

1,3-Dioxo-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines as potent caspase-3 inhibitors

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Abstract—Synthesis, biological evaluation and structure–activity relationships for a series of novel nonpeptide small molecule inhibitors of caspase-3 are described. Among the studied compounds, 8-sulfamide derivatives of 1,3-dioxo-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines have been identified as potent inhibitors of caspases-3. The most active compound within this series (**8f**) inhibited caspase-3 with IC₅₀ = 4 nM.

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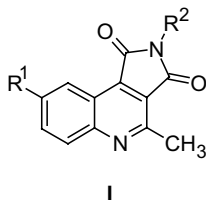
The caspase family comprises a family of highly homologous cysteine proteases that play key roles in inflammation and apoptosis.¹ Among several different groups of caspase enzymes, caspases-3 play a key role in apoptosis.² Therefore, they are attractive targets for therapeutic intervention in several diseases because of the central role played by apoptosis in those conditions. For instance, inhibitors of caspase-3 were described as promising cardioprotectants,³ neuroprotectants⁴ and hepatoprotectants.⁵ Recently, we reported the discovery of a novel class of potent small molecule inhibitors of caspase-3.⁶ In this paper, we describe synthesis, biological evaluation and structure–activity relationships for this series of

novel nonpeptide small molecule inhibitors of caspase-3 having general formula **I**. The target 4-methyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines of general formula **I** were synthesized using a previously reported synthetic method based on Pfitzinger reaction.⁷

According to this approach depicted in [Scheme 1](#), isatins **1a–e** were suspended in water to an approximate concentration of 0.5 M and hydrolyzed with NaOH to give oxoacetates **2a–e**; the latter were then treated in situ with methyl acetoacetate (2 mol. equiv) to afford the corresponding dicarboxylic acids **3a–e**. The acids **3a–e** were converted into furan-2,5-diones **4a–e** upon the reaction with an excess of acetic anhydride in dry pyridine. Reactions of 1 M solutions of anhydrides **4a–e** in pyridine with equimolar amounts of different primary amines **5a–f** smoothly led to imides **6a–t**.

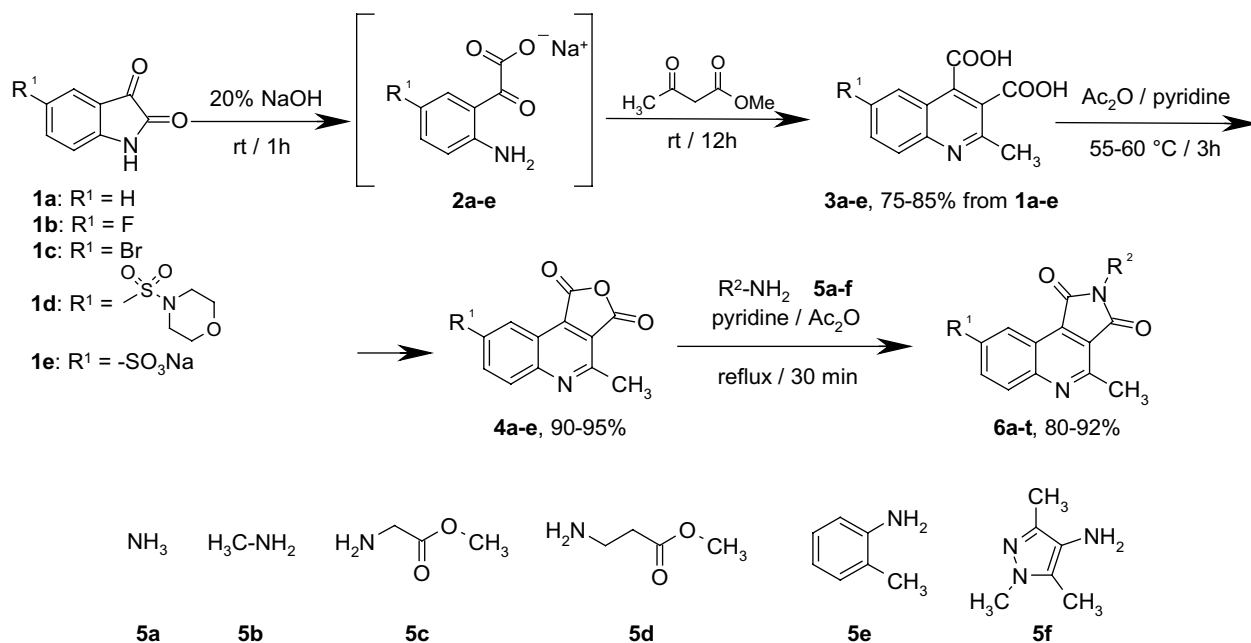
Using the described synthetic scheme, we have obtained a series of novel compounds, which have not been previously reported in the literature. Thus, compound **6s** was synthesized from **1e** as outlined in [Scheme 1](#) in 67% yield and used to synthesize an additional compound series as outlined in [Scheme 2](#).

Chlorosulfonate **6w** was synthesized using reaction of **6s** with POCl₃ at a temperature of 100 °C. Pyridinium

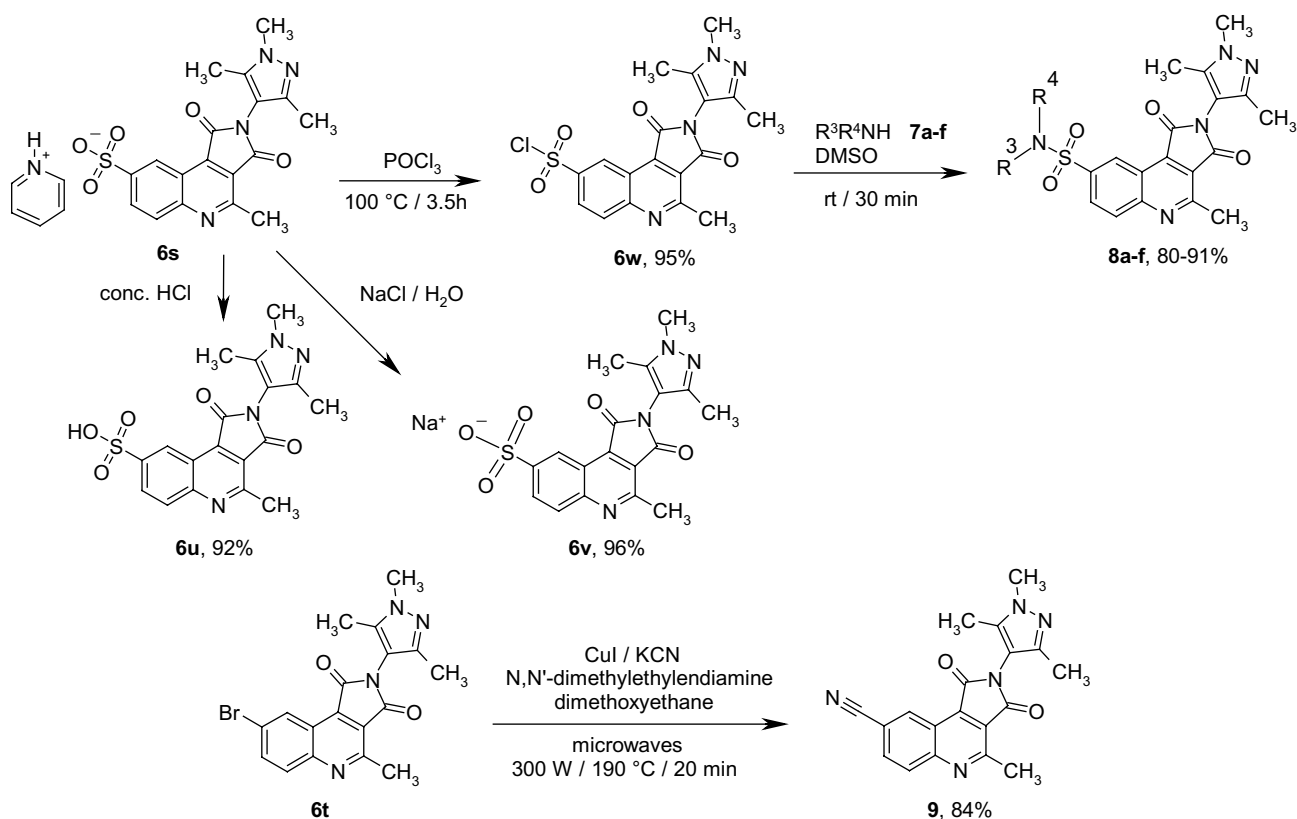


Keywords: Caspase-3; Inhibitor; 1*H*-pyrrolo[3,4-*c*]quinoline.

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Scheme 1.



Scheme 2.

sulfonate **6s** was also converted into acid **6u** and sodium salt **6v** upon treatment with concd HCl or aqueous NaCl, correspondingly; these compounds have also been used in the following biological experiments. Chlorosulfonate **6w** appeared to be a convenient intermediate for synthesis of a small combinatorial library of 8-sulfamide derivatives. The reaction between equi-

molar amounts of **6w** and six linear and cyclic aliphatic amines **7a–f** dissolved in DMSO to an approximate concentration of 1 M in each component proceeded at room temperature and afforded individual sulfonamides **8a–f** in good yields. In addition, the 8-bromo substituted compound **6t** was reacted with KCN in the presence of CuI, *N,N'*-dimethylethylenediamine and

dimethoxyethane under microwave irradiation conditions to afford the 8-cyano derivative **9**. The microwave instrument used was a commercial household microwave oven (Moulinex FM1935G, frequency: 2450 MHz).

All the synthesized compounds were characterized by ^1H NMR; LCMS and HRMS spectral data. Satisfactory analytical data consistent with the shown molecular structures were obtained for all compounds.

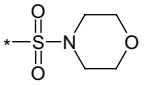
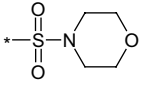
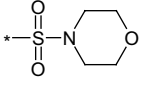
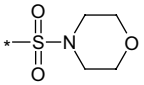
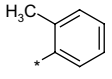
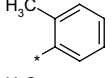
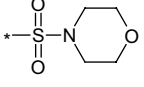
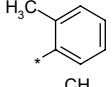
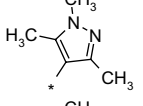
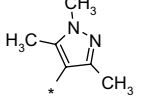
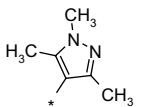
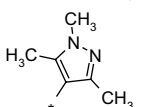
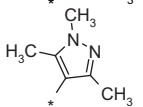
Compounds **6a–v**, **8a–f** and **9** have been tested on their ability to inhibit caspase-3 catalyzed proteolytic breakdown of its fluorogenic substrate, Ac-DEVD-AMC. The caspase-3 activity with and without inhibitors was measured in accordance with the reported protocol⁸ using VICTOR²V (Perkin–Elmer) multimode 96/384-well plate reader by the rate of fluorescence increase (λ_{ex} 360 nm, λ_{em} 460 nm) due to the liberation of a methylcoumarin moiety with concomitant increase in its quantum yield. For all the compounds that exhibited more than 50% inhibition at a concentration of 100 μM , the dose-dependent caspase-3 inhibition curves were registered and the IC_{50} values were calculated using PRISM 4 (GraphPad) software. The most active compounds displayed dose–response curves with a Hill slope close to unity, which indicates a high probability of the compounds being real inhibitors and not promiscuous ones.

The synthesized 4-methyl-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines of general formula **I** displayed high activity in this in vitro caspase-3 inhibition assay (Tables 1 and 2). The activity strongly depends on the nature of substituents in position 2 and especially in the position 8 of this heterocyclic system. In the general case, the activity increases with the increase of electron-withdrawing capacity of the 8-substituent. Thus, for a group of 2-unsubstituted compounds **6a–d**, compound **6a** with $\text{R}^1 = \text{H}$ has $\text{IC}_{50} > 100 \mu\text{M}$; 8-fluoro derivative **6b** and 8-bromo derivative **6c** have IC_{50} equal to 62.8 and 37.1 μM , correspondingly; and 8-(morpholine-4-sulfonyl)-substituted compound **6d** has $\text{IC}_{50} = 0.21 \mu\text{M}$. In this group, the activity changed by three orders of magnitude. Similar dependencies were observed within all other congeneric series with identical 2-substituents.

The observed correlations between the electron-withdrawing ability of the 8-substituent (R^1) and the potency of inhibition are shown in Figure 1. These data suggest that electrophilicity of the imide carbonyls plays a definite role in activity of the studied compounds. The mechanism of inhibition has been studied for a large variety of compounds possessing the electrophilic carbonyls, such as peptidealdehydes,⁹ isatins,¹⁰ homophthalimides,¹¹ quinazolinones,¹² etc. In the reported cases, the mechanism involved addition of the enzyme's catalytic cysteine residue to carbonyl moiety (Fig. 2).

The ability of thiols to reversibly interact with phthalimide-like compounds¹³ in a similar manner suggests that the caspase enzyme could also be reversibly inactivated by electrophilic carbonyls of compounds of gen-

Table 1. In vitro caspase-3 inhibition assay results for compounds **6a–v** and **9**

Compd	R^1	R^2	IC_{50} (μM)
6a	H	H	>100
6b	F	H	62.80
6c	Br	H	37.10
6d		H	0.21
6e	H	CH_3	6.36
6f	Br	CH_3	1.58
6g		CH_3	0.044
6h	H	$\text{CH}_2\text{--CO}_2\text{CH}_3$	4.65
6i	F	$\text{CH}_2\text{--CO}_2\text{CH}_3$	2.50
6j	Br	$\text{CH}_2\text{--CO}_2\text{CH}_3$	0.46
6k		$\text{CH}_2\text{--CO}_2\text{CH}_3$	0.016
6l	H	$\text{CH}_2\text{CH}_2\text{--CO}_2\text{CH}_3$	23.3
6m	F	$\text{CH}_2\text{CH}_2\text{--CO}_2\text{CH}_3$	5.5
6n	Br	$\text{CH}_2\text{CH}_2\text{--CO}_2\text{CH}_3$	1.08
6o		$\text{CH}_2\text{CH}_2\text{--CO}_2\text{CH}_3$	0.037
6p	H		8.11
6q	Br		2.54
6r			0.015
6s	$\text{SO}_3^- \text{PyH}^+$		0.1
6t	Br		0.36
6u	SO_3H		0.09
6v	$\text{SO}_3^- \text{Na}^+$		0.14
9	CN		0.016

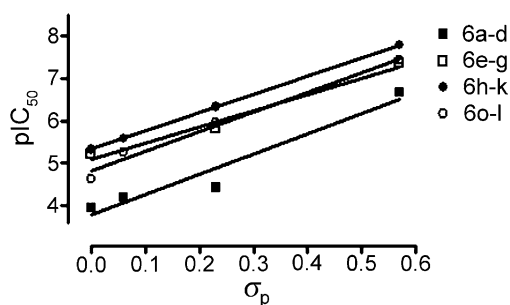
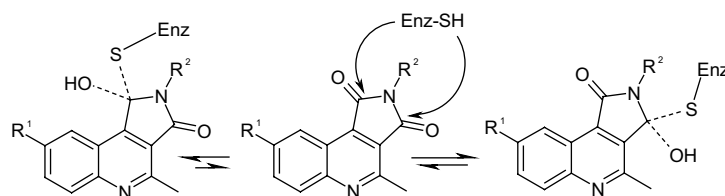
eral formula **I** given appropriate substituents R^1 and R^2 are present.

The nature of the 2-substituents also influences the activity of the synthesized compounds against caspase-3. Thus, in all the studied congeneric series with identical 8-substituents, minimal activity was observed for 2-unsubstituted compounds. The most active compounds

Table 2. In vitro caspase-3 inhibition assay results for 8-(morpholin-4-ylsulfonyl) and 1,3,5-trimethyl-1*H*-pyrazol-4-yl substituted compounds

Compd	R ¹	R ²	IC ₅₀ (μM)
8a			0.033
8b			0.020
8c			0.021
8d			0.023
8e			0.055
8f			0.004

have methoxycarbonylmethyl (e.g., **6j** and **6k** with IC₅₀ = 0.46 and 0.016 μM, correspondingly), 2-methylphenyl (e.g., **6r** with IC₅₀ = 0.015 μM) and 1,3,5-trimethyl-1*H*-pyrazol-4-yl (e.g., **8f** with IC₅₀ = 0.004 μM) substituents in the position 2. Submicromolar activity was observed for two 2-bromo substituted derivatives **6j** and **6t** (IC₅₀ equal to 0.46 and 0.36 μM, correspondingly). Sulfonates **6s**, **6u** and **6v** inhibited caspase-3 in the 0.09–0.14 μM range.

**Figure 1.** Relationship between inhibitory activities (pIC₅₀) and σ_p Hammett constants for R¹ substituents in the series **6**.**Figure 2.** Possible modes of caspase nucleophilic attack on the 'phthalimide' carbonyls.

Among the studied compounds, 8-sulfamide and 8-cyano derivatives of 1,3-dioxo-4-methyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines have been identified as potent inhibitors of caspases-3. The most active compounds within this series, such as **6k**, **6r**, **8b**, **8f** and **9**, inhibited caspase-3 in the 4–20 nM range. Compound **8f** was the most potent inhibitor with IC₅₀ value equal to 4 nM. Parallel experiments demonstrated that the IC₅₀ values for Ac-DEVD-CHO, a potent tetrapeptide inhibitor of caspase-3, was equal to 3.1 nM under the same experimental conditions.

In summary, here we have described the synthesis and activity of a novel class of potent caspase-3 inhibitors based on pyrrolo[3,4-*c*]quinoline-1,3-dione molecular scaffold. Caspase-3 inhibitory activity of the synthesized compounds is highly dependent on the substitutions on the core scaffold, especially at the 8-position. Compound **8f** with a morpholinesulfonyl moiety at the 8-position and 1,3,5-trimethyl-1*H*-pyrazol-4-yl group at the 2-position is the lead compound with potent inhibitory activity (IC₅₀ = 4 nM). Evaluation against other caspases involved in apoptosis, as well as further SAR studies, is continuing.

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