Available online at www.sciencedirect.com
SCIENCE DIRECT
IL FARMACO
Il Farmaco 60 (2005) 804–809
http://france.elsevier.com/direct/FARMAC/

Original article

Synthesis and caspase-3 inhibitory activity of 8-sulfonyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines

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, Chemical Diversity Research Institute, Khimki, Moscow Region, Russia*
[b] *Chemical Diversity Labs, Inc., 11558 Sorrento Valley Rd., Suite 5, 92121 San Diego, CA, United States*
[c] *Department of Medicinal Chemistry, Chemical Diversity Research Institute, Khimki, Moscow Region, Russia*
[d] *Department of Molecular Biology and HTS, Chemical Diversity Research Institute, Khimki, Moscow Region, Russia*

Received 20 January 2005; received in revised form 18 July 2005; accepted 2 August 2005

Available online 21 September 2005

Abstract

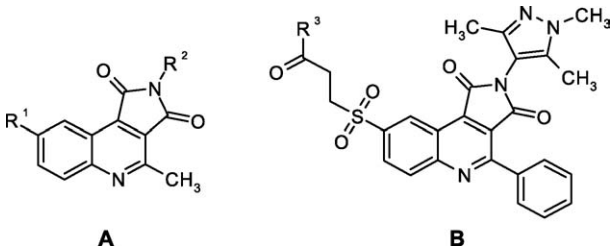
A convenient synthesis of novel 8-sulfonyl-1,3-dioxo-4-methyl-2,3-dihydro-1H-pyrrolo[3,4-c]quinolines is described. As key steps to assemble the target molecular scaffold, our method features (a) Pfitzinger reaction of isatin-5-sulfonate **1** with methyl 3-oxo-3-phenylpropanoate, (b) formation of 1-(1H-pyrazol-4-yl)-1H-pyrrole-2,5-dione intermediate **5**, and (c) reaction of sulfinic acid **9** with acrylate or methylacrylate leading to the corresponding sulfonyl propionates. Two compounds, ester **11** and morpholide **13**, have been identified as potent inhibitors of caspase-3 with IC_{50} = 6 nM. Our primary data suggest noncompetitive and reversible character of caspase-3 inhibition.

Keywords: 8-Sulfonyl-1,3-dioxo-4-methyl-2,3-dihydro-1*H*-pyrrolo[3; 4-*c*]quinolines; Heterocycles; Libraries; Caspase-3; Inhibitor

1. Introduction

Caspases, a family of cysteine-dependent aspartate-directed proteases, comprises highly homologous enzymes that play important role in apoptotic cell death [1–4]. Caspase-3 (apopain) situates at a key junction in the apoptosis, mediating apoptotic cascade from both the intrinsic and extrinsic activation pathways [5,6]. Therefore, caspase-3 is an attractive target for therapeutic intervention. For instance, inhibitors of caspase-3 were described as promising cardioprotectants [7], neuroprotectants [8] and antiarthritic agents [9].

1,3-Dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines (general structure **A**) constitute an interesting group of physiologically active molecules. For instance, they were described as potential antimalarial [10] and cytotoxic [11] agents. This interesting behavior led to the development of a number of methods for the synthesis of variously substituted 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines [10–16]. In our recent works, we have shown that 8-sulfonylamide derivatives of this heterocyclic system ($R^1 = SO_2NR'R''$) exhibited nanomolar inhibitory activity against caspase-3 [12,13]. It was also demonstrated that aryl substituents in position 4 [12] and particularly 1,3,5-trimethylpyrazolyl substituent in position 2 [13] contribute to effective caspase-3 inhibition. These observations have prompted us to explore synthetic routes to modified sulfonyl propionate analogs of active molecules described in the mentioned works, which can lead to novel potent inhibitors of caspase enzymes.



A B

In this paper, we describe synthesis and biological activity of 4-phenyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quino-

* Corresponding author. Tel.: +1 858 794 4860; fax: +1 858 794 493.
E-mail addresses: dk@chemdiv.com (D.V. Kravchenko), av@chemdiv.com (A.V. Ivachtchenko).

0014-827X/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2005.08.001

lines of general formula **B**, which were not previously described in literature. The synthetic method for assembly of the core heterocyclic moiety is based on Pfitzinger reaction [17]. According to this approach depicted in Scheme 1, sodium isatin-5-sulfonate **1** was initially hydrolyzed with aqueous NaOH to give oxoacetate **2**; the latter was then treated with methyl 3-oxo-3-phenylpropanoate under the conditions of Pfitzinger reaction to afford the corresponding dicarboxylic acid **3** (yield 88%).

Acid **3** was converted into furan-2,5-dione **4** upon the reaction with acetic anhydride in dry pyridine (yield 56%). Anhydride **4** readily reacted with primary alcohols to give a mixture of isomeric monoesters. Thus, in LCMS spectrum of a mixture of **4** in methanol, two distinct peaks in ~1:1 ratio were observed corresponding to two isomers **7a** and **7b**. Compound **4** was treated with 4-amino-1,3,5-trimethylpyrazole in pyridine at 70 °C to afford the corresponding imide **5** in 92% yield. Chlorosulfonate **6** was then synthesized using reaction of **5** with $POCl_3$ in tetramethylene sulfone (sulfolane). The reaction proceeded at the temperature 100 °C and smoothly afforded the desired product in 95% yield.

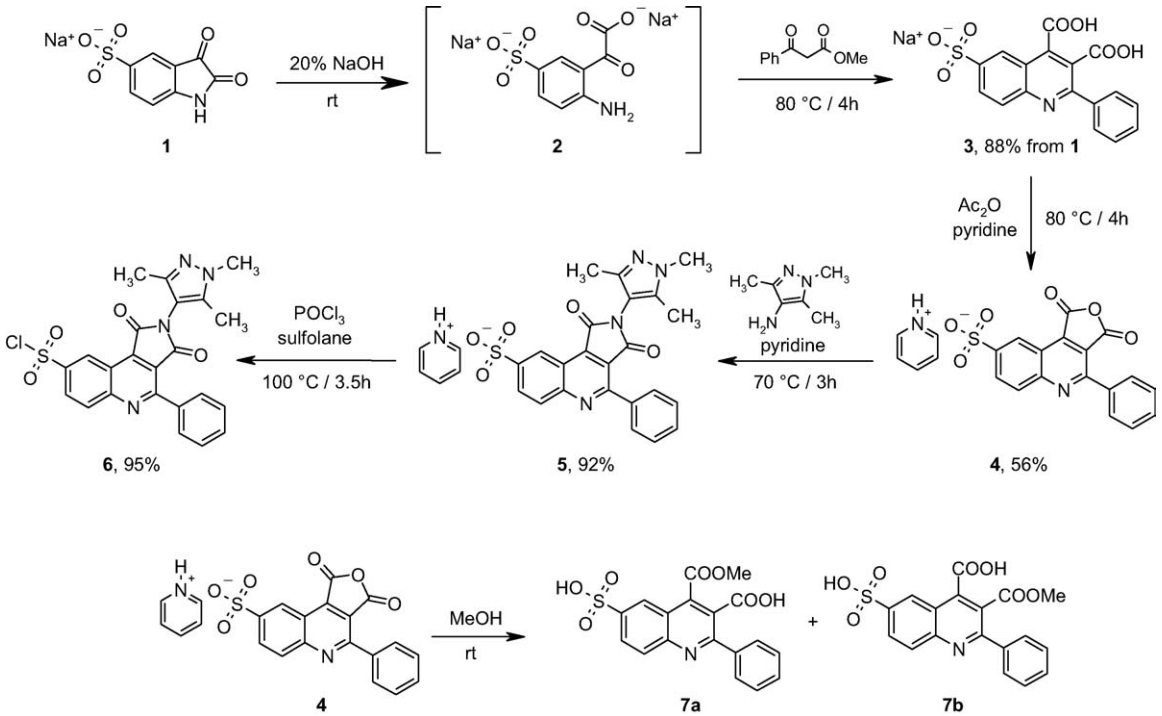
Chlorosulfonate **6** was then converted into sodium sulfinate **8** upon the treatment with aqueous solution of sodium sulfite and sodium bicarbonate (Scheme 2).

Sulfinate **8** was reacted with acetic acid in water to give sulfinic acid **9**; the latter was treated in situ with acrylic acid or with methylacrylate to furnish the corresponding sulfonyl propionates **10** and **11** (yields 65% and 61%, respectively). Acid **10** was smoothly converted into the corresponding chloride **12** upon the reaction with $SOCl_2$ in the presence of DMSO (yield 90%). Finally, morpholide **13** was obtained using the reaction of chloride **12** with morpholine in 1,4-dioxane at room temperature (yield 31% from **10**). We also attempted to hydrolyze ester **11** under mild alkali conditions in order to obtain acid **10**. Somewhat unexpectedly, the desired process was accompanied by opening of the pyrrole-2,5-dione ring. Spectral data (^{1}H NMR and HRMS) indicated the presence of only one individual isomer **14a** or **14b** as the main product of this reaction. The exact assignment of its structure can be made on the basis of crystallographic data and will be reported in due course.

The developed approach to the final carboxamide **13** is compatible with the high-throughput parallel synthesis format, and a wide variety of different amino components can be used in this reaction. The protocols are straightforward and can easily be reproduced on a 10–50 g scale. All the synthesized compounds were characterized by ^{1}H NMR; LCMS and HRMS spectral data. Satisfactory analytical data consistent with the shown molecular structures were obtained for all compounds.

The synthesized 4-phenyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines **10**, **11** and **13** displayed high activity in an in vitro caspase-3 inhibition assay. The most active compounds within this series, **11** and **13**, inhibited caspase-3 with IC_{50} = 6 nM. Similar inhibitory potency was recently observed by us for the corresponding 8-sulfamoyl derivatives of general formula **A** ($R^1 = SO_2NR'R''$) with the IC_{50} values in the range of 6–20 nM [12,13].

The mechanism of inhibition has been assessed for compound **13**, which is the most active and hydrolytically stable in this series. The Lineweaver–Burk plots (Fig. 1), which are the linearized transformations of the Michaelis–Menten curves, demonstrate noncompetitive character of inhibition, as the inhibitor decreases V_{max} without affecting the apparent



Scheme 1. Synthesis of 1,3-dioxo-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline structures.