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Full Length Research Paper

Synthesis and biological properties of Enantiomers of 1-Allyl-4-Hydroxy-6,7-Dimethoxy-2-Oxo-1,2-Dihydroquinoline-3-Carboxylic Acid (1-Ethylpyrrolidin-2-Ylmethyl)-Amide Hydrochloride

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Being guided by the methodological principles of "chiral switches", the synthesis of *S*- and *R*-enantiomers of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (1-ethylpyrrolidin-2-ylmethyl)-amide hydrochloride has been carried out. According to the results of the biological research, it has been found that the ability of the optical isomers obtained to block opioid receptors remains at the racemate level. It is important for future research conclusion – the asymmetrical carbon atom in these compounds is not the site of binding with the target receptor.

Key words: amidation, 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides, opioid receptors antagonists, "chiral switch"

INTRODUCTION

It is known in medicinal chemistry for a long time that optically active substances and their enantiomers can differ greatly by the strength and the character of their action on a living organism. But in the last 25-30 years, racemates have undergone a valid criticism as substances "containing 50% of admixtures" (Ariëns, 1984). This problem has attracted attention of many researchers. As a result, assembling of complex compounds based on available enantiomer pure semi-products of the natural or synthetic origin (chiral pool synthesis) has received a new development (Núñez et al., 2009). Modern technologies of asymmetric synthesis (Fuwa et al., 2011; Umezawa et al., 2011), as well as new methods of effective separation of racemic mixtures at the industrial scale (Hsu et al., 2011; Schuur et al., 2011) have systematically appeared. It is clear that in the papers of such kind, the efforts spent by no means always produce the desired results. However, there are a lot of positive examples by the present time, resulting in a separate direction in medicinal chemistry dealing with prolongation of "life" for medicinal substances that contain asymmetrical carbon atom. According to this methodology which is known as "chiral switches", instead of drugs-racemates applied for a long time and thus losing the patent protection, their more biologically active optically pure enantiomers are available at the pharmaceutical market (Agranat et al., 2002; Agranat and Wainschtein, 2010; Kubinyi, 2006; Tucker, 2000). We tried to use the same strategy for modifying antagonists of opiod receptors previously described that had been revealed among 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (Ukrainets et al., 2010). The most promising of the entire series of compounds studied were 2-ethylaminoethyl-, 3-dimethylaminopropyl-, 1-ethylpyrrolidin-2-ylmethyl-, 3-morpholin-4-ylpropyl- and 3-piperidin-1-ylpropyl-derivatives.

MATERIALS AND METHODS

In view of specificity of the structure of objects studied according to the methodology of "chiral switches", only

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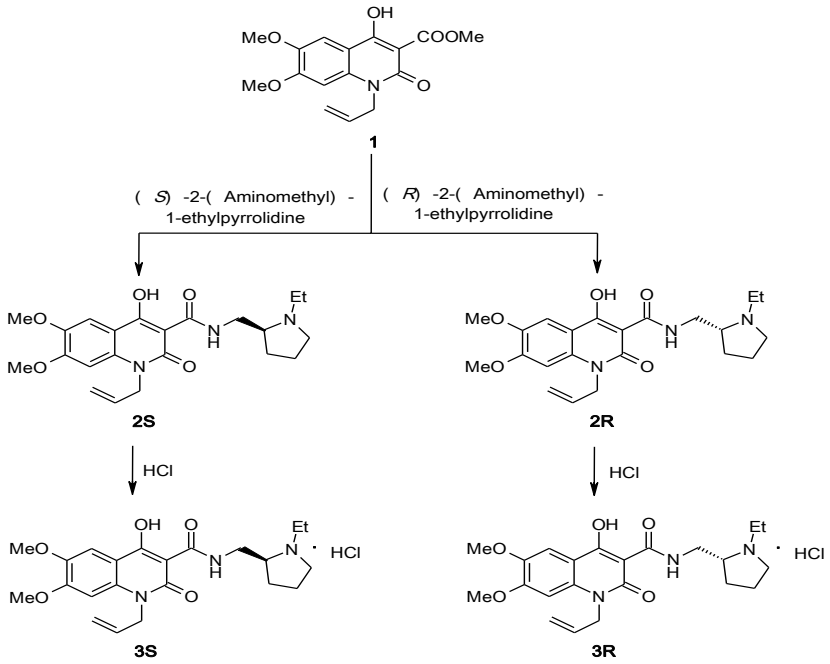

Scheme 1: Synthesis of *S*- and *R*-enantiomers of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (1-ethylpyrrolidin-2-ylmethyl)-amide hydrochloride. At the same time, the target products of the reaction can be isolated as amides-bases **2S** и **2R** or as water soluble hydrochlorides **3S** и **3R**, which are more suitable for pharmacological research.

one of the compounds with a high activity, namely racemic hydrochloride of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (1-ethylpyrrolidin-2-ylmethyl)-amide (Ukrainets et al., 2010), appeared to be suitable for the planned research. The synthesis of its optical antipodes has been carried out independently using different asymmetric reagents, i.e., by interaction of methyl ester **1** with the commercially available chiral 2-(aminomethyl)-1-ethylpyrrolidines in boiling methanol (Scheme 1).

Chemistry

The ^{1}H NMR spectra of the compounds synthesized and COSY 2D ^{1}H NMR experiments were recorded on Varian Mercury-400 spectrometer (400 MHz). The solvent was DMSO-d_6 or $CDCl_3$ and the internal standard TMS. The specific rotations of amides **3S** and **3R** were determined on Polamat A polarimeter. These compounds were synthesized using commercial samples of *S(–)*- and *R(+)*-2-(aminomethyl)-1-ethylpyrrolidines with optical purity of at least 98.5 and 96.0%, respectively, supplied by Acros.

*(S)-(1-ethylpyrrolidin-2-ylmethyl)-amide of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**2S**).* *S(–)*-2-(Aminomethyl)-1-ethylpyrrolidine (1.28 g, 0.01 mol) was added to the solution of ester **1** (3.19 g, 0.01 mol) in methanol (30 ml) and heated at reflux for 3 h. The reaction mixture was cooled and diluted with cold water. The solution was purified with activated charcoal and then left for 7-8 h at 0°C. The crystalline precipitate of *(S)*-(1-ethylpyrrolidin-2-ylmethyl)-amide **2S** was filtered off, washed with cold water, and dried to give 3.65 g (88%) **2S** (it is transferred into hydrochloride without additional purification). M. p. of the crude product is 167-169°C. ^{1}H NMR ($CDCl_3$), δ, ppm (*J*, Hz): 10.39 (t, *J* = 5.1, 1H, CONH); 7.53 (s, 1H, H-5); 6.72 (s, 1H, H-8); 5.95 (m, 1H, C<u>H</u>=CH_2); 5.25 (d, *J* = 10.4, 1H, NCH_2CH=C<u>H</u>-*cis*); 5.10 (d, *J* = 17.2, 1H, NCH_2CH=C<u>H</u>-*trans*); 4.93 (m, 2H, NCH_2); 3.97 (s, 3H, OCH_3); 3.96 (s, 3H, OCH_3); 3.71 (m, 1H, NHC<u>H</u>); 3.28 (m, 1H, NHC<u>H</u>); 3.21 (m, 1H, 5'-CH); 2.93 (m, 1H, C<u>H</u>CH_3); 2.66 (m, 1H, 2'-CH); 2.34 (m, 1H, C<u>H</u>CH_3); 2.18 (m, 1H, 5'-CH); 1.99 (m, 1H, 3'-CH); 1.80 (m, 1H, 3'-CH); 1.72 (m, 2H, 4'-CH_2); 1.15 (t, *J* = 6.9, 3H, NCH_2C<u>H_3</u>).

*(R)-(1-ethylpyrrolidin-2-ylmethyl)-amide of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**2R**).* Obtained by the similar method. The yield is 83 %. M. p. of the crude product is 165-167 °C. ^{1}H NMR ($CDCl_3$), δ, ppm (*J*, Hz): 10.42 (t, *J* = 5.1, 1H, CONH); 7.53 (s, 1H, H-5); 6.73 (s, 1H, H-8); 5.95 (m, 1H, C<u>H</u>=CH_2); 5.25 (d, *J* = 10.4, 1H, NCH_2CH=C<u>H</u>-*cis*); 5.10 (d, *J* = 17.2, 1H, NCH_2CH=C<u>H</u>-*trans*); 4.93 (m, 2H, NCH_2); 3.97 (s, 3H, OCH_3); 3.96 (s, 3H, OCH_3); 3.70 (m, 1H, NHC<u>H</u>); 3.28 (m, 1H, NHC<u>H</u>); 3.22 (m, 1H, 5'-CH); 2.93 (m, 1H, C<u>H</u>CH_3); 2.68 (m, 1H, 2'-CH); 2.34 (m, 1H,

C<u>H</u>CH$_3$); 2.18 (m, 1H, 5'-CH); 1.99 (m, 1H, 3'-CH); 1.80 (m, 1H, 3'-CH); 1.72 (m, 2H, 4'-CH$_2$); 1.15 (t, *J* = 6.9, 3H, NCH$_2$C<u>H</u>$_3$).

Hydrochloride of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid S(–)-(1-ethylpyrrolidin-2-ylmethyl)-amide ***(3S)***. 2-Propanol saturated with gaseous HCl was added to the solution of compound **2S** (4.15 g, 0.01 mol) in 2-propanol (25 ml) adjusting to pH 3. Then, dry diethyl ether (20 ml) was added and left for 5 h at 0°C. The precipitate of hydrochloride **3S** was filtered off, washed with dry diethyl ether, and dried to give 4.37 g (97%) **3S**. M. p. 241-243 °C (anhydrous EtOH). $[\alpha]_D^{20}$ −11.6° (*c* = 5, H$_2$O). ^{1}H NMR (DMSO-d$_6$), δ, ppm (*J*, Hz): 16.65 (s, 1H, 4-OH); 10.54 (t, *J* = 5.3, 1H, CONH); 10.33 (br. s, 1H, NH$^+$); 7.39 (s, 1H, H-5); 6.92 (s, 1H, H-8); 5.93 (m, 1H, C<u>H</u>=CH$_2$); 5.16 (d, *J* = 10.6, 1H, NCH$_2$CH=C<u>H</u>-*cis*); 5.05 (d, *J* = 17.6, 1H, NCH$_2$CH=C<u>H</u>-*trans*); 4.96 (m, 2H, NCH$_2$); 3.90 (s, 3H, OCH$_3$); 3.82 (s, 3H, OCH$_3$); 3.77-2.98 (m, 7H, NHC<u>H$_2$</u>C<u>H</u> + C<u>H$_2$</u>CH$_3$ + 5'-CH$_2$); 2.19-1.70 (m, 4H, 3'-CH$_2$ + 4'-CH$_2$); 1.24 (t, *J* = 7.0, 3H, NCH$_2$C<u>H$_3$</u>). Found, %: C 58.55; H 6.76; N 9.27. C$_{22}$H$_{29}$N$_3$O$_5$ · HCl. Calculated, %: C 58.47; H 6.69; N 9.30.

Hydrochloride of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid R(+)-(1-ethylpyrrolidin-2-ylmethyl)-amide ***(3R)***. Obtained by the similar method. The yield is 96%. M. p. 241-243 °C (anhydrous EtOH). $[\alpha]_D^{20}$ +11.5° (*c* = 5, H$_2$O). ^{1}H NMR (DMSO-d$_6$), δ, ppm (*J*, Hz): 16.68 (s, 1H, 4-OH); 10.54 (t, *J* = 5.3, 1H, CONH); 10.32 (br. s, 1H, NH$^+$);7.36 (s, 1H, H-5); 6.91 (s, 1H, H-8); 5.92 (m, 1H, C<u>H</u>=CH$_2$); 5.16 (d, *J* = 10.6, 1H, NCH$_2$CH=C<u>H</u>-*cis*); 5.05 (d, *J* = 17.6, 1H, NCH$_2$CH=C<u>H</u>-*trans*); 4.95 (m, 2H, NCH$_2$); 3.90 (s, 3H, OCH$_3$); 3.82 (s, 3H, OCH$_3$); 3.77-2.97 (m, 7H, NHC<u>H$_2$</u>C<u>H</u> + C<u>H$_2$</u>CH$_3$ + 5'-CH$_2$); 2.19-1.70 (m, 4H, 3'-CH$_2$ + 4'-CH$_2$); 1.24 (t, *J* = 7.0, 3H, NCH$_2$C<u>H$_3$</u>). Found, %: C 58.58; H 6.75; N 9.25. C$_{22}$H$_{29}$N$_3$O$_5$ · HCl. Calculated, %: C 58.47; H 6.69; N 9.30.

Biological investigation

While carrying out the biological experiments, the animals were treated in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

The ability of hydrochlorides **3S** and **3R** to block opioid receptors was studied in white mice with the weight of 20-30 g by the method involving the elimination of the analgesic effect of narcotic analgesics, in particular, tramadol, a well-known synthetic opioid. The tested animals were separated into the groups of six for each dose and the substance studied. The initial pain threshold was determined for all the animals using a "hot plate" test (Eddy and Leimbach 1953; Tzschentke et al. 2007).

Then, all animals were given tramadol intramuscularly in the thigh in the dose of 10 mg/kg. The predicted analgesic effect was induced in 60 min and confirmed by retesting of the pain threshold. Then, 60 min after tramadol introduction, the animals of the control group were treated with the reference compound – racemic hydrochloride of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydro-quinoline-3-carboxylic acid (1-ethylpyrrolidin-2-ylmethyl)-amide (Ukrainets et al. 2010) in the dose of 1 mg/kg in the same manner, while the animals of other groups were treated with hydrochlorides **3S** and **3R** in the dose of 1 mg/kg. In 30 min all experiments fixed the return of the pain threshold to the initial level testifying the elimination of the analgesic effect of tramadol.

RESULTS AND DISCUSSION

As could be expected, hydrochlorides **3S** и **3R** have the similar melting temperatures and completely identical NMR ^{1}H spectra. It should be noted that as a whole NMR ^{1}H spectra registered in DMSO-d$_6$ provide a rather complete picture of the structure of the samples analysed. However, because of coincidence of a number of signals, the attempts to analyse in detail the ”aliphatic” spectral regions, in which signals of practically all protons of the amide fragment are concentrated at the short site, failed. Besides, hydrochlorides **3S** и **3R** appeared to be substances that are comparatively poorly soluble in DMSO, and it prevented to obtain the satisfactory spectra of homonuclear COSY correlations, which could facilitate significantly the solution of the given analytical task, for them.

The NMR-research of amide-base **2S** has become more conclusive. Thus, in particular, we succeeded to read all the signals of the COSY spectrum of this compound recorded in deuterochloroform. The key for interpretation of the problem ”aliphatic” part of the spectrum was the signal of amide proton NH absorbing in a weak field at 10.39 ppm. Its correlations with the nearest protons allowed to identify definitely the signals of protons of the neighbour methylene group at first, and then the signals of all protons of the pyrrolidine nucleus. The most important of the COSY correlations found are shown below by arrows (Fig. 1).

The synthesis of hydrochlorides **3S** и **3R** is carried out from 2-(aminomethyl)-1-ethylpyrrolidines with the known *S*- and *R*- configuration of asymmetric centres respectively; they were not involved in the process of the chemical reaction. On the other hand, when amidating alkyl 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates by chiral primary amines, neither racemization nor inversion of configuration has been observed (Ukrainets et al. 2000a, Ukrainets et al. 2000b). Therefore, there is every reason to state that optical antipodes **3S** and **3R** keep the space configuration of the initial amines.

Moreover, *S(–)*- and *R(+)*-2-(aminomethyl)-1-

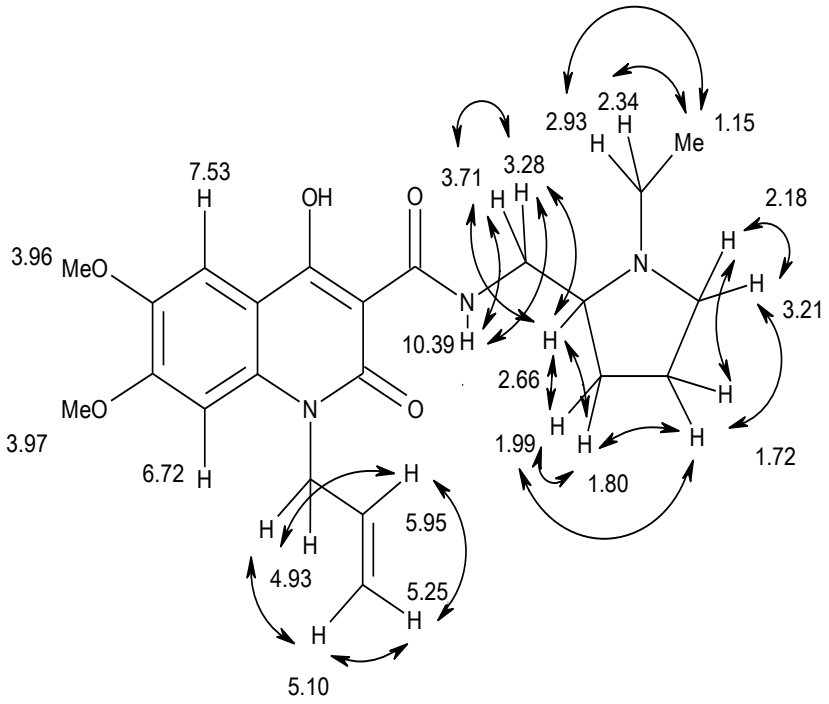

Figure 1. The most important of the COSY correlations for the amide-base **2S**.

Table 1. Biological characteristics of enantiomers **3S** and **3R.**

Compound	Pain threshold level, sec		
	Initial	60 min after tramadol introduction	30 min after introduction of test compound*
3S	14.75 ± 1.11	28.57 ± 2.59	12.27 ± 1.03 (–16.8 %)
3R	18.33 ± 1.32	32.60 ± 3.28	15.37 ± 1.22 (–16.1 %)
Racemate	16.51 ± 1.37	31.25 ± 3.24	13.81 ± 1.19 (–16.3 %)

The changes of the pain threshold level in % relative to the initial data are given in parentheses.

ethylpyrrolidines used by us initially differ slightly from each other by their optical purity (see Materials and methods). That is why it is not surprising that hydrochlorides **3S** и **3R** obtained on their basis are not completely pure stereochemically. However, differences in the absolute values of their optical rotation appeared to be quite insignificant. And this fact, as is known (Potapov 1988), is in itself a good sign of rather high optical purity of the products studied, and it is all sufficient for performing the biological research.

The study of a specific biological activity of the synthesized hydrochslorides of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid *S(–)*- and *R(+)*-(1-ethylpyrrolidin-2-ylmethyl)-amides has shown (Table 1) that in this case, the "chiral switch" did not give the expected result – the ability of both optical isomers **3S** and **3R** to block opioid receptors remains at the racemate level (Ukrainets et al. 2010). It should be noted that such similarity between the biological properties of enantiomers and their racemic mixture is quite rare (Kaneko 1997). For most drugs characterized the close relationship between their space structure and pharmacological activity, that is stereo specificity of action (Thacker 2007; Hardikar 2008; Gordon et al. 2010; Matera et al. 2011). The observed effect testifies that the asymmetrical carbon atom in 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-1,2-dihydroquinoline-3-carboxamides does not have contact with the active site of the target receptor. This fact is important for the subsequent retrieval of new, highly efficient opioid receptor antagonists among the synthetically available quinoline-3-carboxamides.

Our research has demonstrated that *S*- and *R*-enantiomers of 1-allyl-4-hydroxy-6,7-dimethoxy-2-oxo-

1,2-dihydroquinoline-3-carboxylic acid (1-ethylpyrrolidin-2-ylmethyl)-amide hydrochloride can block opiate receptors effectively. Thanks to their pharmacological properties, these compounds can be used as the base of medicines for the complex treatment of opioid drug dependence (addiction). However, the high cost of chiral 2-(aminomethyl)-1-ethylpyrrolidines makes the commercial manufacture of such medicines inexpedient economically, since much more available and cheap in its manufacture racemate is highly competitive with them in the potency of the specific activity.

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