C_6H_{11}	46.5
25c	4-Py	34.9	**26**	–	40.3
25d	Furan-2-yl	39.5	**27**	–	16.2
25e	5-Me-furan-2-yl	39.5	**20e**		69.8

Table 4. Analgesic properties of aminoquinolines **25**-**27** on the model of "acetic acid induced writhing" ($p \leq 0.05$)

Cyclohexylmethylamine substituted acid **25g** deserves individual attention, first of all, because it maintains rather strong influence on the pain reaction in spite of a significant conformation rearrangement of 4-N-fragment subjected to modification as compared to a flat benzyl

prototype. This example testifies the possible perspectives of the given direction development involving hydrogenized analogs of other molecular systems, including heterocyclic ones, in the range of the objects studied.

We came to the conclusion of necessity to continue our research after testing 4-N-R,R'-aminoquinoline-2-ones **26** and **27**. The reason for this was a surprisingly high analgesic activity of 4-(benzylmethylamino)-1*H*-quinoline-2-one (**26**). As previously thought [53], removal of the carboxy group from the molecule inevitably resulted in the essential decrease of analgesic properties. However, as it happens, the presence of 3-carboxy group is already not always necessary for 4-aminoquinoline-2- ones with two substituents in 4-amino group.

5. (4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)acetic acid and its esters

Pain and inflammation belong to the most widespread signs accompanying numerous pathological states. To eliminate these manifestations NSAIDs are currently widely used; among them derivatives of aryl- and hetarylacetic acids – Diclofenac, Aceclofenac, Indometacin, Clinoril, Etodolac, etc., occupy an important place [14, 52]. In this regard, involvement of (4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl)acetic acid and its derivatives in searching new pain-killers conducted by us is logical and regular.

The synthesis of the initial (4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)acetic acid (**28**, Figure 13) has been carried out by acylation of methyl *N*-methylanthranilate with β-methoxycarbonylpropionyl chloride with subsequent treatment of the intermediate sodium anilide by methylate in methyl alcohol. The mixture of methyl esters of quinolin-3-yl)acetic and benzoazepine-4-carboxylic acids formed in the course of this reaction is subjected to hydrolysis and recyclization into the same final product – (quinolin-3-yl)acetic acid **28** when treating with the aqueous solution of KOH [64]. Esterification of this compound catalyzed by acids gives alkyl (4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)acetates (**29**) with high yields; they are also of interest for pharmacological testing.

Figure 13. (4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)acetic acid and its esters

One of the characteristic criteria of efficiency for anti-inflammatory drugs is the anti-exudative action. In this regard we began testing the biological properties of the compounds synthesized with studying their effect on the exudative phase of acute aseptic inflammation. The research was conducted on the model of carrageenan edema in mice [65]. As a reference drug the classic nonsteroidal anti-inflammatory drug – Diclofenac in the dose of 8 mg/kg (ED_{50}) was used. The results obtained show that the initial quinoline acetic acid **28** in the equimolar dose to Diclofenac can decrease the carrageenan edema size by 23.1% (Table 5). Esterification affects the anti-exudative properties especially successful. Among the compounds synthesized the substances, which do not practically yield Diclofenac in their activity (esters **29b**,**f**,**h**) and even exceed it somehow (allyl ester **29c**) have been found. In this range of compounds the interesting dependence has been revealed – transfer from esters with the normal *O*-alkyl chains to derivatives of the *iso*-structure is accompanied almost complete loss of the anti-inflammatory action.

But for the analgesic properties of quinolinylacetic acid **28** and its esters **29** ("acetic acid induced writhing", $p \leq 0.05$, details see Quinoline-3-carboxamides **12**) this structural biological regularity is not already characteristic. Although here most of esters appeared to be much more active than the initial acid.

Compound	R	Anti-inflammatory activity(edema reduction, %)	Analgesic activity (decrease in the amount of "acetic acid writhing", %)
28	–	23.1	28.5
29a	Me	12.7	64.2
29b	Et	45.5	54.4
29c	All	52.5	24.1
29d	Pr	20.4	33.9
29e	*i*-Pr	3.1	39.3
29f	Bu	46.2	50.2
29g	*i*-Bu	27.3	50.2
29h	C_5H_{11}	44.5	35.9
29i	*i*-C_5H_{11}	9.6	22.1
Diclofenac (8 mg/kg)		49.8	–
Diclofenac (5 mg/kg)		–	51.6

Table 5. Anti-inflammatory and analgesic properties of quinolinylacetic acid **28** and its esters **29** ($p < 0.05$)

The X-ray diffraction study of the spatial structure of the most powerful pain-killer from the esters group – methyl quinolinylacetate **29a –** has allowed to determine that the quinolone ring in the molecule of this compound is incompletely planar: the torsional angle $C_{(1)}$–$N_{(1)}$–$C_{(9)}$–$C_{(8)}$ is -5.8(2)° (Figure 14). Hence, a shortened intramolecular contact of $H_{(5)}$…$O_{(2)}$ 2.40 Å (the

sum of van der Waal radii 2.46 Å) appears. The methoxycarbonyl fragment of the substituent at atom $C_{(8)}$ is located orthogonally to the plane of the bicycle and turn a little in relation to $C_{(8)}$–$C_{(10)}$ bond [torsional angles are $C_{(7)}$–$C_{(8)}$–$C_{(10)}$–$C_{(11)}$ 93.9(1)° and $C_{(8)}$–$C_{(10)}$–$C_{(11)}$–$O_{(3)}$ -19.7(2)°]. The methyl group is in *ap*-conformation in relation to $C_{(10)}$–$C_{(11)}$ bond [the torsional angle is $C_{(12)}$–$O_{(4)}$–$C_{(11)}$–$C_{(10)}$ 178.3(1)°].

A rather strong repulsion has been detected between atoms of the methyl group at atom $N_{(1)}$ and adjacent atoms of the carbonyl group $C_{(9)}$–$O_{(1)}$ and hydrogen atom in peri-position of the benzene ring; shortened intramolecular contacts of $H_{(2)}$…$C_{(13)}$ 2.53 Å (2.87 Å), $H_{(2)}$…$H_{(13c)}$ 2.27 Å (2.34 Å), $H_{(13c)}$…$C_{(2)}$ 2.74 Å (2.87 Å) and $H_{(13a)}$…$O_{(1)}$ 2.24 Å (2.46 Å) testify about it.

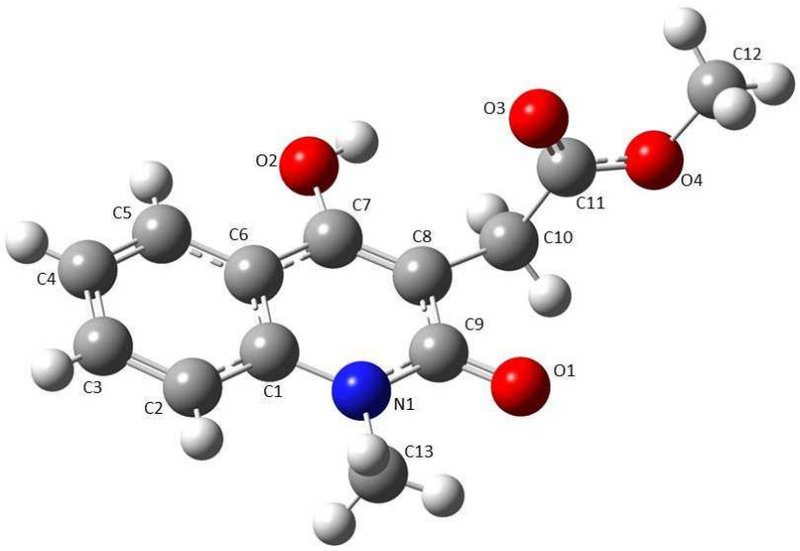

Figure 14. Structure of the methyl quinolinylacetate **29a** molecule with numbering of the atoms

Molecules of methyl quinolinylacetate **29a** form endless zigzag chains in the crystal (Figure 15) along the crystallographic line [0 0 1] owing to intermolecular H-bonding of $O_{(2)}$–H…$O_{(1)'}$ (x, 0.5 - y, 0.5 + z) H…O 1.76 Å, O–H…O 160°. It seems that formation of this hydrogen bond stipulates $C_{(9)}$–$O_{(1)}$ 1.251(1) Å bond lengthening comparing to its mean value 1.210 Å. The system of intermolecular C–H…π hydrogen bonds: $C_{(12)}$–$H_{(12a)}$…$C_{(5)'}$ (x, 0.5 - y, -0.5 + z) H…π 2.78 Å, C–H…π 172°; $C_{(13)}$–$H_{(13a)}$…$C_{(11)'}$ (x, 0.5 - y, -0.5 + z) H…π 2.84 Å, C–H…π 138° and $C_{(13)}$–$H_{(13b)}$…$C_{(5)'}$ (-x, 1 - y, 1 - z) H…π 2.81 Å, C–H…π 148° has also been found in the crystal.

A comparative analysis of X-ray diffraction data of methyl quinolinylacetate **29a** and its ethyl analog **29b** [66] reveals a remarkable resemblance not only the peculiarities of the spatial structure of these compounds, but their crystalline packing as well. In this connection and taking into account the abovementioned examples of a significant influence of the crystalline structure of 4-hydroxyquinolin-2-ones on their biological activity, the related analgesic properties of esters **29a** and **29b** are quite logical. In addition, significant differences in the anti-inflammatory action of these substances are an eloquent evidence of the fact that the crystalline structure is though important, but not the only factor determining the pharmacological properties of a substance.

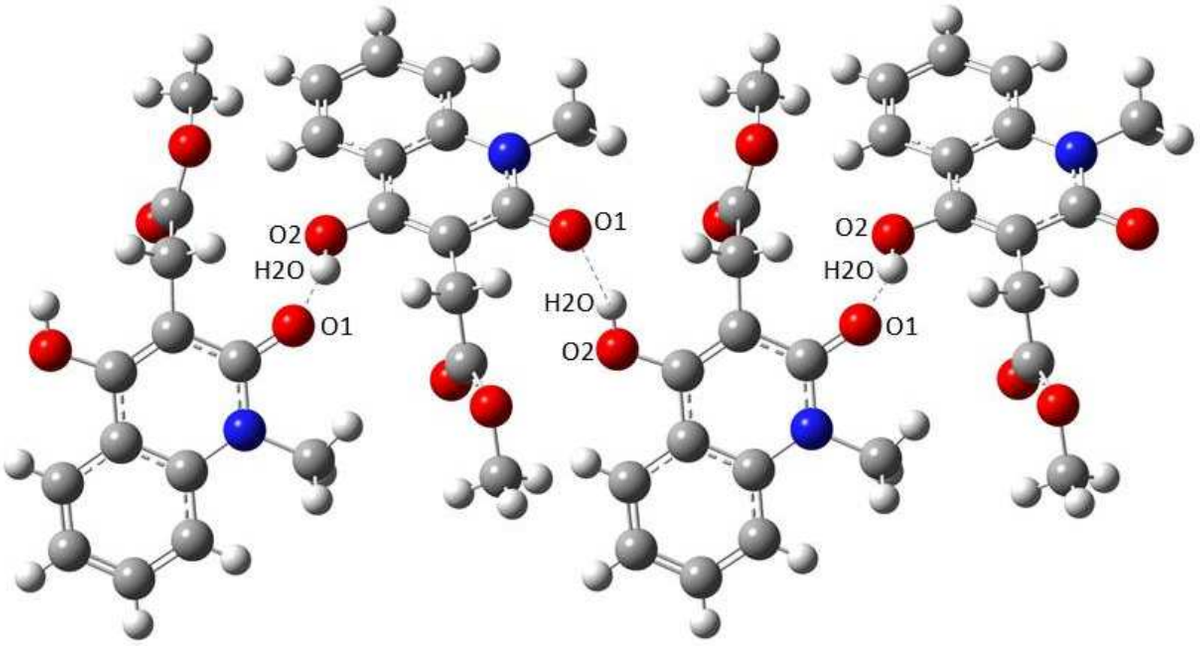

Figure 15. Endless zigzag chains formed in the crystal by molecules of methyl quinolinylacetate **29a**. The dotted lines indicate the intermolecular hydrogen bonds

6. The study of *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide as a promising pain-killer

According to the results of the primary pharmacological screening only one compound – *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**12**, R = CH_2Py-3) has been selected as a lead compound from the large group of 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides, their closest structural analogs and some derivatives. After oral introduction to white mice in the dose of 20 mg/kg this compound is able to reduce the number of writhings caused by intraperitoneal injection of acetic acid by 75.3 % (see Table 1). Picolyl-3-amide **12** has also demonstrated a high activity – 81.1% ($p < 0.05$) – on the model of "kaolinic writhings" used for research of the peripheral component of the analgesic effect [65].

The effect of the lead compound on the central component of the nociceptive system has been studied *in vivo* on the models involving the central mechanisms of the pain formation: thermal or electric irritation of the murine paw, as well as thermal irritation of the rat's tail and electric stimulation of the rat's tailhead [65]. All experiments have been carried out according to the same scheme: *1* – determination of the initial level of algesthesia in all animals induced by the appropriate nociceptive irritator; *2* – oral introduction of picolyl-3-amide **12** to the experimental animals in the dose of 20 mg/kg and the solvent to the control group of animals; *3* – monitoring of the pain threshold in every 30 minutes during 5 hours; *4* – calculation of the analgesic activity comparing to control.

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It has been found that on the model of the thermal irritation of paws ("hot plate") sensitivity of mice to pain already decreases by 39.4% in 30 minutes after the beginning of the experiment. In general the analgesic effect lasts about 4.5 hours reaching its maximum in 75.7% ($p < 0.05$) at the point of 2.0 hours. After the change of the thermal irritator by the electric one the picture observed is practically the same – with the maximum of 90.1% ($p \leq 0.05$) during the second hour and further with smooth decline in activity.

On the model of the thermal irritation of the rat's tail ("tail flick") the maximum analgesic effect – 101.0% ($p < 0.05$) already develops in 1 hour after introduction of picolyl-3-amide **12** and retains at ths level during the hour. By the end of testing, i.e. by the 5-th hour, the analgesic activity consistently decreases though its level still remains rather noticeable (32.4%).

When using electrostimulation of the rat's tailhead the pain threshold increases not so rapidly – during the first 30 minutes its growth is only 10.5%. However, further the potency of the analgesic action quickly grows and by the second hour of the experiment it exceeds the control indices by 90.9% ($p < 0.05$), after that it gradually decreases.

A high activity of picolyl-3-amide **12** shown on the models of pains of the central origin allow to suggest about the receptor mechanism of its analgesic effect. To confirm or dispose this assumption we carried out a series of experiments in studying the influence of the lead compound on opioid, adrenergic and dopaminergic receptors. Besides, a possible participation of GABA-ergic links of the central nociceptive system in the mechanism of its analgesic action was checked. All investigations of this series were conducted on the model of the thermal irritation of the rat's tail ("tail flick") according the scheme described above with the only difference that another two groups of animals were added – those taken the known reference drug and its combination with the new substance under research. In all experiments Picolyl-3-amide **12** was introduced orally in the dose of 20 mg/kg as a fine aqueous suspension stabilized by Tween-80. The reference drugs were introduced orally or intraperitoneally in the doses recommended for each of them [67]. When working with combinations of substances at first a reference drug was introduced, then in 20 minutes the lead compound was introduced.

As the experiments showed, analgesic effects of picolyl-3-amide **12** demonstrated by it when taken alone and on background of the preliminary introduction of Naloxone (3.0 mg/kg) differ slightly (Figure 16). Therefore, the lead compound does not have a substantial effect on opioid receptors.

The study of the possible participation of the adrenergic system in the mechanism of the analgesic action of picolyl-3-amide **12** was conducted with the help of α_2-adrenoceptor agonist Clonidine (0.02 mg/kg) and β-adrenergic blocking agent Propranolol (14.5 mg/kg). Analysis of the data obtained testifies that the lead compound in combination with Clonidine losses the most part of its initially high analgesic properties – especially during the first 2.5 hours of the experiment (Figure 17). The same picture can be observed in the case of its combination with Propranolol (Figure 18). It entitles us to believe that picolyl-3-amide **12** exerts its analgesic activity through, at least, partial blocking of central α_2-adrenoreceptors and activation of β-adrenoreceptors.

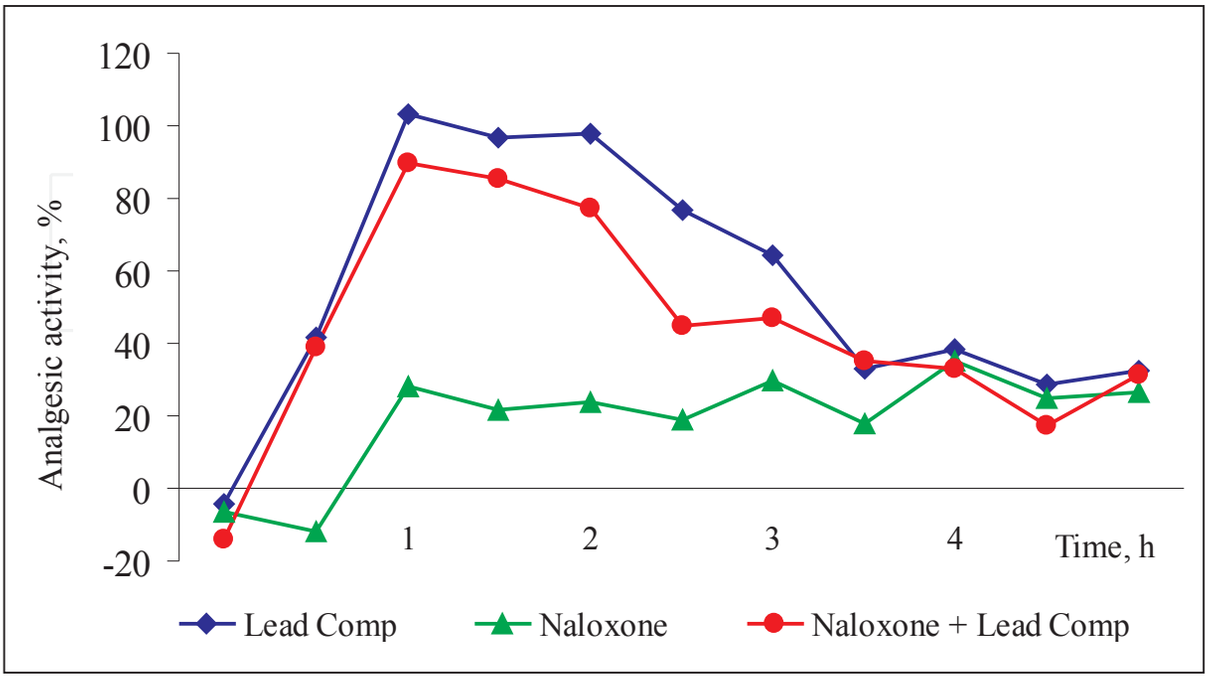

Figure 16. Lead compound & Naloxone

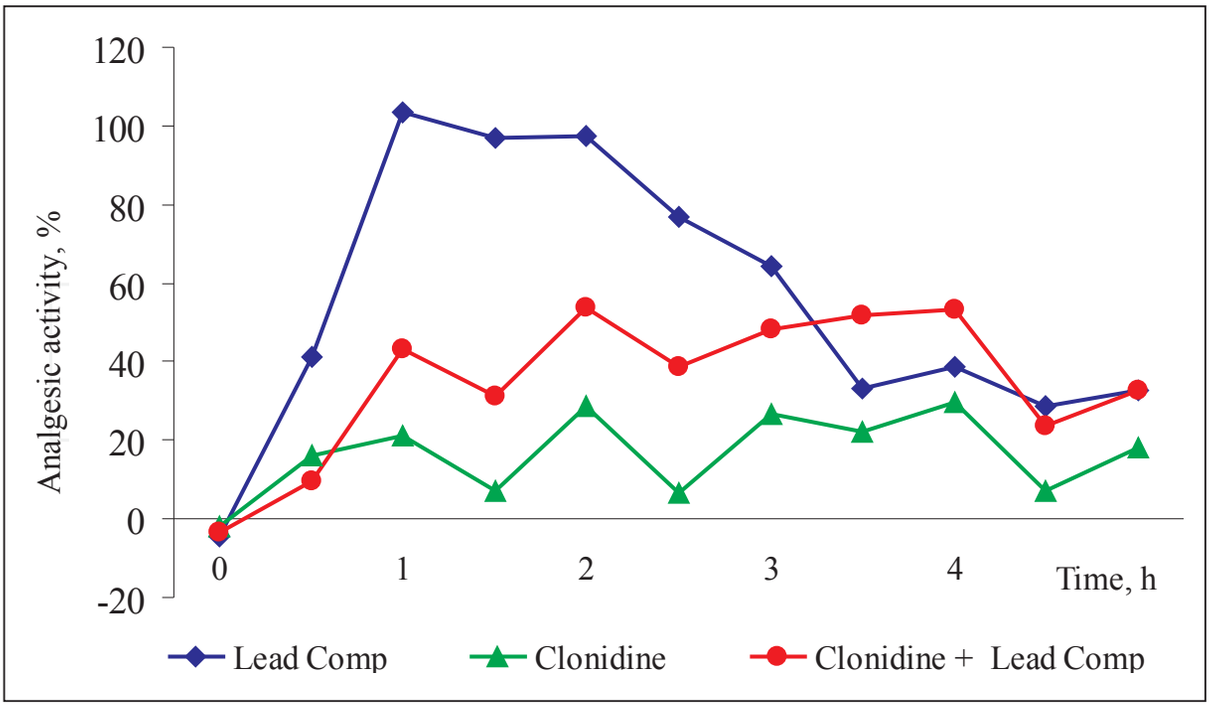

Figure 17. Lead compound & Clonidine

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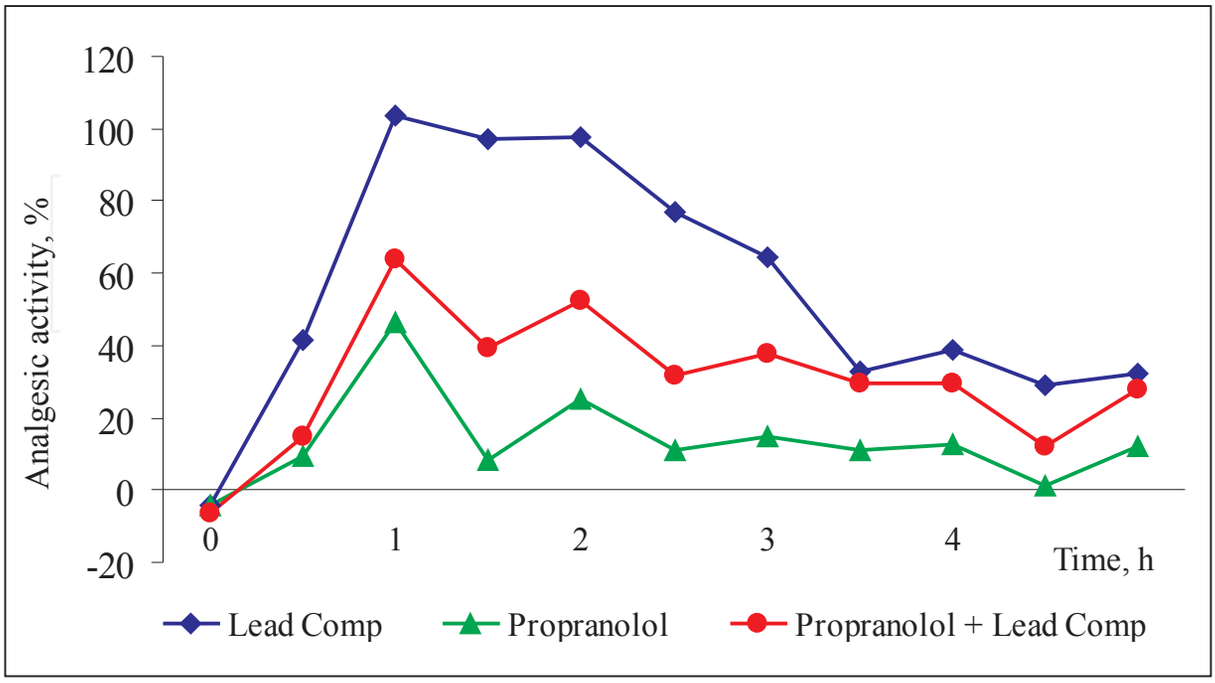

Figure 18. Lead compound & Propranolol

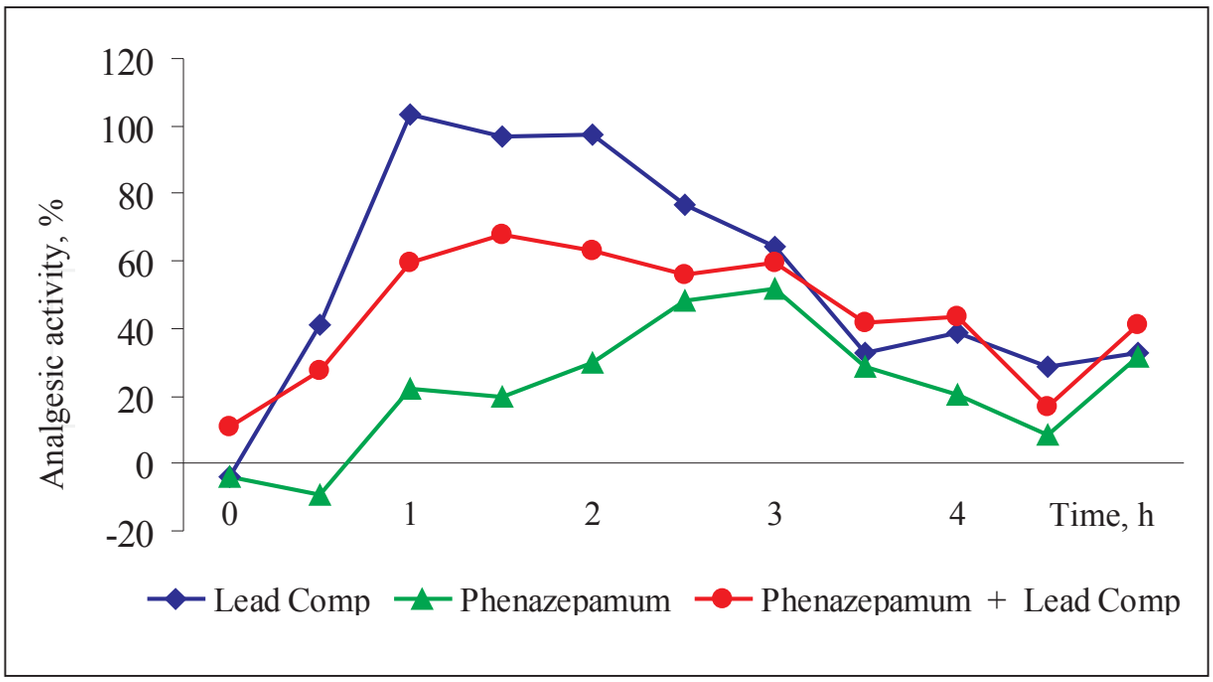

Figure 19. Lead compound & Phenazepamum

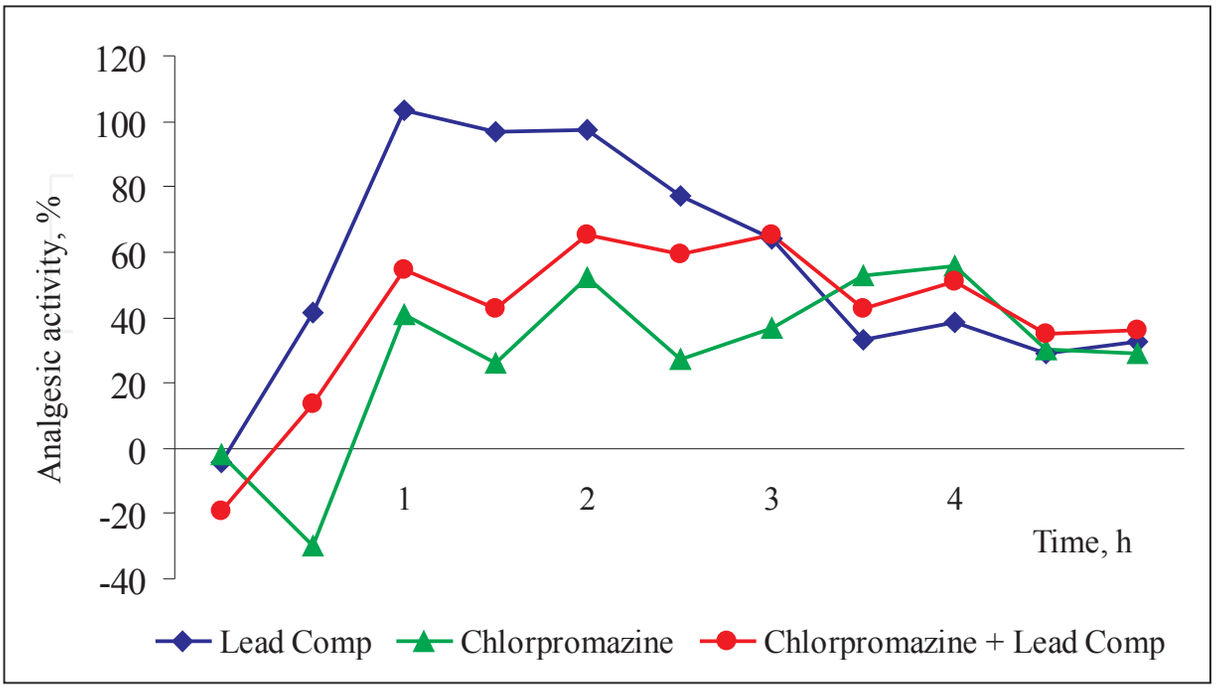

Figure 20. Lead compound & Chlorpromazine

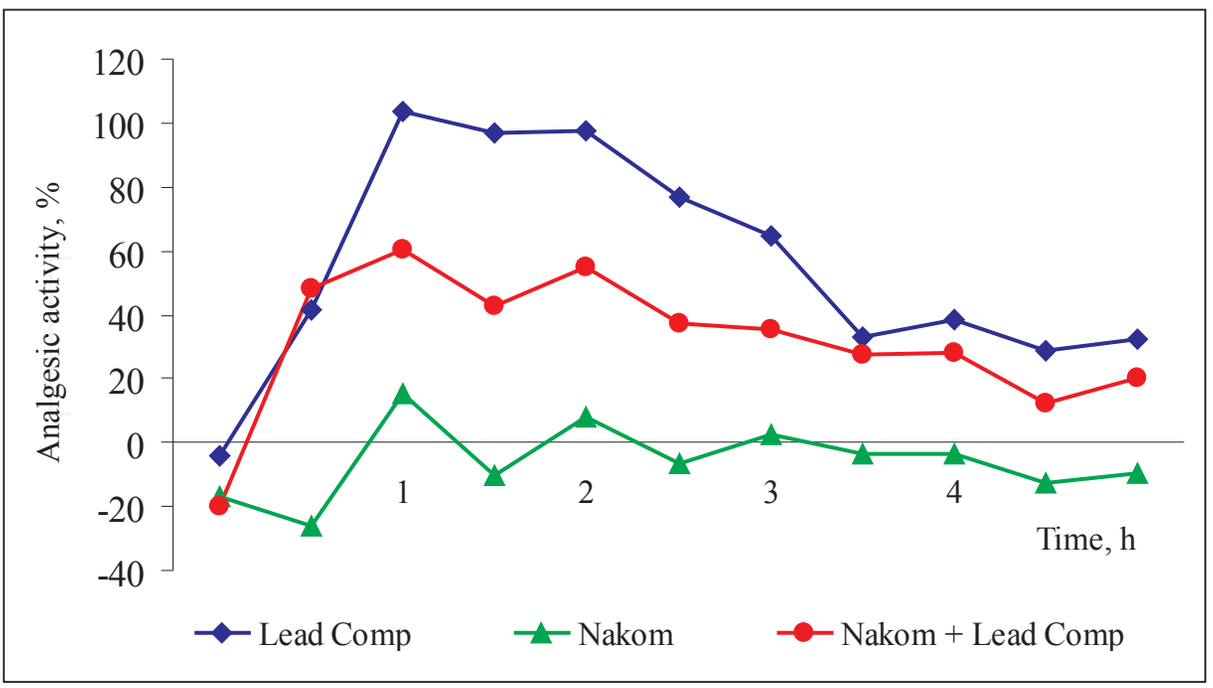

Figure 21. Lead compound & Nakom®

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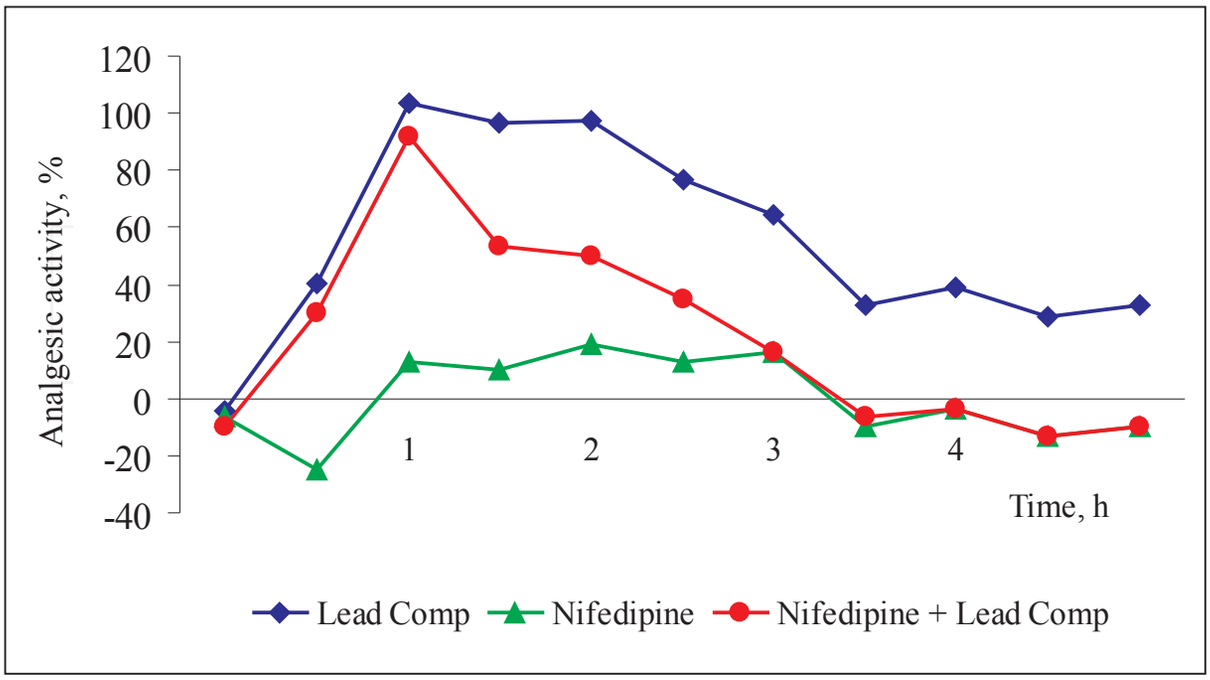


Figure 22. Lead compound & Nifedipine

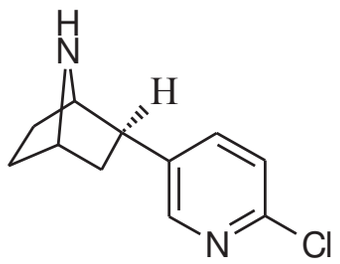
Differences in analgesic properties of the lead compound, which it exerts with independent introduction and in combination with Phenazepamum (0.19 mg/kg), in general appeared to be not so expressed (Figure 19). Thus, the conclusion can be made about its insignificant effect on the GABA-ergic system.

Participation of picolyl-3-amide **12** dopamine receptors in the mechanism of the analgesic action has been studied with the help of their blocking agent Chlorpromazine (14.0 mg/kg). In this case the effect is more expressed than in the previous test. But in general it appeared to be brief – after gradual increase during the first hour of the experiment it reaches the maximum, retains this level for about 30 minutes, and then begins to fade (Figure 20).

To study the influence of the lead compound on release of dopamine and noradrenaline in the CNS the combined medicinal form Nakom® containing Levodopa, a precursor of dopamine, together with Carbidopa, an inhibitor of its peripheral decarboxylation, was used. If when introduced alone picolyl-3-amide **12** provides a rapid enhancement of the analgesic properties till the maximum value during an hour, on the background of Nakom® (24.0 mg/kg) in 30 minutes after the start of testing the growth of activity is sharply discontinued (Figure 21). By the first hour blocking of the analgesic action of the lead compound achieves approximately 40% and lasts about two hours.

Recently the question about possibilities of creating new pain-killers based on agonists of neuronal nicotinic acetylcholine receptors (*n*AChR) is being actively discussed in scientific literature [68-70]. Epibatidine alkaloid (**30**, Figure 23) isolated from the extract of the Ecuadorean tree frog skin (*Epipedobates tricolor*) became the incentive for development of this

approach. In the experiments in mice this compound revealed 200–500 times higher analgesic activity than morphine on various experimental models. It is of great importance that analgesia caused by Epibatidine is not relieved by Naloxone, an opioid receptor antagonist. By its mechanism of action this natural alkaloid appeared to be a powerful agonist of neuronal nicotinic acetylcholine receptors regulating different functions of the nervous system [71]. Therefore, it is not surprising that a lot of attention is paid to synthetic representatives of this group of biologically active substances. The search has been carried out among derivatives of various nitrogen heterocycles [71]. One of the successful findings was 5-(trifluoromethyl)-6-(1-methylazepan-4-yl)methyl-1*H*-quinolin-2-one (**31**), which exhibited a potent agonist activity on several human *n*AChRs [72]. Its structural similarity with 4-hydroxyquinolin-2-ones studied served as a theoretical prerequisite to testing the influence on *n*AChR and picolyl-3-amide **12** offered as a new pain-killer. In this testing a specific nicotinic antagonist Nifedipine being capable to block effectively the analgesic activity of Epibatidine [71] was used. During the first hour Nifedipine (102.2 mg/kg) practically had no effect on the analgesic action of picolyl-3-amide **12** (Figure 22). Then, however, the marked inhibiting effect developed rapidly and preserved till the end of the experiment.



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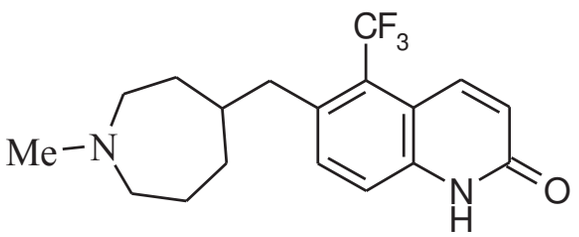

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Figure 23. Natural (**30**) and synthetic (**31**) agonists of nicotinic acetylcholine receptors

Reviewing the preliminary results of this piece of our work it is worth mentioning the ability of picolyl-3-amide **12** to arrest effectively the pains of central and peripheral origin. By its mechanism of the analgesic action this compound can not be named a selective inhibitor of one type of receptors. Having no effect on opioid receptors picolyl-3-amide **12** reveals its analgesic properties mainly via interaction with the adrenergic system and activation of nicotinic acetylcholine receptors. Other mediator systems, in particular the catecholaminergic one, are involved to much lesser extent. The GABA-ergic link of the central nociceptive system participates little in the mechanism of the analgesic action of the lead compound.

The antipyretic action of the lead compound studied on the model of fever in rats caused by subcutaneous injection of Brewer's yeast suspension [65] is classified as mild. Picolyl-3-amide **12** did not exert any clinically significant anti-inflammatory effect (the paw edema in mice induced by subcutaneous injection of 1% formalin solution [65]).

Taking into account the fact that for many drug of the group of nonnarcotic analgesics the nonselective inhibition of prostaglandin biosynthesis is characteristic we have studied a

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possible ulcerogenic action of the lead compound by the known method [65]. As it turned out, picolyl-3-amide **12** caused visible changes of the gastric mucosa in the half of experimental mice with a single introduction in very high dose exceeding the therapeutic ones: UD_{50} =1582 mg/kg.

Any new potential drug must comply with current high requirements not only by the specific activity, but safety as well. The study of acute toxicity conducted in white mice has shown that picolyl-3-amide **12** refers to practically nontoxic substances – its median lethal dose (LD_{50}) taken orally is 9527 mg/kg.

Thus, *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**12**, R = CH_2Py-3) has realistic chances to become a medicine and it is recommended to wide preclinical trials as a promising pain-killer.

7. The latest ideas and findings when creating highly active pain-killers on the basis of 4-hydroxyquinolin-2-ones and related heterocycles

With the beginning of comprehensive preclinical trials of *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide the search of new promising compounds, which are suitable for creation of effective analgesics on their basis, does not naturally stop. Using the gathered experience we continued working in this direction attracting mathematical methods in addition to such traditional methods of this sort of investigations as synthetic, physicochemical and pharmacological ones. Besides, to introduce principally new substituents in the quinoline ring, as well as diversification of the objects under study due to heterocycles related in their structure appeared to be very useful and reasonable. In particular, the extremely interesting direction of searching new pain-killers among 4-hydroxyquinolin-2-one derivatives was replacement of carbonyl in position 2 to the sulfo group, i.e. transfer to 4-hydroxy-2,1-benzothiazine 2,2-dioxides.

7.1. QSAR-analysis of the analgesic activity and toxicity of 4-hydroxyquinolin-2-one derivatives

The search of regularities for the "structure – action" relationship in the range of biologically active substances is an important stage on the way of purposeful design of new drugs with the targeted complex of pharmacological properties. In this connection we attempted to generalize the results of the chemical and biological research conducted with the help of QSAR-analysis. For this purpose the dependence of the analgesic activity of various 4-hydroxyquinolin-2-one derivatives on their molecular structure was analyzed according to the definite scheme consisting of some successive steps.

Formation of learning and test samples. The learning sample is formed from 89 compounds of various chemical classes: 4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (**12**), some *N*-(3-pyridylmethyl)-4-hydroxy-2-oxoquinoline-3-carboxamides (**14**), 4-N-R,R'-aminoquinolin-2-ones (**20e**, **25-27)** and alkyl (4-hydroxy-1-methyl-2-oxo-1,2-

dihydroquinolin-3-yl)acetates (**29**). For external testing of models 17 *N*-(3-pyridylmethyl)-4-hydroxy-2-oxoquinoline-3-carboxamides (**14-16**) and 1-(2-carbamoylethyl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**17h**) have been used. The analgesic properties of all compounds have been studied under the same conditions on the model of "acetic acid induced writhing".

Calculation of structural descriptors for all tested compounds. To calculate descriptors two varients of the molecular structure representation – simplex method (Simplex Representation of Molecular Structure or SIRMS) [73] and circulation model (CM) [74] were used. Within the scope of SIRMS the structure is in the form of a set with tetratomic fragments of the fixed composition, topology and symmetry. The values of physical and chemical characteristics of atoms, which are important for displaying a property (lipophilicity, particle charges, etc.), are taken into account when differentiating atoms on simplexes. The structure's descriptor is the number of fragments (simplexes) of a certain type. The circulation model of a molecule is a structure of arbitrary construction in the form of pseudocycle, for which similarity parameters of Cremer-Pople cycle [75] are calculated; they act as descriptors.

Statistical data processing, selection of significant descriptors. During preprocessing of the whole array of descriptors those that do not correlate with the property are excluded. Then analysis of intercorrelating descriptors is carried out. It is evident that pairs of descriptors correlating among themselves contain the same structural information; therefore, one of these descriptors can be excluded. Further selection of only significant descriptors is performed from the rest array with the help of the trend-vector procedure [73].

QSAR models building, their validation and verification with the help of the moving control procedure and test sample. For building QSAR models the method of partial least squares (PLS) [73] was used. Its undoubtful advantage is quantitative interpretability of the "structure – property" dependences obtained. For each group of the descriptors selected (circulation and simplex) two models were obtained. Their approximation possibilities were estimated on the basis of determination coefficients (R^2), statistical stability (Q^2) – with the help of the procedure of external five-fold cross-validation [73]. The predictive capability of models was esrimated on the basis of determination coefficients (R^2_{test}) for test samples and the mean-squared prediction error (S_{test}).

Statistical characteristics of both models are rather high – for simplex descriptors: $R^2 = 0.95$, $Q^2 = 0.75$, $R^2_{test} = 0.86$, $S_{test} = 5.6$; for descriptors of the circulation model: $R^2 = 0.93$, $Q^2 = 0.75$, $R^2_{test} = 0.81$, $S_{test} = 5.1$.

These two models are combined in final consensus QSAR model validated by the external test sample (Table 6) formed from 18 compounds, which have not taken part in model building.

The analysis of the data given in Table 6 shows that the mean forecast error for the analgesic activity is only 8%. Therefore, the predictive force of the QSAR model obtained is quite satisfactory and suitable to use for interpretation, out of experimental screening of compounds previously unstudied and molecular design.

Interpretation of models, estimation of contributions of the physicochemical factors and structural fragments in the analgesic activity. Application of the PLS method allows to

Compound	Analgesic activity, %		Compound	Analgesic activity, %	
	Experimental	Calculated		Experimental	Calculated
6 (R = All)	56	47	**14m**	50	70
12 (R = CH2Py-3)	75	72	**14n**	75	71
14a	64	68	**14o**	74	75
14b	60	69	**14p**	63	72
14g	78	76	**14q**	59	71
14h	54	63	**14r**	58	71
14j	63	64	**15**	45	39
14k	70	74	**16**	81	61
14l	61	73	**17h**	60	66

Table 6. The estimation of the predictive force of the QSAR on the external test set

estimate quatitatively the contribution of each particular descriptor in the biological activity. And since a descriptor is the molecule's fragment taking into account the physicochemical characteristics of atoms, the possibility to estimate their relative importance appears. This information is needed for further estimation of the supposed mechanisms of the activity demonstration, as well as design of new highly active agents.

In the sector diagram (Figure 24) the results of analysis of relative contribution of various physical and chemical factors to the analgesic activity of 1,2-dihydroquinolin-2-ones derivatives are presented.

As seen from the given diagram, the electrostatic factors such as partial charges on atoms, polarizability and lipophilicity have the greatest influence on the analgesic effect. On this basis it can be assumed that the analgesic activity of 1,2-dihydroquinolin-2-ones is mainly determined by their electrostatic interaction with biological targets. The substantial influence of lipophilicity is obviously connected with transmembrane transfer of molecules to the sites of their binding with a receptor.

Similarly in the framework of the symplex approach contributions of individual structural fragments can be calculated and it is possible to determine those that make the maximum positive contribution in the analgesic activity of compounds from the learning sample. However, we think, it is much more interesting to perform computation on totally new and unstudied structures, i.e. to use for the molecular design.

Molecular design of new potentially activepain-killers. With the help of the consensus QSAR model we performed a purposeful design of new promising analgesics of 4-hydroxyquinoline-2-one range. As it is clear from Table 7 where some virtual structures with computed values of their analgesic action are given, the mathematical assessment of the biological activity exceeds greatly the minimal (i.e. 50%) efficiency criterion for the pain syndrome relief. We

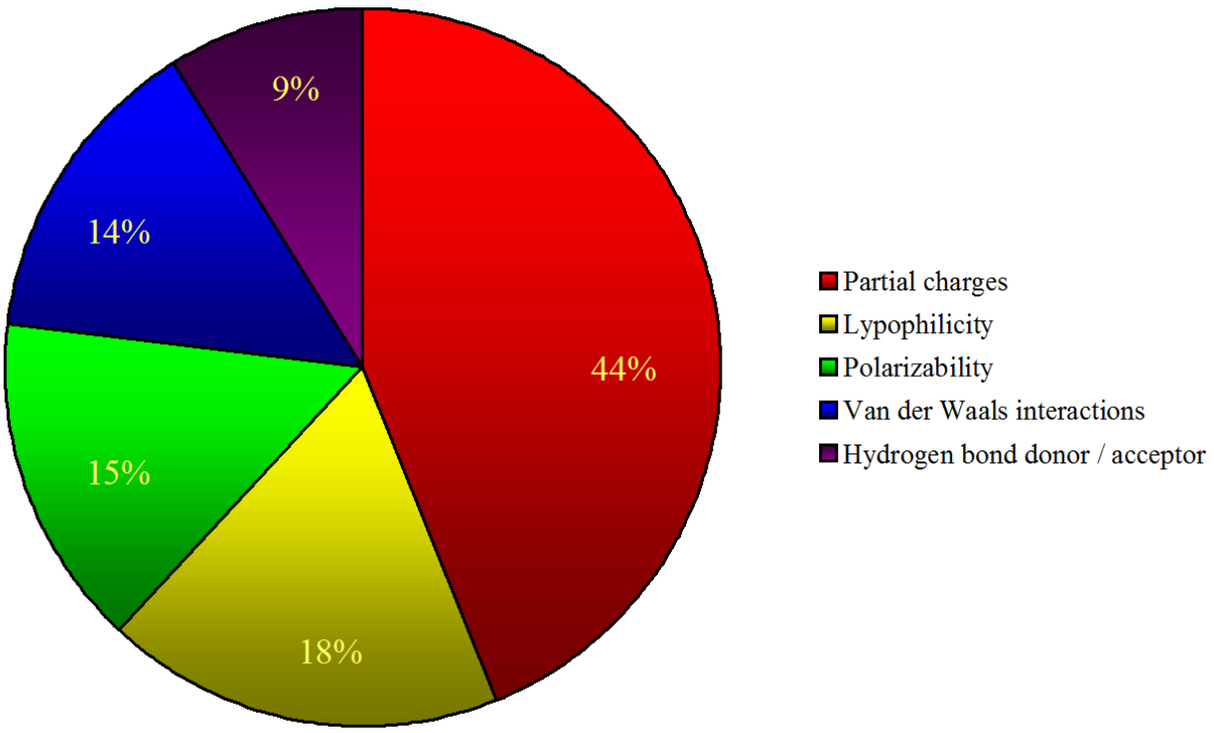

Figure 24. The relative impact of physical and chemical factors on the analgesic activity of 1,2-dihydroquinolin-2-ones

hope the information will be useful for many medical chemists engaged in the problem of effective analgesic agents creation.

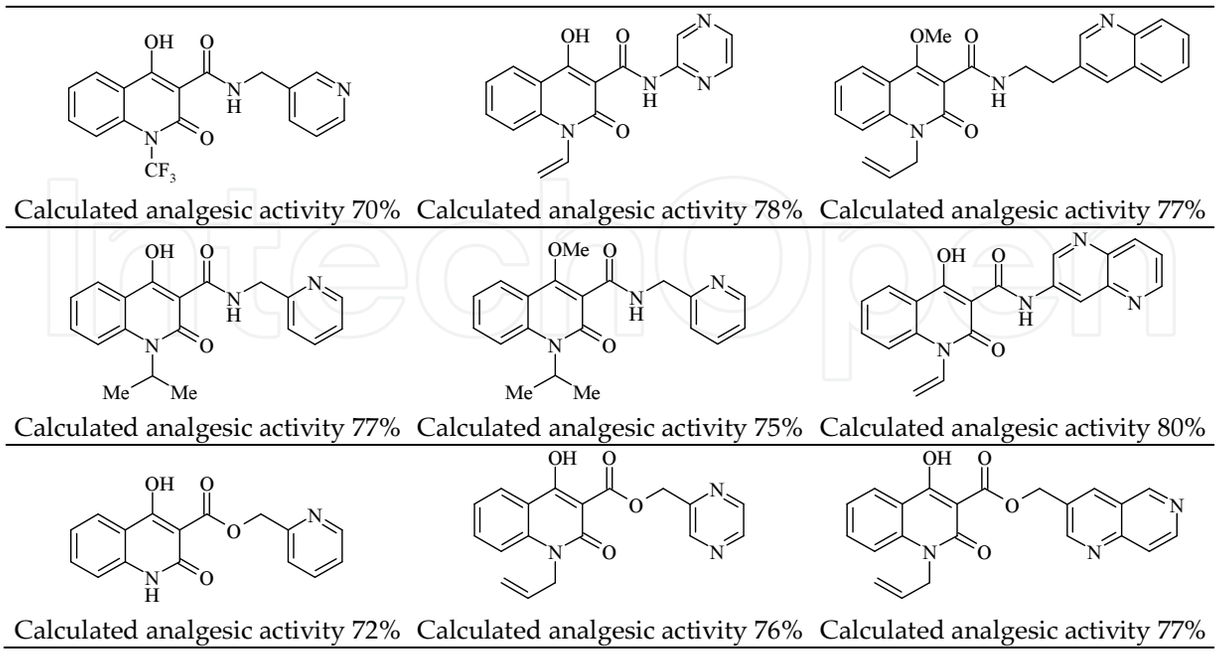

Table 7. Virtual quinoline-3-carboxamides and quinoline-3-carboxylates with potentially high activity

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Analysis of toxicity and mutagenicity of highly active compounds. Toxicity and mutagenicity are the most important characteristics of any biologically active substance. How powerful specific action a pretender to drug does possess, but without conformance to the current requirements of safety it can never be allowed to medical application. Hence attempts of researchers to manage toxicity and mutagenicity of a new potential drug at the early stages of its development become clearer. Computer prognosis may be quite useful in such cases. For this purpose the already known QSAR models are suitable [73, 75]. One of them allows estimating a possible toxicity of compounds in relation to the model infusoria *Tetrahymena pyriformis*, another one – their mutagenicity within a framework of Ames test. To characterize toxicity the following scale has been suggested: *-2 < low toxic ≤ 0; 0 < moderately toxic < +1; +1 ≤ high toxic*. The results of calculation of mutagenicity are of two classes: *0 – non-mutagenic substances, 1 – mutagenic substances*.

According to the mathematical prognosis the most active pain-killers found among the derivatives of 1,2-dihydroquinolin-2-ones belong to low toxic substances (Table 8). Although calculations confirm our assumption (see section 3.3) about a noticeable increase of toxicity when introducing a bromine atom in the benzene moiety of the quinolone ring, but the presence of the atom is still permitted. But the second bromine atom in the molecule is extremely undesirable – besides enhancement of toxicity it promotes appearance of mutagenicity.

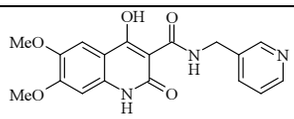
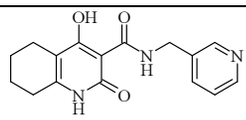
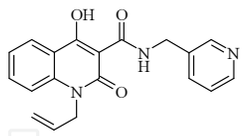
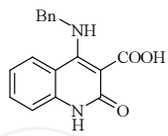
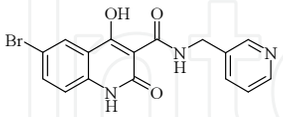
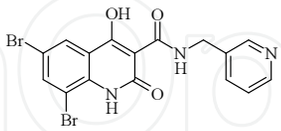
Compound	Toxicity	Mutagenicity	Compound	Toxicity	Mutagenicity
	- 1.61	0		- 1.12	0
	- 0.88	0		- 1.05	0
	+ 0.15	0		+ 0.23	1

Table 8. Calculated toxicity and mutagenicity of certain 1,2-dihydroquinolin-2-ones

Modeling of active compounds metabolism is one more example of using the obtained QSAR model in chemical and biological research. Modeling itself is performed by another method, of course, – in this case transformation of the most active 1,2-dihydroquinolin-2-ones into virtual metabolites under the influence of the rat liver enzymes has been calculated with the help of QSAR ToolBox 3.0 software [76]. Only after this the QSAR model suggested by us is used; with its help the analgesic properties prediction for all theoretically possible metabolites (Table 9 presents only the small part of them) is performed.

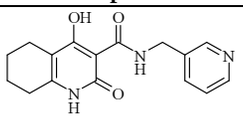
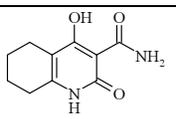
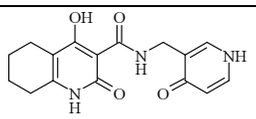
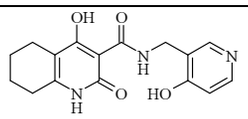
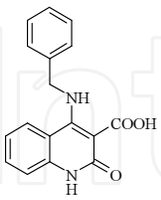
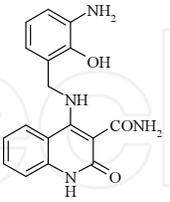
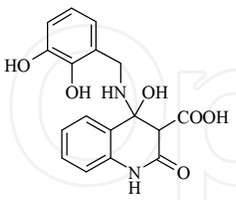
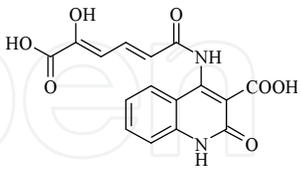
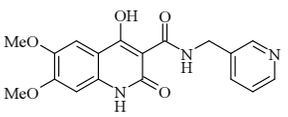
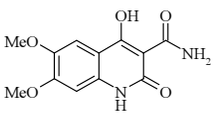
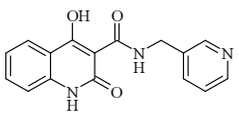
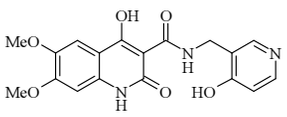
Compound	Possible metabolites		
			
Experimental AA 81%	Calculated AA 64%	Calculated AA 71%	Calculated AA 67%
			
Experimental AA 75%	Calculated AA 76%	Calculated AA 60%	Calculated AA 58%
			
Experimental AA 75%	Calculated AA 68%	Calculated AA 74%	Calculated AA 66%

Table 9. Highly active 1,2-dihydroquinolin-2-ones and their theoretically possible metabolites (AA – analgesic activity)

Such information is rather interesting and important for screening, especially if it is completed by calculations of the possible toxicity and mutagenicity, and not only leading structures, but their virtual metabolites as well. It allows to exclude substances, which are capable to transform into highly toxic or mutagenic products, from candidates to drugs at early stages of screening. Thus, efficiency of the purposeful search of new pain-killers increases significantly.

7.2. 3-(3-R-Carbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanenitriles and their functional derivatives

Aryl- (hetaryl) propanoic acids and their derivatives have an extremely wide spectrum of biological properties, due to which they have become the base of numerous vital drugs of different pharmacological group [14, 52]. For example, only among NSAIDs permitted to medical application and belonging to nonnarcotic analgesics there are about several dozens of such compounds [46]. Therefore, it is not surprising that further we studied the structures combined two pharmacologically important fragments in one molecule, namely 4-hydroxyquinolin-2-one and propanoic acid. As one of the variants for the practical solution of this task we suggested 3-(3-alkylcarbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanenitriles (**32**) that are easily avaliable synthetically [77, 78]; as a rule, in the conditions of alkaline hydrolysis they give the corresponding 3-(3-alkylcarbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanoic acids with good yields (**33**, Figure 25) [78].

The analgesic activity for the synthesized compounds of this great series was measured on the "acetic acid induced writhing" test. The test substances and the reference drug Diclofenac were administered *per os* in the form of a thin aqueous suspension stabilized by Tween-80 in the dose of 5 mg/kg. This dose corresponds to ED_{50} of Diclofenac exactly for the model of "acetic acid induced writhing" [39]. The analysis of the research data obtained shows that the great

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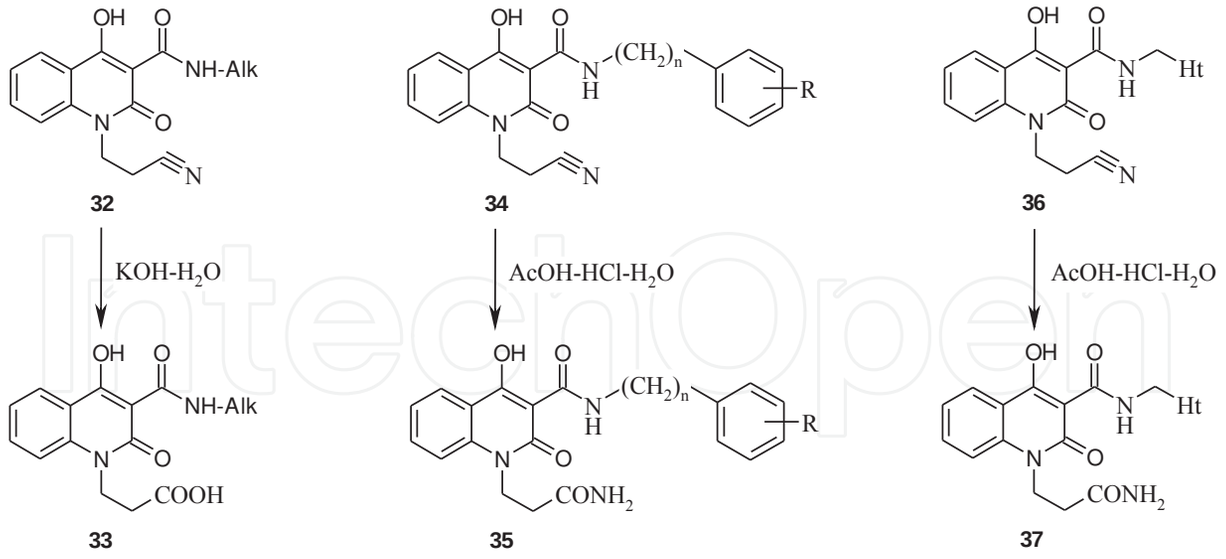

Figure 25. Quinolinyl-propanenitriles **32**, **34**, **36**, quinolinyl-propanoic acids **33** and quinolinyl-propaneamides **35**, **37**

majority of the substances investigated actually reveal the marked and statistically valid ($p \leq 0.05$) analgesic properties.

Thus, from the group of 3-alkylcarbamoyl substituted quinolinyl-propanenitriles **32** some compounds such as propyl- (**32d**), *iso*-butyl- (**32g**), *sec*-butyl- (**32h**), 2-hydroxyethyl- (**32u**), 3-chloropropyl- (**32x**) and 3-methoxypropyl- (**32y**) amides are of immediate interest, their analgesic effect does not yield Diclofenac and even exceeds it (Table 10). In general, transfer from nitriles **32** to the corresponding propanoic acids **33** affects analgesic properties negatively. However, there some positive exceptions – in the case of allylamide **33c**, for example, the transformation mentioned is accompanied with the substantial intensification of activity. If the fact that the synthetic precursor of this compound is also highly active is taken into account, then nitrile **32c** → acid **33c** bunch can be of interest for further more detailed study.

Such approach for studying 3-(3-R-carbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanenitriles appears to be quite logical and reasonable. However, as a minimum, one important moment was still omitted – intermediate quinolinyl-propaneamides, which form inevitably during trasformation of nitriles into acids, stay out of sight. Meanwhile, interest to these compounds rises many times if it is taken into account that in a living organism metabolism of nitriles can be by different ways, including that by the primary hydration to amides [79, 80]. With regard to the issues in focus it means that the efficiency of any nitrile or amide as a pain-killer increases greatly if their metabolites also reveal analgesic properties. Therefrom the idea appeared to involve by all means the intermediate link – quinolinyl-propaneamides together with initial quinolinyl-propanenitriles and final propanoic acids in the range of the investigations conducted. This allows to select, first of all, those compounds that besides the own high analgesic effect will have a rather active metabolite as promising leading structures from the chain of nitrile → amide → acid.

Compound	Alk	Analgesic activity (decrease in the amount of "acetic acid writhing", %)	
		32 (-$CH_2CH_2C{\equiv}N$)	**33** (-CH_2CH_2COOH)
a	Me	36.3	35.6
b	Et	44.2	28.7
c	All	59.5	73.3
d	Pr	51.0	0
e	*i*-Pr	38.8	40.4
f	Bu	18.6	33.2
g	*i*-Bu	62.1	0
h	*s*-Bu	64.3	10.5
i	C_5H_{11}	38.5	40.7
j	*i*-C_5H_{11}	42.1	20.4
k	C_6H_{13}	47.0	17.9
l	C_7H_{15}	45.3	16.7
m	C_8H_{17}	49.4	22.5
n	C_9H_{19}	42.6	31.3
o	$C_{10}H_{21}$	40.2	16.8
p	*cyclo*-C_3H_5	43.3	0
q	*cyclo*-C_5H_9	40.5	22.9
r	*cyclo*-C_6H_{11}	48.7	15.2
s	*cyclo*-C_7H_{13}	46.4	12.6
t	Adamantan-1-yl	31.1	10.5
u	CH_2CH_2OH	51.2	–
v	$CH_2CH_2CH_2OH$	47.3	–
w	CH_2CH_2Cl	24.9	–
x	$CH_2CH_2CH_2Cl$	63.0	–
y	$CH_2CH_2CH_2OMe$	50.6	–
z	$CH_2CH_2CH_2OPr$-*i*	45.4	–
Diclofenac (5 mg/kg)		52.0	

Table 10. The analgesic activity of alkylcarbamoyl substituted quinolinyl-propanenitriles **32** and the corresponding quinolinyl-propanoic acids **33**

To implement this idea the method of selective hydration of 3-(3-R-carbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanenitriles to the corresponding propaneamides is re-

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quired. The task is not so simple than it may seem at first sight since amides primarily formed are usually subjected to hydrolysis much easier than initial nitriles. As a result, it is not always possible to stop the reactions of this type at the stage of amides formation. It is for this reason that the aforementioned alkaline hydrolysis of nitriles **32** to acids **33** is intentionally unsuitable for obtaining propaneamides.

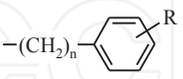
Compound		Analgesic activity (decrease in the amount of "acetic acid writhing", %)	
		34 (-$CH_2CH_2C{\equiv}N$)	**35** (-$CH_2CH_2CONH_2$)
a	$PhCH_2$	54.4	–
b	*cyclo*-$C_6H_{11}CH_2$	29.3	–
c	2-$FC_6H_4CH_2$	20.3	38.3
d	4-$FC_6H_4CH_2$	67.1	36.4
e	2-$ClC_6H_4CH_2$	16.5	0
f	4-$ClC_6H_4CH_2$	0	56.0
g	2-$MeC_6H_4CH_2$	39.2	40.9
h	3-$MeC_6H_4CH_2$	18.0	41.1
i	4-$MeC_6H_4CH_2$	0	28.6
j	2-$MeOC_6H_4CH_2$	38.1	–
k	4-$MeOC_6H_4CH_2$	34.8	35.5
l	3,4-$(MeO)_2C_6H_3CH_2$	0	39.2
m	Piperonyl	0	14.7
n	(±) PhCH(Me)	16.1	47.8
o	*S*(-) PhCH(Me)	10.6	46.1
p	*R*(+) PhCH(Me)	21.7	47.0
q	(±) 4-$MeOC_6H_4CH(Me)$	46.6	–
r	*S*(-) 4-$MeOC_6H_4CH(Me)$	17.3	–
s	*R*(+) 4-$MeOC_6H_4CH(Me)$	22.5	–
t	$PhCH_2CH_2$	55.3	–
u	3-$ClC_6H_4CH_2CH_2$	42.6	–
v	4-$ClC_6H_4CH_2CH_2$	23.4	–
w	4-$MeOC_6H_4CH_2CH_2$	64.6	35.7
x	3,4-$(MeO)_2C_6H_3CH_2CH_2$	39.5	20.8
y	$PhCH_2CH_2CH_2$	46.6	
Diclofenac (5 mg/kg)		57.2	

Table 11. The analgesic activity of arylalkylcarbamoyl substituted propanenitriles **34** and the corresponding propaneamides **35**

We succeeded to find the effective method of transformation of 3-(3-arylalkylcarbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-1-yl)propanenitriles (**34**) into the corresponding pro-

paneamides **35** with the help of a simple and available reagent – the mixture of hydrochloric and acetic acids with the low content of water [81]. The method is interesting by the fact that, if required, it allows to perform more profound chemical transformations – for example, hydrolysis of nitriles in amides – only by increasing the reaction duration.

Compound	CH_2-Ht	Analgesic activity (decrease in the amount of "acetic acid writhing", %)	
		36 (-$CH_2CH_2C{\equiv}N$)	**37** (-$CH_2CH_2CONH_2$)
a	Picolyl-2	72.3	47.0
b	Picolyl-3	36.6	10.2
c	Picolyl-4	21.0	31.2
d	Furfuryl	0	–
e	5-Me-furfuryl	59.8	–
f	Tetrahydrofurfuryl	15.4	0
g	Thiophen-2-ylmethyl	0	–
Diclofenac (5 mg/kg)		44.3	

Table 12. The analgesic activity of hetarylalkylcarbamoyl substituted propanenitriles **36** and the corresponding propaneamides **37**

Comparison of analgesic properties of the obtained triad of arylalkylcarbamoylsubstituted propanenitriles, propaneamides and propanoic acids allows to assert that, as a rule, the acid appears to be the least active in the chain of nitrile → amide → acid. Thus, further we focused our efforts on studying only nitriles and amides. It follows from the data given in Tables 11 and 12 that often quinolinyl-propaneamides actually demonstrate higher analgesic properties than their synthetic precursors. Therefore, it is expedient to perform the further search of potential pain-killers in the range of the compounds studied among 1-(2-cyanoethyl)- and 1-(2-carbamoylethyl)-quinolines. Furthermore, with transfer from acids to amides or nitriles acidity decreases essentially, as well as probability of manifestation of the ulcerogenic action being a serious drawback of many modern analgesics.

By the available data from the whole group of quinolinyl-propanoic acids derivatives studied so far, in addition to the abovementioned allyl substituted nitrile **32c**, 1-(2-cyanoethyl)-*N*-(2-pyridylmethyl)-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (**36a**) deserves attention. The basis for this conclusion is a high analgesic activity of not only these nitriles themselves, but of their possible metabolites as well – acid **33c** and amide **37a**, respectively.

7.3. 1-R-4-Hydroxy-2,2-dioxo-1*H*-2λ6,1-benzothiazine-3-carboxamides

Oxicams are an integral part of the range of modern non-steroidal anti-inflammatory drugs with the marked analgesic effect in the range of their biological activities [14, 46, 52]. Piroxicam (**38**, R = 2-Py, Figure 26) became the first commercially successful drug of this group. Later its more effective analogs – Isoxicam (**38**, R = 5-Me-isoxazol-3-yl), Meloxicam (**38**, R = 5-Me-

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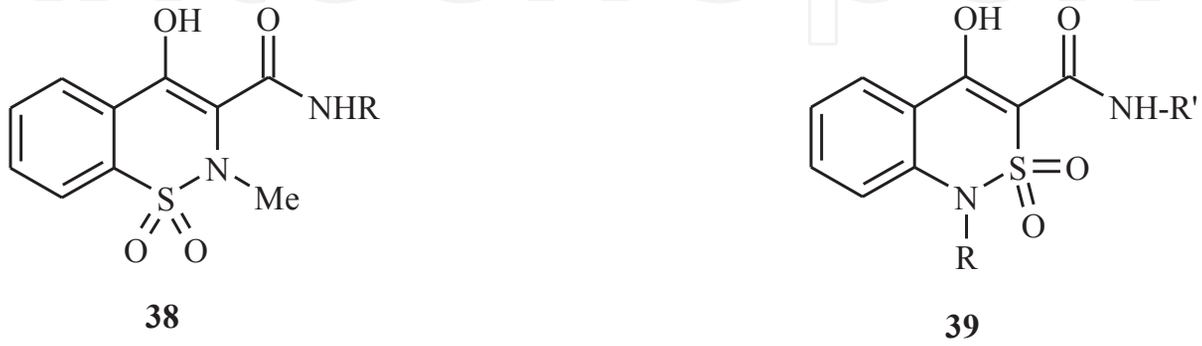
thiazol-2-yl), etc., appeared at the pharmaceutical market. Today they are widely used by practical medicine in treating numerous rheumatic and autoimmune human diseases under the common name of selective inhibitors of cyclooxygenase-2. It is interesting that isomeric oxicams of 4-hydroxy-1-R-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides (**39**), which are different only by reverse mutual arrangement of atoms of nitrogen and sulfur in the thiazine cycle, remain practically completely unstudied at present. The cause of the existing situation is known – it is the absence of effective preparative methods for the synthesis of compounds of this chemical group.



38 **39**

Figure 26. Oxicams (**38**) and isomeric 4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides (**39)**

It should be noted that almost half a century ago some 1-*N*-methyl-substituted carboxanilides **39** were obtained by the reaction of l-methyl-3,4-dihydro-lH-2,l-benzothiazin-4-one 2,2-dioxide with isocyanates in dimethyl sulfoxide solution with the yields from 28 to 100% [82]. However, because of the low yields at the first two stages of obtaining the initial l-methyl-3,4-dihydro-lH-2,l-benzothiazin-4-one 2,2-dioxide this four-step synthetic scheme of 1-methyl-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **39** appeared to be unattractive. Furthermore, its application is greatly limited by the necessity of using isocyanates – they are often expensive or almost unavailable reagents, and it significantly complicates the research for purposeful search of "structure – property" regularities. As a result, unfortunately, this undoubtedly interesting work [82] has not got its further development.

Taking these circumstances into account we offer a fundamentally different three-step scheme for the synthesis of the target 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **39** suggesting the initial obtaining of alkyl 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylates; with their subsequent amidation a practically unlimited and freely available range of various alkyl-, aryl- and hetarylamides can be used.

As was shown earlier, lower alkyl 1-R-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates are easily and rapidly amidated by primary and even secondary alkyl-, aryl- and hetarylamines. At the same time for their high reactivity it is necessary their simultaneous presence in the pyridinic part of the molecule of both 4-OH and 2-C=O groups [32]. With the transfer to alkyl 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylates the powerful acidifying effect of the sulfo group so greatly increases 4-OH-acidity that the ordinary salt formation

begins to prevent amidation. By comparison – the salts of alkyl 1-R-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates are extremely unstable with amines and rapidly decompose even by carbon dioxide of the air [83]; and, as a rule, they do not cause problems in amidation. On the contrary the similar salts of their 2-sulfo analogs can be readily isolated and characterized. When heating them in the medium of a highly boiling inert solvent they can be transformed with the high yields into the corresponding 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **39**. Although for this purpose several hours are needed, whereas in case of 2-carbonyl derivatives the similar procedure takes only 3-5 min.

In general, the method offered appeared to be quite effective and with its help we succeeded in synthesizing a great series of the target alkyl-, arylalkyl-, aryl- and hetarylamides of 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxylic acids. To confirm their structure NMR (^{1}H and ^{13}C) spectroscopy, mass spectrometry, and in some cases X-ray structural analysis have been used.

The screening study of analgesic properties of 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **39** was performed in white nonlinear male rats using the standard model of "tail flick" thermal irritation [65]. The substances under research and reference-drugs were introduced in the dose of 20 mg/kg orally in the form of a fine aqueous suspension stabilized by Tween-80. The antinociceptive effect was estimated by comparing the duration of the latent period (the time before tail flick) and in one hour after introduction of the substances studied.

According to the results of the pharmacological research among 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides **39** synthesized the substances, which exceed greatly the known drugs both of oxicam range (Piroxicam and Meloxicam) and other chemical groups (Diclofenac, Ketorolac and even Nalbuphine, narcotic analgesic introduced intraperitoneally) by their analgesic properties have been found. On this basis some of them are recommended for further profound study as new potential analgesics.

Therefore, the results obtained has demonstrated clearly and convincingly that optimization of the known drugs by creation of their close structural analogs differing only by inverse mutual arrangement of atoms or substituents, which we have called “flip-flop drugs” methodology, is rather interesting, productive and promising for the future.

8. Conclusion

Reviewing the preliminary results of the complex research, which is far from its completion as yet, even now it is possible to state with certainty that 4-hydroxyquinolin-2-ones have actually appeared to be practically the inexhaustible source of highly effective pain-killers. One of these compounds – *N*-(3-pyridylmethyl)-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamide – possesses important analgesic properties on various experimental models; it is practically nontoxic, does not have the ulcerogenic action in therapeutic doses, greatly exceeds many currently known medicines by these parameters and thanks to these facts it is recommended to wide preclinical trials. Besides, according to the results of QSAR-

analysis not only relative contributions of some physical and chemical factors and the structural fragments to the analgesic activity of 1,2-dihydroquinolin-2-ones have been determined, but new potentially highly active virtual substances, which are suitable enough for synthesis and further testing, have been suggested. The primary pharmacological screening has also found some promising analgesics, but among 3-(3-R-carbamoyl-4-hydroxy-2-oxo-1,2-dihydroquinolin-1-yl)propanenitriles already obtained and 1-R-4-hydroxy-2,2-dioxo-1*H*-2λ^6,1-benzothiazine-3-carboxamides that are structurally related to them.

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