# NEW VARIANT OF THE PFIZINGER REACTION. SYNTHESIS AND CHEMICAL TRANSFORMATIONS OF SUBSTITUTED 2-AMINOMETHYL-QUINOLINE-3,4-DICARBOXYLIC ACIDS

Dmitri V. Kravchenko,[a] Volodymyr M. Kysil,[b] Sergey E. Tkachenko,[b,c] Sergey Maliarchouk,[b,d] Ilya M. Okun,[b,d] Alexandre V. Ivachtchenko*[,b]

[a]Department of Organic Chemistry, [c]Department of Medicinal chemistry, [d]Department of Molecular Biology and HTS, Chemical Diversity Research Institute, Khimki, Moscow Reg., Russia, [b]ChemDiv, Inc., San Diego, CA USA

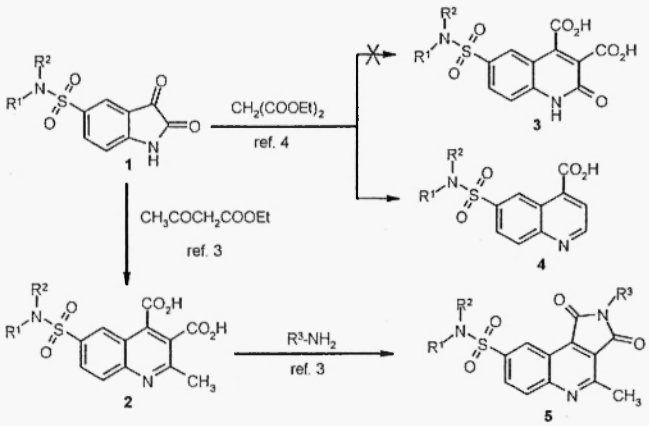
*To whom correspondence should be addressed. Phone: (858) 794-4860. Fax: (858) 794-4931

E-mail: av@chemdiv.com

**Abstract:** We present a convenient synthesis of novel 8-sulfonyl-4-(morpholin-4-ium-4-ylmethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines using a modification of the classical Pfitzinger reaction. According to our method, the 5-sulfonylated isatins were reacted with a mixture of ethyl 4-chloro-3-oxo-butanoate and morpholine. The resulting quinoline-3,4-dicarboxylic acids were then successively converted into the corresponding furo[3,4-*c*]quinoline-1,3-diones and pyrrolo[3,4-*c*]quinoline-1,3-diones. The latters appeared to be effective nonpeptide inhibitors of caspase-3 enzyme.

## Introduction

In a series of our recent works, we studied the behavior of 5-sulfamoylisatins under the conditions of Pfitzinger reaction (1,2). Thus, we reported a novel effective synthetic route to 6-sulfamoylquinoline-3,4-dicarboxylic acids (3). In a follow-up study of this synthetic method, we have found that the presence of sulfamoyl group in position 5 of isatin moiety increases the susceptibility of the isatin carbonyl group to nucleophilic attack and, thus, facilitates the formation of various reactive intermediates (4). The subsequent reactions of these intermediates are kinetically controlled and are often favorable over the classical Pfitzinger pathway. Thus, 6-sulfamoylquinoline-4-carboxylic acid **4** was isolated as the major product of reaction between *N*-substituted 5-sulfamoylisatins **1** with diethyl malonate instead of the anticipated 2-oxo-1,2-dihydroquinoline-4-carboxylic acid **3** (Scheme 1).



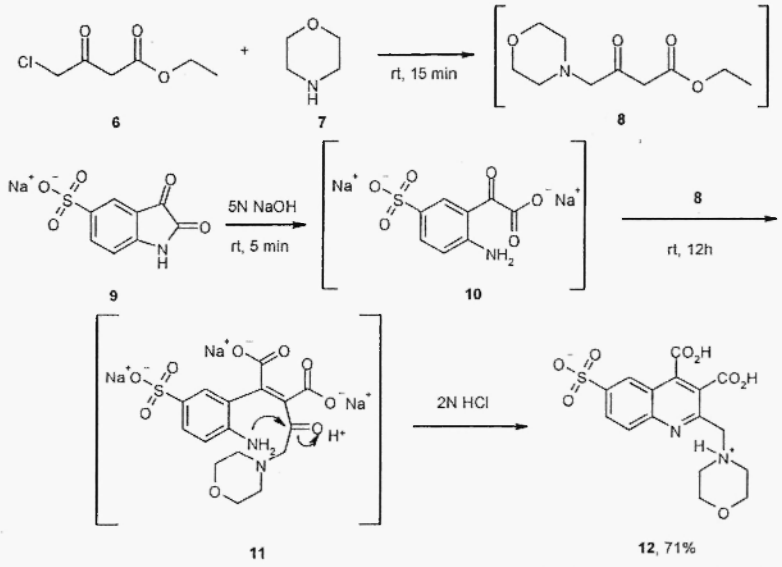

Transformations of 5-sulfamoylisatins (3,4).

**Scheme-1**

In the mentioned work (3), we succeded to obtain dicarboxylic acids **2** using the reaction of 5-sulfamoyl isatins **1** with ethyl acetoacetate under strong alkali conditions. Acids **2** were then converted into the corresponding 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines **5**. The latters represent a novel pharmacophoric heterocyclic scaffold with interesting biological activity (5,6).

**Results and Discussions**

Reported herein are our continuing studies on the synthesis and characterization of novel derivatives of 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline heterocyclic system. According to our approach depicted in Scheme 2, sodium isatin-5-sulfonate **9** was initially hydrolized with aqueous NaOH to give oxoacetate **10**. The latter was then treated with a mixture of methyl 4-chloro-3-oxo-butanoate **6** and morpholine **7** (1:4, mol/mol), which actually contained *in situ* formed ethyl 4-morpholin-4-yl-3-oxobutanoate **8**. The 3-(morpholin-4-ylacetyl)but-2-enedioate **11** formed in this reaction underwent rapid conversion into the corresponding 3,4-dicarboxylic acid **12** upon acidification of the reaction mixture. It is worthy of note that a very complex, inseparable mixture of products was formed when we treated oxoacetate **10** with methyl 4-chloro-3-oxo-butanoate, without addition of morpholine.

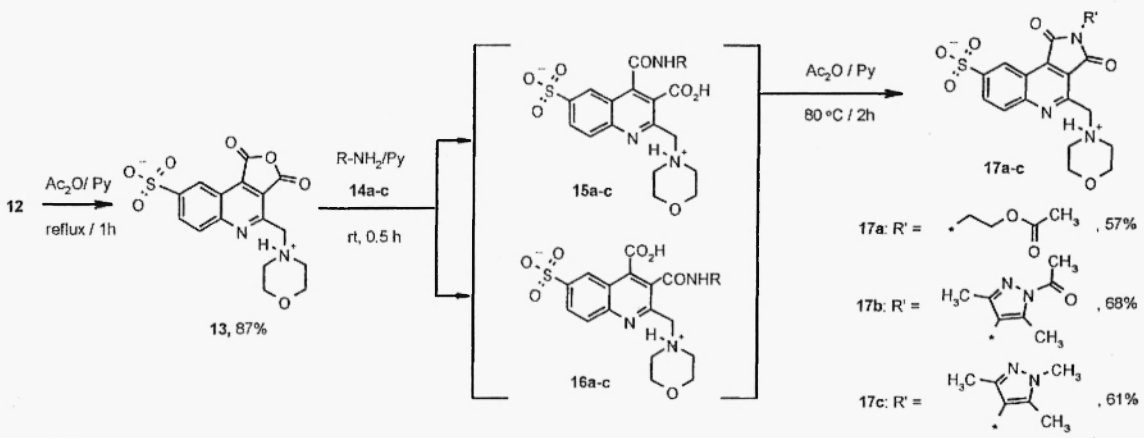



Synthesis of 3,4-dicarboxy-2-(morpholin-4-ium-4-ylmethyl)quinoline-6-sulfonate **12**.

**Scheme-2**

3,4-Dicarboxylic acid **12** was converted into furan-2,5-dione **13** upon the reaction with acetic anhydride in dry pyridine (Scheme 3). According to LCMS data, reaction of anhydride **13** with primary amines **14a-c** initially led to a mixture of two isomers **15a-c** and **16a-c** in 1:1 ratio. This mixture was converted into imides **17a-c** upon the treatment with acetic anhydride (yield 57-68%). In the case of 2-aminoethanol **14a** and 4-amino-3,5-dimethyl-1*H*-pyrazole **14b**, the assembly of the pyrrolo[3,4-*c*]quinoline-1,3-dione heterocyclic system is accompanied by acetylation leading to the corresponding acetylated products. Interestingly, imides **5** (Scheme 1) could be obtained in a similar manner, but without addition of $Ac_2O$ (3). These experimental data suggest that the intermediates **15a-c** and **16a-c** are probably stabilized by intramolecular electrostatic interaction between the carboxylic (carboxamide) group and the positively charged morpholinium nitrogen, and addition of the dehydrating agent is required for their conversion into the desired imides **17a-c**.

The assignment of these structures was made on the basis of LCMS, $^1$H NMR, $^{13}$C NMR and high-resolution mass-spectroscopy data. Satisfactory analytical data consistent with the shown molecular structures were obtained for all compounds.




Synthesis of 8-sulfonyl-4-(morpholin-4-ium-4-ylmethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines.

**Scheme-3**

Recently, we have discovered that 4-alkyl-substituted 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines display potent inhibitory activity against caspase-3 and represent a new chemotype of nonpeptide small molecule inhibitors of this enzyme (5,6). In a similar way, compounds **17a-c** have been tested on their ability to inhibit caspase-3 catalyzed proteolytic breakdown of its fluorogenic substrate. In this *in vitro* assay, the obtained compounds displayed high inhibitory activity with $IC_{50}$ in the range of 0.5-1.26 μM ($IC_{50}$ = 0.50 μM for **17a**, $IC_{50}$ = 1.00 μM for **17b** and $IC_{50}$ = 1.26 μM for **17c**). These preliminary biological data suggest that further structure-activity relationship studies around the synthesized scaffold can provide compounds with valuable physiological activities.

## Conclusions

We have developed an efficient synthetic approach to novel 4,8-disubstituted derivatives of 1,3-dihydro-pyrrolo[3,4-*c*]quinoline-1,3-diones. As key step, the method features Pfitzinger reaction of 5-sulfonylated isatins with a mixture of ethyl 4-chloro-3-oxo-butanoate and morpholine. Subsequent reactions amenable to high-throughput combinatorial protocol provide a convenient synthetic way to the target compounds, which represent a valuable pharmacophoric chemotype. Thus, the synthesized compounds are effective inhibitors of caspase-3 enzyme, which plays a key role in apoptosis and, therefore, represents attractive target for therapeutic intervention in several diseases (7).

## Experimental

**3,4-Dicarboxy-2-(morpholin-4-ium-4-ylmethyl)quinoline-6-sulfonate 12**. Morpholine (6.96 mL, 80 mmol) was slowly added to a solution of methyl 4-chloro-3-oxo-butanoate (3.01 g, 20 mmol) in dioxane (15 mL) at 10-15 °C, and the mixture was stirred at r. t. for 1.5 h. The resulting mixture was added to a solution of sodium isatin-5-sulfonate (2.49 g, 10 mmol) in 5N NaOH (25 mL). The reaction mixture was stirred at r. t. overnight, then washed with ether (2×40 mL). The water layer was acidified with 2N HCl until pH 2-3 was reached, and the resulting suspension was kept at 0 °C for 3 h. The formed precipitate was filtered out, washed with cool 2N HCl and lyophilized to afford pure acid **12** as light-grey solid. Yield 2.81 g (71%). HRMS: m/z [M+H$^+$] calcd for $C_{16}H_{16}N_2O_8S$: 397.0700. Found: 397.0681; $^1$H-NMR (400 MHz, DMSO-$d_6$): δ 14.3 (br.s, 2H), 10.3 (br.s, 1H), 8.23 (d, $J_m$ = 1.8 Hz, 1H), 8.11-8.13 (m, 2H), 5.02 (s, 2H), 3.97-4.04 (m, 8H); $^{13}$C NMR (DMSO-$d_6$): δ 167.29, 166.14, 149.90, 148.06, 146.25, 144.23, 130.48, 128.69, 122.04, 121.22, 120.97, 63.34, 59.21, 52.50.

**4-(Morpholin-4-ium-4-ylmethyl)-1,3-dioxo-1,3-dihydrofuro[3,4-*c*]quinoline-8-sulfonate 13.** A mixture of 12 (1.98 g, 5 mmol), acetic anhydride (15 mL) and pyridine (1 mL) was stirred at 80 °C for 2 h. The obtained solution was cooled to 0 °C and kept at this temperature for 4 h. The formed precipitate was filtered off, washed with $Ac_2O$ and ether, and dried in vacuo to give 1.65 g (87%) of **13**, which was used at the next step without further purification.

**General Procedure for Preparation of Compounds 17a-c.** Anhydride **13** was added to a solution of amine **14a-c** (0.25 mmol) in pyridine (2 mL), and the mixture was stirred at r. t. for 0.5 h. Acetic anhydride (1 mL) was added and the resulting mixture was stirred at 80 °C for 2 h and then cooled to r. t. Ether (5 mL) was added and the formed precipitate was collected by centrifugation, washed with EtOAc and dried to afford imides **17a-c** as colorless solids.

2-[2-(Acetyloxy)ethyl]-4-(morpholin-4-ium-4-ylmethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-8-sulfonate **17a**, yield 57%; HRMS: m/z [M+H$^+$] calcd for $C_{20}H_{21}N_3O_8S$: 464.1122. Found: 464.1120; $^1$H-NMR (400 MHz, DMSO-$d_6$): δ 10.6 (br.s, 1H), 9.04 (d, $J_m$ = 1.8 Hz, 1H), 8.36 (d, $J_o$ = 9.1 Hz, 1H), 8.19 (dd, $J_o$ = 9.1 Hz, $J_m$ = 1.8 Hz, 1H), 4.27 (t, $J$ = 1.55 Hz, 2H), 3.90 (t, $J$ = 5.5 Hz, 2H), 3.65-3.59 (m, 4H), 3.41-3.33 (m, 2H), 3.02-2.81 (m, 4H), 2.99 (s, 3H). 2-(1-Acetyl-3,5-dimethyl-1*H*-pyrazol-4-yl)-4-(morpholin-4-ium-4-ylmethyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-8-sulfonate **17b**, yield 68%; HRMS: m/z [M+H$^+$] calcd for $C_{23}H_{23}N_5O_7S$: 514.1391. Found: 514.1394. 4-(Morpholin-4-ium-4-ylmethyl)-1,3-dioxo-2-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline-8-sulfonate **17c**, yield 61%; HRMS: m/z [M+H$^+$] calcd for $C_{22}H_{23}N_5O_6S$: 486.1442. Found: 486.1440.

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