Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1841–1845

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

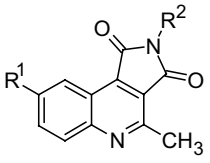
Dmitri V. Kravchenko,[a] Vladimir V. Kysil,[b] Alexey P. Ilyn,[b] Sergey E. Tkachenko,[b,c] Sergey Maliarchouk,[b,d] Ilya M. Okun[b,d] and Alexandre V. Ivachtchenko[b,*]

[a]*Department of Organic Chemistry, Chemical Diversity Research Institute, 114401 Khimki, Moscow Region, Russia*
[b]*ChemDiv, Inc., 92121 San Diego, CA, USA*
[c]*Department of Medicinal Chemistry, Chemical Diversity Research Institute, 114401 Khimki, Moscow Region, Russia*
[d]*Department of Molecular Biology and High-Throughput Screening, Chemical Diversity Research Institute, 114401 Khimki, Moscow Region, Russia*

Received 27 October 2004; revised 7 February 2005; accepted 8 February 2005

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 IC_{50} = 4 nM.

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] neuroprotectants[4] 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]



I

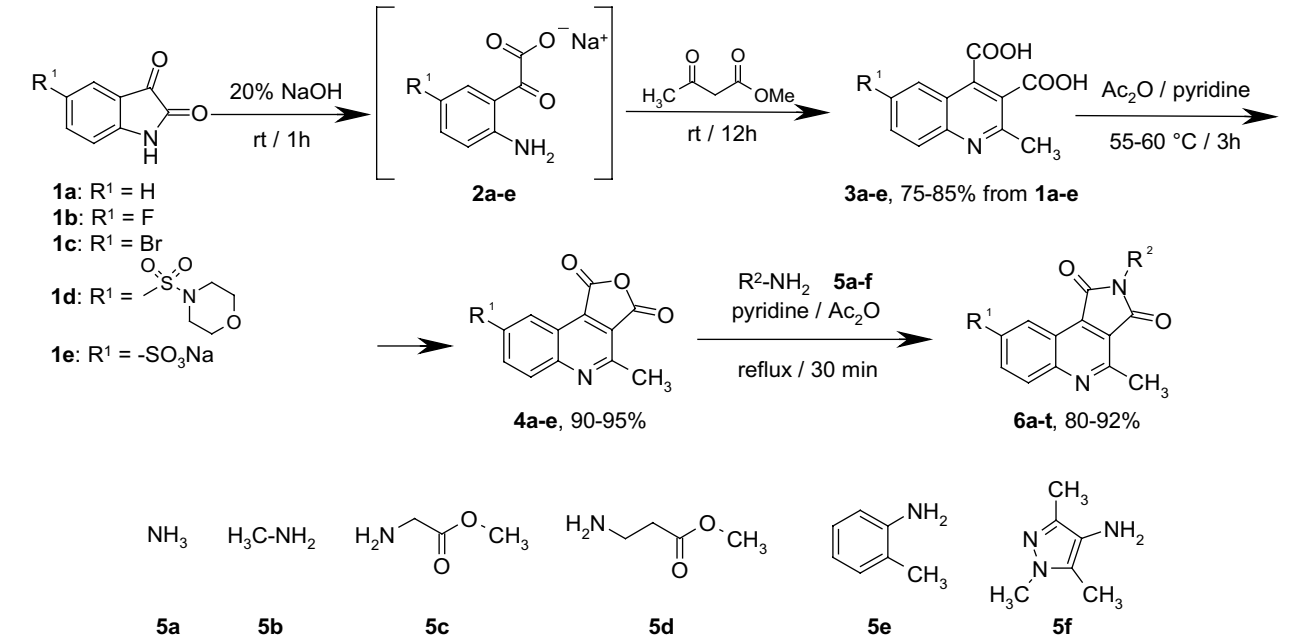
According to this approach depicted in Scheme 1, isatins **1a**–**e** were suspended in water to an approximate concentration of 0.5 M and hydrolyzed with NaOH to give oxoacetates **2a**–**e**; the latter were then treated in situ with methyl acetoacetate (2 mol. equiv) to afford the corresponding dicarboxylic 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.

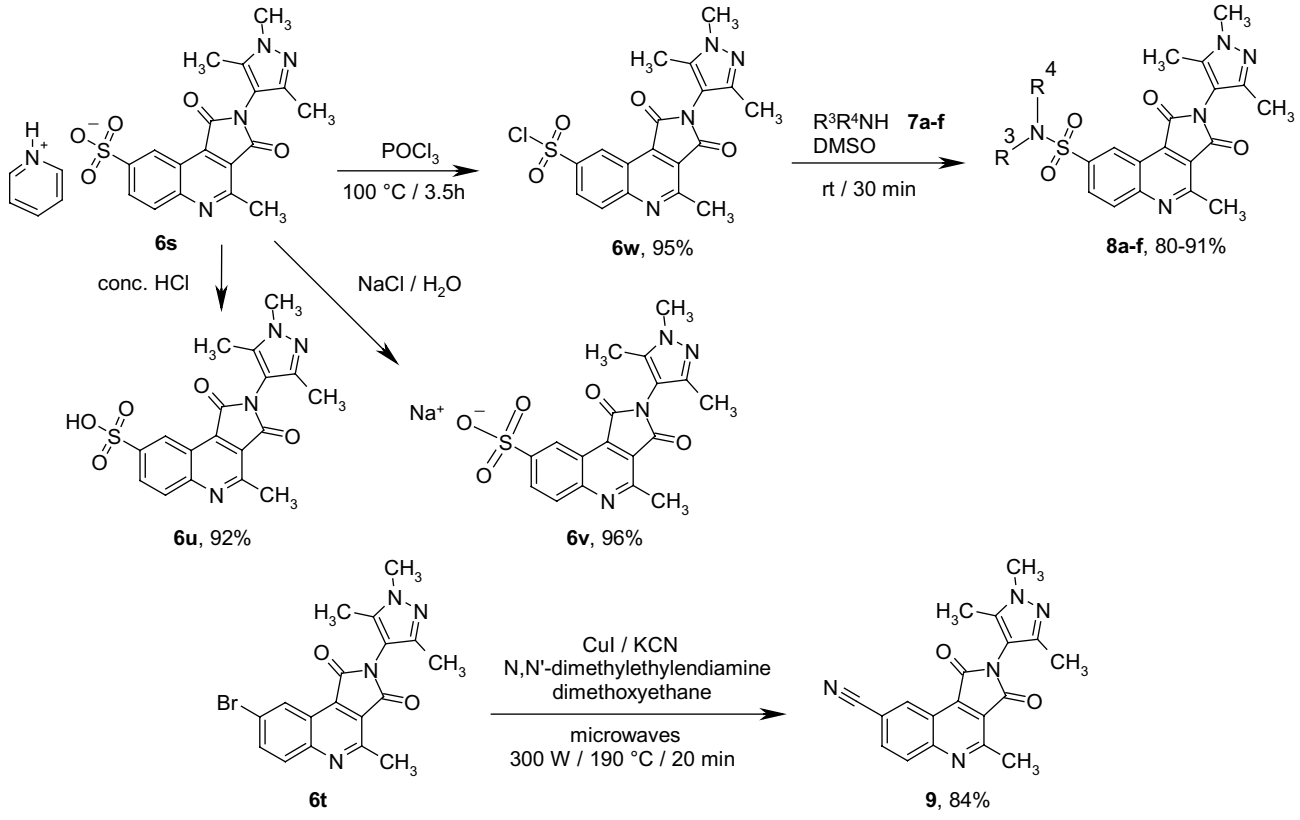
Chlorosulfonate **6w** was synthesized using reaction of **6s** with $POCl_3$ at a temperature of 100 °C. Pyridinium

Keywords: Caspase-3; Inhibitor; 1*H*-pyrrolo[3,4-*c*]quinoline.
* Corresponding author. Tel.: +1 858 794 4860; fax: +1 858 794 4931; e-mail: av@chemdiv.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.02.027



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 equimolar 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*′-dimethylethylendiamine 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 ^{1}H NMR; LCMS and HRMS spectral data. Satisfactory analytical data consistent with the shown molecular structures were obtained for all compounds.

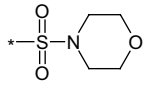
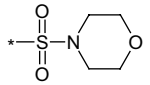
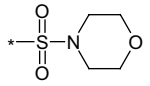
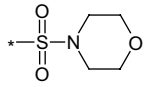
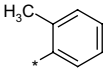
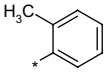
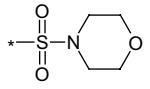
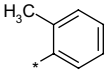
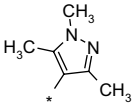
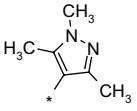
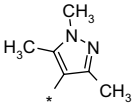
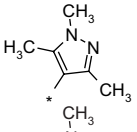
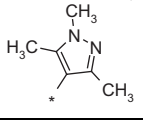
Compounds **6a**–**v**, **8a**–**f** and **9** have been tested on their ability to inhibit caspase-3 catalyzed proteolytic breakdown of its fluorogenic substrate, Ac-DEVD-AMC. The caspase-3 activity with and without inhibitors was measured in accordance with the reported protocol[8] using VICTOR^{2}V (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-substitutent. Thus, for a group of 2-unsubstituted compounds **6a**–**d**, compound **6a** with R^1 = H has IC_{50} > 100 μM; 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 IC_{50} = 0.21 μM. 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).

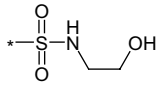
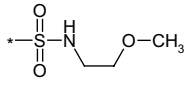
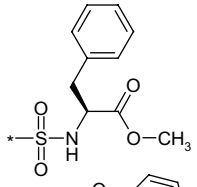
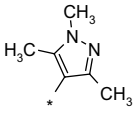
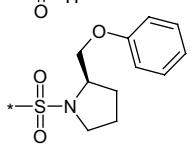
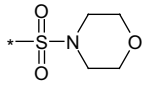
The ability of thiols to reversibly interact with phthalimide-like compounds[13] in a similar manner suggests that the caspase enzyme could also be reversibly inactivated by electrophilic carbonyls of compounds of general formula **I** given appropriate substituents R^1 and R^2 are present.

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	CH_2–CO_2CH_3	4.65
6i	F	CH_2–CO_2CH_3	2.50
6j	Br	CH_2–CO_2CH_3	0.46
6k		CH_2–CO_2CH_3	0.016
6l	H	CH_2CH_2–CO_2CH_3	23.3
6m	F	CH_2CH_2–CO_2CH_3	5.5
6n	Br	CH_2CH_2–CO_2CH_3	1.08
6o		CH_2CH_2–CO_2CH_3	0.037
6p	H		8.11
6q	Br		2.54
6r			0.015
6s	$SO_3^-PyH^+$		0.1
6t	Br		0.36
6u	SO_3H		0.09
6v	$SO_3^-Na^+$		0.14
9	CN		0.016

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 IC_{50} = 0.46 and 0.016 μM, correspondingly), 2-methylphenyl (e.g., **6r** with IC_{50} = 0.015 μM) and 1,3,5-trimethyl-1*H*-pyrazol-4-yl (e.g., **8f** with IC_{50} = 0.004 μM) substituents in the position 2. Submicromolar activity was observed for two 2-bromo substituted derivatives **6j** and **6t** (IC_{50} 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.

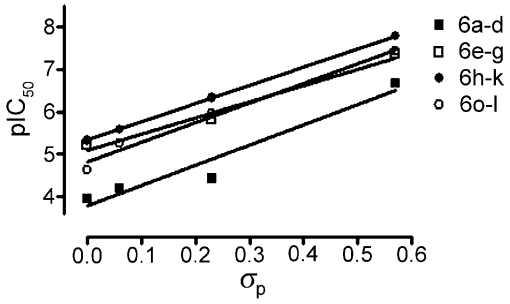


Figure 1. Relationship between inhibitory activities (pIC_{50}) and σ_p Hammett constants for R^1 substituents in the series **6**.

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 IC_{50} value equal to 4 nM. Parallel experiments demonstrated that the IC_{50} 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 (IC_{50} = 4 nM). Evaluation against other caspases involved in apoptosis, as well as further SAR studies, is continuing.

Acknowledgements

The authors thank Caroline T. Williams (Department of Analytical Chemistry, ChemDiv, Inc.) for LCMS and NMR spectral data.

References and notes

1. Stennicke, H. R.; Ryan, C. A.; Salvesen, G. S. *Trends Biochem. Sci.* **2002**, *27*, 94.
2. Porter, A. G.; Janicke, R. U. *Cell Death Differ* **1999**, *6*, 99.
3. Chapman, J.; Magee, W.; Stukenbrok, H.; Beckius, G.; Milici, A.; Tracey, W. *Eur. J. Pharmacol.* **2002**, *456*, 59.
4. Scott, C.; Sobotka-Briner, C.; Wilkins, D.; Jacobs, R.; Folmer, J.; Frazee, W.; Bhat, R.; Ghanekar, S.; Aharony, D. *Pharmacol. Exp. Ther.* **2003**, *304*, 433.
5. Anselmo, D.; Katori, M.; Kaldas, M.; Hoglen, N.; Valentino, K.; Busuttil, R.; Kupiec-Weglinski, W.; Farmer, D. *Am. J. Transplant.* **2002**, *2*, 920.
6. Ivachtchenko, A.; Khvat, A.; Kysil, V.; Maliartchuk, S.; Tkachenko, S.; Okun, I. *Drugs Future* **2004**, *29*, 191.
7. (a) Pfitzinger, W. *J. Prakt. Chem.* **1886**, *33*, 100; (b) Godard, A.; Queguiner, G. *J. Heterocycl. Chem.* **1980**, *17*, 465; (c) Campaigne, E.; Hutchinson, J. H. *J. Heterocycl. Chem.* **1970**, *7*, 655; (d) Ried, W.; Weidemann, P. *Chem. Ber.* **1971**, *104*, 3341; (e) Ganjian, I.; Khorshidi, M.; Lalezari, I. *J. Heterocycl. Chem.* **1991**, *28*, 1173; (f)

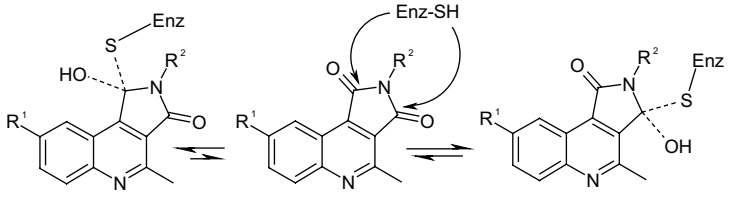


Figure 2. Possible modes of caspase nucleophilic attack on the 'phtalimide' carbonyls.

Ivachtchenko, A. V.; Kobak, V. V.; Il'yin, A. P.; Trifilenkov, A. S.; Busel, A. A. *J. Comb. Chem.* **2003**, *5*, 645.
8. Durrieu, F.; Belloc, F.; Lacoste, L.; Dumain, P.; Chabrol, J.; Dachary-Prigent, J.; Morjani, H.; Boisseau, M. R.; Reiffers, J.; Bernard, P.; Lacombe, F. *Exp. Cell Res.* **1998**, *240*, 165.
9. Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W., III; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T. *J. Med. Chem.* **1998**, *41*, 2786.
10. Webber, S. E.; Tikhe, J.; Worland, S. T.; Fuhrman, S. A.; Hendrickson, T. F.; Matthews, D. A.; Love, R. A.; Patick, A. K.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; DeLisle, D. A.; Ford, C. E.; Binford, S. L. *J. Med. Chem.* **1996**, *39*, 5072.
11. Wang, Q. M.; Johnson, R. B.; Jungheim, L. N.; Cohen, J. D.; Villarreal, E. C. *Antimicrob. Agents Chemother.* **1998**, *42*, 916.
12. Scott, C.; Sobotka-Briner, C.; Wilkins, D.; Jacobs, R.; Folmer, J.; Frazee, W.; Bhat, R.; Ghanekar, S.; Aharony, D. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 433.
13. Oswaldo, N.; Jose, R.; Larry, A. *J. Phys. Org. Chem.* **1994**, *7*, 80.