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Full Length Research Paper

Halogen substitution anilides of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]quinoline-6-carboxylic acid as an attempt of optimization of quinolone diuretics by "me-too" method

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Using the principles of creating "me-too-drugs" halogen substitution anilides of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]quinoline-6-carboxylic acid have been synthesized as potential diuretics. According to the results of biological trials, the substances exceeding the diuretic effect of hydrochlorothiazide in sufficiently less dose have been revealed. It has been shown that like pyrroloquinolines, which were previously studied, 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]quinoline-6-carboxamides can also be the base for new highly effective diuretics and are worthy of further research.

Key words: Amidation, anilides, 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides, diuretics, "me-too".

INTRODUCTION

Imitation of the existing drugs with the simultaneous introduction of insignificant chemical alterations in their structure is well known in medicinal chemistry as "me-too" or "follow-on" technology (Giordanetto et al., 2011; Kubinyi, 2006; Evans, 2010). By definition drugs creating in such way have the structure close to the parent drug of the therapeutic group. At the same time their molecule should be obligatory original. It gives the designer a possibility to enter the market with the legally protected analogue of the innovative drug (Volskaya 2007).

There are two positive moments with "me-too-drugs" method. At first, some of these analogues (Kravchenko and Korzinov, 2008; Cohen et al., 2006; Serrao et al., 2009; Sopko et al., 2008; Reingold et al., 2009) often really exceed "the first-in-class" drug by the complex of properties, replace it completely with time and even become "best-in-class". The obvious example of this is "me-too-diuretic" Hydrochlorothiazide, which displaced its precursor Chlorothiazide. However, it should be remembered that sometimes the differences of the novel drug that is actively introduced may be quite arbitrary; they may be rather marketing than pharmacological ones.

The second positive effect concerning the manufacture of "me-too" drugs is a noticeable decrease in prices within the same therapeutic group (Nusbaum, 2002; Zhao and Guo, 2009; Chow and Liu, 2010). However, according to other data (Hollis, 2004, 2005; DiMasi and Paquette, 2004) expectations with regard to fall in prices have not always come true. As a result, there are debates and discussions about the expediency of creation, indications and application of such drugs at all (Volskaya, 2007; Lee 2004; Austin et al., 2006; Chow et al., 2010).

However, in spite of some biases "me-too" technology remains rather powerful tool for decreasing production costs and reducing the terms of development, clinical trials and market launch of new medicines, owing to that there is an increasing interest in many world countries today (Kravchenko and Korzinov, 2008; Cohen et al., 2006).

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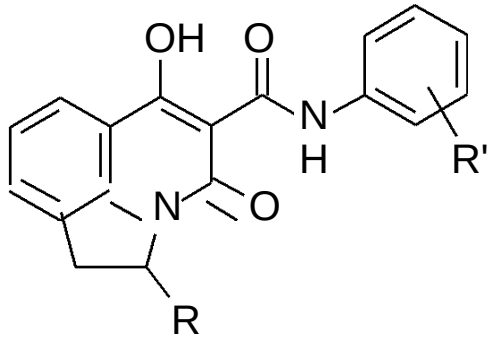


1: a R = H; **b** R = Me

Figure 1. General formula of pyrrolo[3,2,1-*ij*]quinolines revealing diuretic properties.

Taking into account the facts mentioned earlier our attempt to use the principles of creation of "me-too-drugs" for optimization of the previously described diuretics from the range of 6-hydroxy-4-oxo-2,4-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-5-carboxanilides 1a,b (Figure 1) (Ukrainets et al., 2007a, 2007b; Ukrainets et al. 2011a, 2011b; Ukrainets and Bereznyakova, 2012) is quite natural.

As an obvious and easily performed variant of the chemical modification of the compounds with the general formula 1 we studied anilides of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]quinoline-6-carboxylic acid 3 a-m. In this case it is of crucial importance that with transfer from pyrrolo-quinolone heterocyclic systems to pyrido-quinolone ones the spatial structure of a molecule changes insignificantly, and, as a result, both types of substances reveal similar regularities the “structure – diuretic activity” relationship (Ukrainets et al., 2010). In other words, the probability of diuretic properties revealing by anilides 3a-m at least at the level of basic analogues 1a,b remains rather high. What results prove to be in reality is the aim of the present research.

MATERIALS AND METHODS

Chemistry

^{1}H NMR spectra for anilides 3a-m were recorded on a Bruker WM-360 (360 MHz) spectrometer. The solvent was DMSO-d_6 and TMS as an internal standard for all cases. The chemical shifts values were recorded on δ scale and the coupling constants (*J*) in hertz. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, quin = quintet, m = multiplet. Melting points were determined by using the open capillary method and are uncorrected. Mass spectra were obtained on a Varian 1200 L spectrometer in full scanning mode in the range 40 to 450 *m/z* and EI ionization 70 eV. Elemental analysis was done on EuroVector EA-3000 analyzer. The synthesis of ethyl 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1- *ij*]quinoline-6-carboxyate (2) was carried out by the method in the study (Ukrainets et al., 2010).

General procedure for the synthesis of 7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxanilides (3a-m). A mixture of ethyl ester 2 (2.73 g, 0.01 mol), and the corresponding aniline (0.01 mol) was stirred and allow to stand at 130 to 140°C for 5 to 15 min. The reaction mixture was then cooled to about 100°C, ethanol (10 to 15 ml) was carefully added, and thoroughly triturated. The precipitated corresponding anilide 3a–m was filtered off, washed with cold alcohol, dried, and recrystallized from the mixture of dimethylformamide (DMF) and ethanol.

N-(2-Fluorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3a)

Yield: 85%; mp. 211-213°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.19 (1H, s, 7-OH), 12.88 (1H, s, NH), 8.40 (1H, t, *J* = 7.7, H-3'), 7.98 (1H, d, *J* = 8.0, H-8), 7.44 (1H, d, *J* = 7.5, H-10), 7.18 (1H, t, *J* = 7.5, H-9), 7.15-7.08 (3H, m, 4', 5', 6'), 4.17 (2H, t, *J* = 5.7, CH_2-3), 3.01 (2H, t, *J* = 6.1, CH_2-1), 2.14 (2H, quin, *J* = 5.7, CH_2-2); EI-MS (*m/z*): 338 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}FN_2O_3$: C, 67.45; H, 4.47; N, 8.28%; found: C, 67.58; H, 4.56; N, 8.21%.

N-(3-Fluorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3b)

Yield: 94%; mp. 178-180°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.21 (1H, s, 7-OH), 12.79 (1H, s, NH), 7.98 (1H, d, *J* = 8.1, H-8), 7.66 (1H, d, *J* = 10.3, H-2'), 7.44 (1H, d, *J* = 7.1, H-10), 7.33-7.28 (2H, m, H-5', 6'), 7.18 (1H, t, *J* = 7.6, H-9), 6.83 (1H, t.d, *J* = 7.7, *J* = 2.6, H-4'), 4.14 (2H, t, *J* = 5.8, CH_2-3), 3.01 (2H, t, *J* = 6.1, CH_2-1), 2.13 (2H, quin, *J* = 5.8, CH_2-2); EI-MS (*m/z*): 338 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}FN_2O_3$: C, 67.45; H, 4.47; N, 8.28%; found: C, 67.59; H, 4.57; N, 8.19%.

N-(4-Fluorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3c)

Yield: 96%; mp. 193-195°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.37 (1H, s, 7-OH), 12.65 (1H, s, NH), 7.97 (1H, d, *J* = 8.1, H-8), 7.68 (2H, d.d, *J* = 8.6, *J* = 5.2, H-2', 6'), 7.45 (1H, d, *J* = 7.5, H-10), 7.18 (1H, t, *J* = 7.5, H-9), 7.06 (2H, t, *J* = 9.2, H-3', 5'), 4.14 (2H, t, *J* = 5.8, CH_2-3), 3.00 (2H, t, *J* = 6.1, CH_2-1), 2.13 (2H, quin, *J* = 5.8, CH_2-2); EI-MS (*m/z*): 338 $[M]^+$; Elemental analysis: calcd. for

$C_{19}H_{15}FN_2O_3$: C, 67.45; H, 4.47; N, 8.28%; found: C, 67.54; H, 4.52; N, 8.31%.

N-(3,4-Difluorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3d)

Yield: 93%; mp. 187-189°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.09 (1H, s, 7-OH), 12.78 (1H, s, NH), 7.98 (1H, d, *J* = 8.1, H-8), 7.83 (1H, d.d.d, *J* = 12.5, *J* = 7.0, *J* = 2.2, H-2'), 7.46 (1H, d, *J* = 7.1, H-10), 7.30 (1H, d, *J* = 8.5, H-6'), 7.18 (1H, t, *J* = 7.6, H-9), 4.13 (2H, t, *J* = 5.5, CH_2-3), 3.01 (2H, t, *J* = 6.0, CH_2-1), 2.13 (2H, quin, *J* = 5.5, CH_2-2); EI-MS (*m/z*): 356 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{14}F_2N_2O_3$: C, 64.04; H, 3.96; N, 7.86%; found: C, 63.92; H, 3.85; N, 7.94%.

N-(2-Chlorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3e)

Yield: 87%; mp. 203-205°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.23 (1H, s, 7-OH), 13.00 (1H, s, NH), 8.46 (1H, d, *J* = 8.4, H-6'), 7.99 (1H, d, *J* = 8.0, H-8), 7.46 (1H, d, *J* = 7.5, H-10), 7.42 (1H, d, *J* = 8.4, H-3'), 7.29 (1H, t, *J* = 7.8, H-5'), 7.19 (1H, t, *J* = 7.6, H-9), 7.09 (1H, t, *J* = 7.8, H-4'), 4.18 (2H, t, *J* = 5.7, CH_2-3), 3.01 (2H, t, *J* = 6.1, CH_2-1), 2.14 (2H, quin, *J* = 5.7, CH_2-2); EI-MS (*m/z*): 354/356 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}ClN_2O_3$: C, 64.32; H, 4.26; N, 7.90%; found: C, 64.24; H, 4.15; N, 7.81%.

N-(3-Chlorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3f)

Yield: 95%; mp. 176-178°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.17 (1H, s, 7-OH), 12.77 (1H, s, NH), 7.98 (1H, d, *J* = 8.0, H-8), 7.84 (1H, s, H-2'), 7.49 (1H, d, *J* = 8.0, H-6'), 7.44 (1H, d, *J* = 7.4, H-10), 7,30 (1H, t, *J* = 8.0, H-5'), 7.18 (1H, t, *J* = 7.6, H-9), 7.09 (1H, d, *J* = 8.0, H-4'), 4.14 (2H, t, *J* = 5.7, CH_2-3), 3.00 (2H, t, *J* = 6.1, CH_2-1), 2.13 (2H, quin, *J* = 5.7, CH_2-2); EI-MS (*m/z*): 354/356 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}ClN_2O_3$: C, 64.32; H, 4.26; N, 7.90%; found: C, 64.41; H, 4.37; N, 8.03%.

N-(4-Chlorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3g)

Yield: 94%; mp. 193-195°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.26 (1H, s, 7-OH), 12.73 (1H, s, NH), 7.97 (1H, d, *J* = 8.1, H-8), 7.67 (2H, d, *J* = 8.7, H-2', 6'), 7.44 (1H, d, *J* = 7.4, H-10), 7.31 (2H, d, *J* = 8.7, H-3', 5'), 7.18 (1H, t, *J* = 7.7, H-9), 4.13 (2H, t, *J* = 5.8, CH_2-3), 3.00 (2H, t, *J* = 6.0, CH_2-1), 2.13 (2H, quin, *J* = 5.8, CH_2-2); EI-MS (*m/z*): 354/356 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}ClN_2O_3$: C, 64.32; H, 4.26; N, 7.90%; found: C, 64.40; H, 4.35; N, 7.97%.

N-(2,3-Dichlorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3h)

Yield: 84%; mp. 241-243°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.05 (1H, s, 7-OH), 13.19 (1H, s, NH), 8.44 (1H, t, *J* = 5.2, H-5'), 8.00 (1H, d, *J* = 8.0, H-8), 7.48 (1H, d, *J* = 7.3, H-10), 7.29-7.26 (2H, м, H-4', 6'), 7.20 (1H, t, *J* = 7.5, H-9), 4.20 (2H, t, *J* = 5.7, CH_2-3), 3.03 (2H, t, *J* = 6.1, CH_2-1), 2.15 (2H, quin, *J* = 5.7, CH_2-2); EI-MS (*m/z*): 388/390/392 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{14}Cl_2N_2O_3$: C, 58.63; H, 3.63; N, 7.20%; found: C, 58.51; H, 3.54; N, 7.14%.

N-(2,4-Dichlorophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3i).

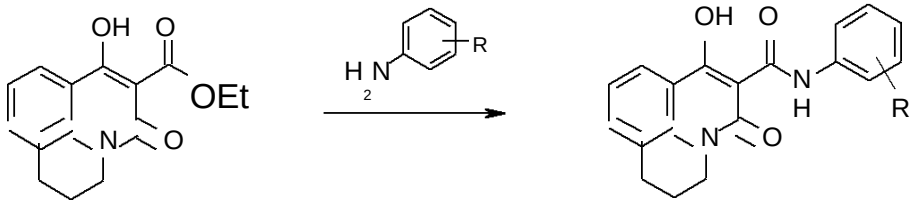
Yield: 89%; mp. 249-251°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 15.96 (1H, s, 7-OH), 13.13 (1H, s, NH), 8.43 (1H, d, *J* = 8.7, H-6'), 7.97 (1H, d, *J* = 8.0, H-8), 7.56 (1H, s, H-3'), 7.49 (1H, d, *J* = 7.3, H-10), 7.36 (1H, d, *J* = 8.7, H-5'), 7.23 (1H, t, *J* = 7.6, H-9), 4.15 (2H, t, *J* = 5.6, CH_2-3), 2.99 (2H, t, *J* = 6.1, CH_2-1), 2.11 (2H, quin, *J* = 5.6, CH_2-2); EI-MS (*m/z*): 388/390/392 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{14}Cl_2N_2O_3$: C, 58.63; H, 3.63; N, 7.20%; found: C, 58.54; H, 3.71; N, 7.32%.

N-(2-Bromophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3j)

Yield: 82%; mp. 202-204°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.25 (1H, s, 7-OH), 12.88 (1H, s, NH), 8.39 (1H, d, *J* = 8.3, H-6'), 7.99 (1H, d, *J* = 8.0, H-8), 7.60 (1H, d, *J* = 8.0, H-3'), 7.46 (1H, d, *J* = 7.4, H-10), 7.34 (1H, t, *J* = 7.8, H-5'), 7.18 (1H, t, *J* = 7.5, H-9), 7.04 (1H, t, *J* = 7.8, H-4'), 4.18 (2H, t, *J* = 5.8, CH_2-3), 3.01 (2H, t, *J* = 6.1, CH_2-1), 2.14 (2H, quin, *J* = 5.8, CH_2-2); EI-MS (*m/z*): 398/400 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}BrN_2O_3$: C, 57.16; H, 3.79; N, 7.02%; found: C, 57.27; H, 3.92; N, 6.94%.

N-(3-Bromophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3k)

Yield: 91%; mp. 193-195°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.18 (1H, s, 7-OH), 12.78 (1H, s, NH), 8.00 (1H, d, *J* = 8.0, H-8), 7.97 (1H, s, H-2'), 7.56 (1H, d, *J* = 8.5, H-6'), 7.46 (1H, d, *J* = 7.4, H-10), 7.27-7.23 (2H, m, H-4', 5'), 7.19 (1H, t, *J* = 7.6, H-9), 4.15 (2H, t, *J* = 5.8, CH_2-3), 3.01 (2H, t, *J* = 6.1, CH_2-1), 2.14 (2H, quin, *J* = 5.8, CH_2-2); EI-MS (*m/z*): 398/400 $[M]^+$; Elemental analysis: calcd.



2 **3a-m**

Figure 2. Synthesis of anilides 3 from ester 2 and anilines.

for $C_{19}H_{15}BrN_2O_3$: C, 57.16; H, 3.79; N, 7.02%; found: C, 57.25; H, 3.88; N, 7.12%.

N-(4-Bromophenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3l)

Yield: 96%; mp. 198-200°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.24 (1H, s, 7-OH), 12.74 (1H, s, NH), 7.98 (1H, d, *J* = 7.9, H-8), 7.62 (2H, d, *J* = 8.5, H-2', 6'), 7.48-7.42 (3H, m, H-10, 3', 5'), 7.19 (1H, t, *J* = 7.6, H-9), 4.14 (2H, t, *J* = 5.6, CH_2-3), 3.01 (2H, t, *J* = 5.9, CH_2-1), 2.13 (2H, quin, *J* = 5.6, CH_2-2); EI-MS (*m/z*): 398/400 $[M]^+$; Elemental analysis: calcd. for $C_{19}H_{15}BrN_2O_3$: C, 57.16; H, 3.79; N, 7.02%; found: C, 57.22; H, 3.85; N, 6.96%.

N-(2-Bromo-4-methylphenyl)-7-hydroxy-5-oxo-2,3-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-6-carboxamide (3 m)

Yield: 83%; mp. 227-229°C; ^{1}H NMR (360 MHz, DMSO-d_6): δ 16.35 (1H, s, 7-OH), 12.76 (1H, s, NH), 8.23 (1H, d, *J* = 8.6, H-6'), 7.99 (1H, d, *J* = 8.1, H-8), 7.46 (1H, d, *J* = 7.3, H-10), 7.42 (1H, s, H-3'), 7.19 (1H, t, *J* = 7.7, H-9), 7.12 (1H, d, *J* = 8.6, H-5'), 4.18 (2H, t, *J* = 5.7, CH_2-3), 3.02 (2H, t, *J* = 6.0, CH_2-1), 2.35 (3H, s, Me), 2.14 (2H, quin, *J* = 5.7, CH_2-2); EI-MS (*m/z*): 412/414 $[M]^+$; Elemental analysis: calcd. for $C_{20}H_{17}BrN_2O_3$: C, 58.13; H, 4.15; N, 6.78%; found: C, 58.26; H, 4.27; N, 6.67%.

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 effect of the compounds synthesized on the excretory renal function was studied in white outbred rats of both genders with the weight of 180 to 200 g by the standard method (Sernov and Gatsura, 2000). All experimental animals were given a water load calculating in 25 ml/kg via a gastric tube. The control group was given only the similar amount of water with Tween-80. The anilides 3a-m tested was introduced *per os* in the form of thin water suspension stabilized by Tween-80. The primary screening was carried out in the dose of 10 mg/kg, which corresponds to ED_{50} of one of the most active 6-hydroxy-4-oxo-2,4-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-5-carboxanilides (1a, R' = 4-OMe). The values of excretion were registered in 4 h comparing them with the control, as well as a known diuretic hydrochlorothiazide (Kleemann et al., 2008) used in its effective dose of 40 mg/kg.

RESULTS AND DISCUSSION

The way for obtaining of anilides of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]quinoline-6-carboxylic acid is known and it consists in long-term (up to 48 hours) refluxion of ethyl ester 2 with 40% excess of the corresponding aniline in a large volume of bromobenzene (Kutyrev and Kappe, 1997). Yields are often low, that is why this method has not been distributed widely. We have found a very simple but quite effective solution of this problem. As it turned out, to achieve high yields of target anilides 3a-m it is sufficient to keep the mixture of ester 2 and the corresponding aromatic amine in equimolar amounts at 130-140 °C allowing the evolving ethanol to distill freely from the reactor (Figure 2). Thereby duration of amidation decreases to 5 to 15 min. Besides, the necessity of using a highly boiling inert solvent and a great amount of aniline not any longer exists.

All anilides 3a-m synthesized are colourless crystalline substances soluble in DMF and dimethyl sulfoxide (DMSO), slightly soluble in ethanol, practically insoluble in water. Their structure is confirmed by ^{1}H NMR spectra, which characteristic feature is common set of signals for protons of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*] quinoline cycle for all compounds. Thus, enolic hydroxyl occurs as a narrow singlet in a weak region δ: in average 16.25 ppm. In the aromatic spectral range there are two doublets δ: 7.99 and 7.45 ppm and triplet δ: 7.19 ppm conditioned by protons of the benzene part of the quinolone nucleus in positions 8, 10

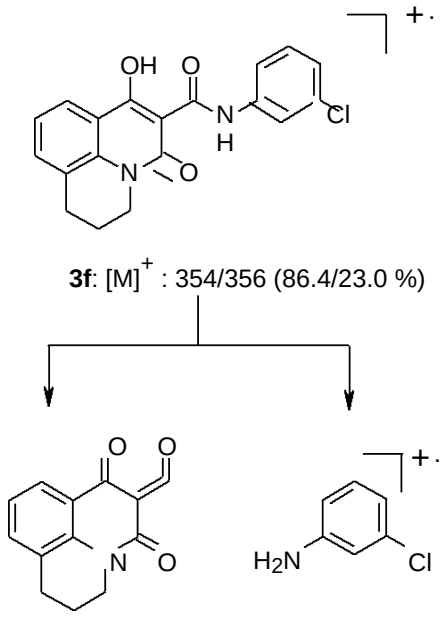


Figure 3. The primary fragmentation of a molecular ion of *meta*-chloroanilide 3f.

and 9, respectively. Methylene groups of tetrahydropyridine fragment of the molecule resonate in the powerful field appearing as two triplets δ: 4.15 and 3.00 ppm and quintet at δ: 2.13 ppm (CH_2-groups in position 3, 1 and 2, respectively). The position of substituents in anilide fragments are easily determined by intensity and multiplicity of signals remaining in the aromatic nucleus of protons (Experimental). But ^{1}H NMR spectroscopy cannot determine which exactly halogen is present in the sample. That is why while studying the structure of all anilides 3a-m we used additionally mass spectrometry.

It should be noted that mass spectrometry allows solving similar analytical problems not only by determining the molecular weight of the substance tested. Multiplicity and relative intensity of separate peaks in the spectrum also provide important information about the halogen presence in the molecule since they are determined by the isotope content of a halogen (Terentiev and Stankyavichus, 1987). For example, as an element in nature chlorine exists in the form of two stable isotopes ^{35}Cl and ^{37}Cl in the ratio of 3:1. As a result, doublet of molecular ion peaks with *m/z* 354/356 is clearly fixed in the mass-spectra of *meta*-chloroanilide 3f with approximately the same ratio of intensities (Figure 3).

The peaks of molecular ions of mono-brominated anilides 3j-m have also the form of doublets, but their intensity is practically the same since occurrence of ^{79}Br and ^{81}Br isotopes are approximately the same. The character of mass spectrum changes with the increase of the number of atoms of chlorine or bromine in the molecule (Terentiev and Stankyavichus, 1987). In particular, the peaks of molecular ions of dichloro-substituted anilides 3h,i are triplets. Fluorine is monoisotopic, that is why all peaks in the mass spectra of fluorine anilides 3a-d, irrespective of the number of atoms of fluorine, are singlets.

The primary fragmentation of molecular ions of the compounds studied caused by electronic impaction is shown on the example of *meta*-chloroanilide 3f, it is accompanied by breaking of the acyclic amide bond with formation of common to all samples tricyclic ketene 4 with *m/z* 227 and a fragment of specific amine 5 (Figure 3). By the way, such behaviour is quite common for many 4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamides (Ukrainets et al., 2009a, 2009b).

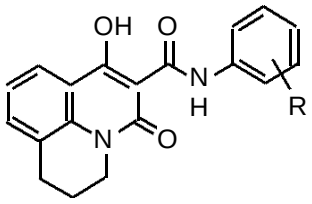
Evaluation of the diuretic activity

The experimental data (Table 1) obtained when studying the effect of halogen-substituted anilides 3a-m on the excretory function of the kidneys testify that the common for all group regularities of the "structure – activity" relationship are rather difficult to reveal. But in narrower ranges the dependence of the diuretic effect revealed on the nature and position of halogen in the anilide fragment can be seen quite clearly. For example, one may confidently state that the presence of the bromine atom in the anilide fragment impacts negatively on the diuretic properties. Thus, its transfer from *ortho*-position to *meta*- and further to *para*-position is accompanied by appearance and gradual intensification of the antidiuretic activity.

The influence of the chlorine atom is not so unambiguous. If *ortho*- and *para*-isomers 3e,g inhibit diuresis slightly, then, on the contrary, *meta*-chloroanilide 3f is intensifies markedly. A positive effect of *meta*-substituent is also observed in the case of dichloroanilides 3h,i.

Fluoro-substituted derivatives are of the most interest of all the substances studied. They exert the diuretic properties irrespective of the position of the fluorine atom. However, this factor certainly influence on the potency of the diuretic effect – of the whole group only *para*-fluoroanilide of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido [3,2,1-*ij*] quinoline-6-carboxylic acid 3c call attention to itself. This compound exceeds in much less dose hydrochlorothiazide by its specific activity, and, therefore, is worth of further research. Interestingly that *para*-fluoroanilide appeared to be the most powerful diuretic even among halogen-substituted 6-hydroxy-2-methyl-4-oxo-2,4-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-5-carboxanilides (1b, R' = 4-F) (Ukrainets et al., 2011a) previously researched. But in the group of 6-hydroxy-4-oxo-2,4-dihydro-1*H*-pyrrolo[3,2,1-*ij*]quinoline-5-carboxanilides (1a) the presence of the fluorine atom in *para*-position of the anilide fragment has been considered

Table 1. The diuretic activity for anilides **3a-m** and Hydrochlorothiazide



Compound	R	Diuresis in 4 h, ml	%*
3a	2-F	4.07 ± 0.21	+ 19
3b	3-F	4.79 ± 0.28	+ 40
3c	4-F	6.67 ± 0.30	+ 95
3d	3,4-F_2	3.79 ± 0.24	+ 11
3e	2-Cl	3.08 ± 0.15	– 10
3f	3-Cl	4.75 ± 0.32	+ 39
3g	4-Cl	2.94 ± 0.13	– 14
3h	2,3-Cl_2	4.89 ± 0.25	+ 43
3i	2,4-Cl_2	2.49 ± 0.17	– 27
3j	2-Br	3.55 ± 0.24	+ 4
3k	3-Br	2.43 ± 0.16	– 29
3l	4-Br	2.32 ± 0.20	– 32
3m	2-Br-4-Me	2.87 ± 0.22	– 16
Hydrochlorothiazide	–	5.16 ± 0.31	+ 51
Control	–	3.42 ± 0.27	–

* "+" Indicates increase and "-" inhibition of diuresis when compared with the control taken as 100%.

exceptionally undesirable (Ukrainets et al., 2007b).

Conclusions

Amidation of ethyl 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido [3,2,1-*ij*] quinoline-6-carboxyate by anilines has been suggested to carry out by heating of equimolar amounts of reagents without a solvent. The given method allows reducing the duration of the reaction to some minutes, to cease using the excess of aniline and at the same time to obtain target anilides with high yields. The influence of all halogen substitution anilides of 7-hydroxy-5-oxo-2,3-dihydro-1*H,5H*-pyrido[3,2,1-*ij*]-quinoline-6-carboxylic acid synthesized by this method on the excretory function of the kidneys has been studied. As a result, the substances, which exceed in a lower dose the known drug hydrochlorothiazide in their diuretic properties, have been found. The regularities of the “structure – activity” relationship revealed are discussed.

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