Letters

Synthesis and Structure−Activity Relationship of 4-Substituted 2-(2-Acetyloxyethyl)-8-(morpholine-4-sulfonyl)pyrrolo[3,4-*c*]quinoline-1,3-diones as Potent Caspase-3 Inhibitors

Dmitri V. Kravchenko,[†] Yulia A. Kuzovkova,[†] Volodymyr M. Kysil,[‡] Sergey E. Tkachenko,[‡,§] Sergey Maliarchouk,[‡,#] Ilya M. Okun,[‡,#] Konstantin V. Balakin,[‡,§] and Alexandre V. Ivachtchenko*[,‡]

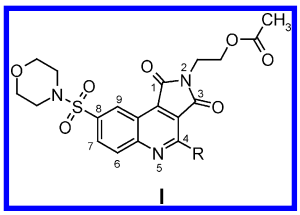
Departments of Organic Chemistry, Medicinal Chemistry, and Molecular Biology and HTS, Chemical Diversity Research Institute, Khimki, Moscow Reg., Russia, and ChemDiv, Inc., 11558 Sorrento Valley Road, Suite 5, San Diego, California 92121

Received December 14, 2004

Abstract: Synthesis, biological evaluation, and SAR dependencies for a series of novel 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline inhibitors of caspase-3 are described. The inhibitory activity of the synthesized compounds is highly dependent on the nature of 4-substituents on the core scaffold. 4-Methyl- and 4-phenyl-substituted derivatives, which were the most active compounds within this series, inhibited caspase-3 with IC_{50} of 23 and 27 nM, respectively.

Caspases, a family of cysteine-dependent aspartate-directed proteases, comprise highly homologous enzymes that play an important role in apoptotic cell death.[1] Caspase-3 (apopain) is situated at a key junction in the apoptosis, mediating apoptotic cascade from the intrinsic and extrinsic activation pathways.[2] Therefore, caspase-3 is an attractive target for therapeutic intervention. For instance, inhibitors of caspase-3 were described as promising cardioprotectants,[3] neuroprotectants,[4] and antiarthritic agents.[5]

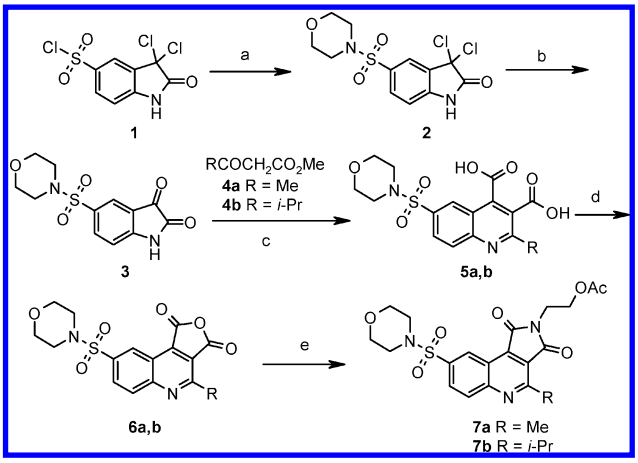
1,3-Dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines represent a relatively little explored class of heterocyclic structures with promising physiological activities. Thus, they were reported as cytotoxic agents[6] and central nervous system active agents, selective agonists, antagonists, or inverse agonists for $GABA_A$ (γ-aminobutyric acid A) brain receptors.[7] Recently, we described an efficient synthesis and biological activity of 8-sulfamide derivatives of 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines.[8] Synthesis of 5*H*-pyrrolo[3,4-*c*]quinoline-1,3,4-triones was also reported.[7] In this paper, we describe synthesis and biological evaluation for a series of novel nonpeptide small-molecule inhibitors of caspase-3 having general formula **I**.



I

The synthesis of the 4-substituted pyrrolo[3,4-*c*]-quinoline-1,3-dione ring system was accomplished according to a sequence of reactions shown in Scheme 1. Commercially available chloride **1** was reacted with morpholine in 1,4-dioxane to give sulfonyl amide **2**. The latter was refluxed in a AcOH/water (1:1 v/v) mixture to afford 8-sulfamoyl isatin **3**. Dicarboxylic acids **5** were prepared via a Pfitzinger reaction[9] of **3** with keto esters **4a**,**b** under strong alkali conditions. Acids **5** were then converted into furandiones **6a**,**b** upon the reaction with acetic anhydride. Reactions of anhydrides **6a**,**b** with 2-aminoethanol smoothly led to imides **7a**,**b**.

Scheme 1[a]



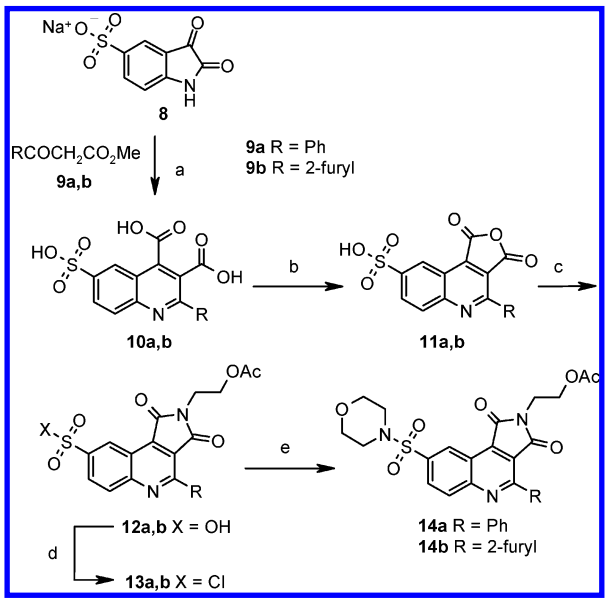
[a] Reagents and conditions: (a) morpholine, 1,4-dioxane, room temp, 1 h, 92%; (b) AcOH/H_2O (1:1), reflux, 12 h, 89%; (c) KOH in H_2O, room temp, 12 h, 60−87%; (d) Ac_2O, 100 °C, 3 h, 80−88%; (e) 2-aminoethanol, pyridine, room temp, 1 h, then Ac_2O, 80 °C, 30 min, 60−65%.

The second group of 4-substituted pyrrolo[3,4-*c*]quinoline-1,3-diones was obtained utilizing the commercially available 2,3-dioxo-2,3-dihydro-1*H*-indole-5-sulfonate **8** as starting reactant. According to this approach depicted in Scheme 2, **8** was treated with keto esters **9a**,**b** under the conditions of Pfitzinger reaction to afford the corresponding dicarboxylic acids **10a**,**b**. Acids **10a**,**b** were converted into furandiones **11a**,**b** upon reaction with acetic anhydride in dry pyridine. Compounds **11a**,**b** were successively treated with 2-aminoethanol and acetic anhydride in pyridine to afford the corresponding imides **12a**,**b**. Sulfonyl chlorides **13a**,**b** were then synthesized from **12a**,**b** by using $POCl_3$ in tetramethylene

* To whom correspondence should be addressed. Phone: (858) 794-4860. Fax: (858) 794-4931. E-mail: av@chemdiv.com.
[†] Department of Organic Chemistry, Chemical Diversity Research Institute.
[‡] ChemDiv, Inc.
[§] Department of Medicinal Chemistry, Chemical Diversity Research Institute.
[#] Department of Molecular Biology and HTS, Chemical Diversity Research Institute.

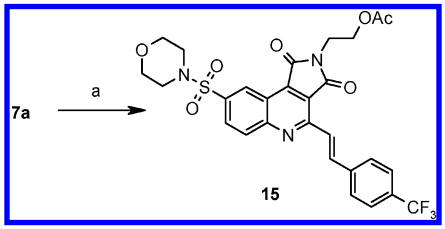
10.1021/jm048987t CCC: $30.25
Published on Web 04/29/2005

Scheme 2[a]



[a] Reagents and conditions: (a) LiOH in H_2O, room temp, 12 h, 60−85%; (b) Ac_2O, pyridine, 100 °C, 30 min, 80−88%; (c) 2-aminoethanol, pyridine, room temp, 1 h, then Ac_2O, 80 °C, 30 min, 75−97%; (d) $POCl_3$, sulfolane, 80 °C, 2 h, 65−85%; (e) morpholine, 1,4-dioxane, 60 °C, 30 min, 65−95%.

Scheme 3[a]



[a] Reagents and conditions: (a) 4-(trifluoromethyl)benzaldehyde, $ZnCl_2$, Ac_2O, 110 °C, 70 h, 20%.

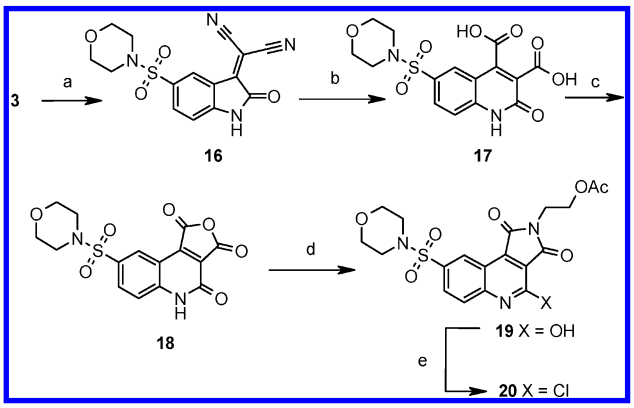
sulfone (sulfolane). The reaction proceeded at 80 °C and smoothly afforded the desired product. Finally, morpholides **14a**,**b** were obtained from the reaction of **13a**,**b** with morpholine in 1,4-dioxane at 60 °C.

Compound **15** was obtained according to a modification of the reported procedure[10] by condensation of 4-methyl derivative **7a** with 4-(trifluoromethyl)benzaldehyde in acetic anhydride in the presence of $ZnCl_2$ (Scheme 3).

Scheme 4 shows the synthesis of 4-chloride intermediate **20**, which was used for the synthesis of 4*S*- and 4*N*-substituted pyrrolo[3,4-*c*]quinoline-1,3-diones. Condensation of **3** with malononitrile led to intermediate **16**, which was converted into dicarboxylic acid **17** by reaction with a mixture of concentrated HCl and AcOH. Acid **17** was converted into furandione **18** upon reaction with acetic anhydride. Compound **18** was treated with 2-aminoethanol and acetic anhydride in pyridine at 80 °C, and the resulting imide **19** was reacted with $POCl_3$ to afford the desired chloride **20**.

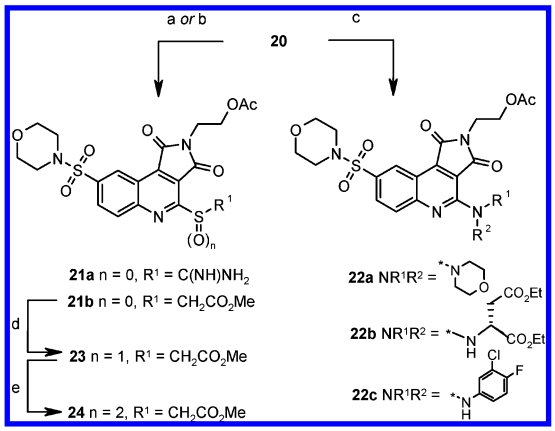
Chloride **20** easily reacted with nucleophilic agents (Scheme 5) such as thiourea, methyl mercaptoacetate, morpholine, diethyl (2*R*)-2-amino-2-methylsuccinate, and 3-chloro-4-fluoroaniline to smoothly afford the corresponding 4*S*- and 4*N*-substituted products **21a**,**b** and **22a**−**c**. Compound **21a** was prone to rapid spontaneous hydrolysis leading to the corresponding thiol; the latter

Scheme 4[a]



[a] Reagents and conditions: (a) $CH_2(CN)_2$, *N*-methylmorpholine, MeOH, reflux, 30 min, 96%; (b) concentrated HCl/AcOH (1:1), reflux, 12 h, 43%; (c) Ac_2O, 100 °C, 30 min, 91%; (d) 2-aminoethanol, pyridine, 80 °C, 15 min, then Ac_2O, 80 °C, 1 h, 86%; (e) $POCl_3$, reflux, 1 h, 96%.

Scheme 5[a]



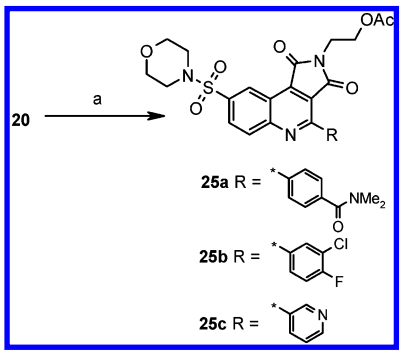
[a] Reagents and conditions: (a) (for **21a**) $(NH_2)_2CS$, *i*-PrOH, reflux, 30 min, 75%; (b) (for **21b**) $HSCH_2CO_2Me$, dimethoxyethane, Cs_2CO_3, ultrasonic bath, room temp, 1 h, 65%; (c) morpholine (for **2a**) or diethyl (2*R*)-2-amino-2-methylsuccinate (for **22b**) or 3-chloro-4-fluoroaniline (for **22c**), *i*-PrOH, reflux, 20−60 min, 45−77%; (d) *m*-CPBA, CH_2Cl_2, 0 °C, 1 h, 70%; (e) *m*-CPBA, CH_2Cl_2, room temp, 4 h, then 40 °C, 1.5 h, 56%.

was also unstable and underwent slow oxidation, which led to a disulfide dimer. Oxidation of sulfide **21b** with *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane at 0 °C afforded sulfinate **23**. When the same reaction was performed at higher temperature (∼40 °C), sulfonate **24** was formed.

Finally, Suzuki coupling[11] of **20** with 3-chloro-4-fluorophenylboronic acid, 4-(*N*,*N*-dimethylaminocarbonyl)phenylboronic acid, or 3-pyridylboronic acid in the presence of $PdCl_2(PPh_3)_2$ afforded the corresponding 4-aryl substituted compounds **25a**−**c** (Scheme 6).

Compounds **7a**,**b**, **14a**,**b**, **15**, and **19**−**25** have been tested for their ability to inhibit caspase-3 catalyzed proteolytic breakdown of its fluorogenic substrate, Ac-DEVD-AMC. The synthesized 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 (Table 1). The most active compounds within this series have alkyl, aryl, and heteroaryl 4-substituents. They demonstrated inhibitory activity (IC_{50}) in the 20−60 nM range. 4-Hydroxy- and 4-chloro-substituted derivatives (**19** and **20**) and 4-sulfanyl derivatives with their oxidized analogues (**21a**,**b**, **23**, and **24**) are significantly

Scheme 6[a]



[a] Reagents and conditions: (a) 3-chloro-4-fluorophenylboronic acid (for **25a**) or 4-[(dimethylamino)carbonyl]phenylboronic acid (for **25b**) or 3-pyridylboronic acid (for **25c**), dimethoxyethane, $PdCl_2(PPh_3)_2$, Na_2CO_3, H_2O, reflux, 4 h, 19−25%.

Table 1. In Vitro Caspase-3 Inhibitory Potency of 4-Substituted 2-(2-Acetyloxyethyl)-8-(morpholine-4-sulfonyl)pyrrolo[3,4-*c*]quinoline-1,3-diones of General Formula **I**

compd	R	IC_{50}, nM
7a	Me	23 ± 2
7b	*i*-Pr	40 ± 3
14a	Ph	27 ± 3
14b	2-furyl	36 ± 4
15	4-CF_3−C_6H_4−CHCH	33 ± 3
19	OH	10900 ± 3800
20	Cl	294 ± 16
21a	S−C(NH)NH_2	255 ± 20
21b	S−CH_2CO_2Me	1290 ± 30
22a	1-morpholyl	15350 ± 3500
22b	EtOC(O)−CH_2−$CH(CO_2Et)$−NH	5190 ± 2700
22c	3-Cl−4-F−C_6H_3−NH	27900 ± 3300
23	SO−CH_2CO_2Me	352 ± 21
24	SO_2−CH_2CO_2Me	362 ± 26
25a	4-(Me_2NCO)−C_6H_4	58 ± 6
25b	3-Cl−4-F−C_6H_3	33 ± 4
25c	3-pyridyl	33 ± 5

less potent (IC_{50} = 250−1300 nM). The less active compounds within the synthesized set have alkyl- and arylamino 4-substituents (IC_{50} = 5−28 μM). It is suggested that electrophilicity of imide carbonyls, which can be affected by different types of 4-substituents, plays an essential role in the activity of the studied compounds. In accordance with this suggestion, we have observed a clear correlation between the inhibitory activity and nucleophilicity of the 4-substituents (see Supporting Information).

The mechanism of inhibition has been assessed for **7a**, which is the most active in this series. The noncompetitive character of the inhibition has been demonstrated in the experiments, in which the inhibitory potency was measured in the presence of increasing concentration of the substrate Ac-DEVD-AMC (Figure 1). The incremental increase in the substrate concentration did not shift the inhibition curves to the right as one would expect for competitive inhibition. An IC_{50} of 23 ± 6 nM was found for **7a** from these curves. Parallel experiments demonstrated that the IC_{50} value for Ac-DEVD-CHO, a potent tetrapeptide inhibitor of caspase-3, was equal to 3.1 ± 0.2 nM under the same experimental conditions. Inhibition by **7a** was reversible, as demonstrated by an experiment in which a 10-fold concentration of the compound was preincubated for 1 h with 10-fold caspase-3 concentration followed by 10-fold dilution of the mixture into a standard assay buffer to a final compound concentration of 30 nM. At 1.5 h

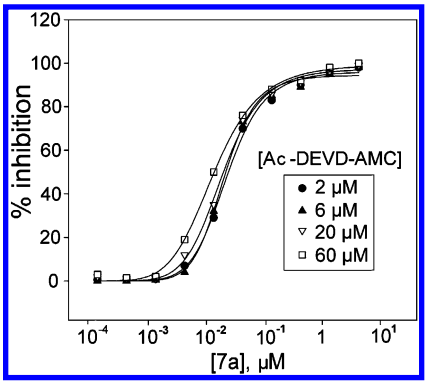


Figure 1. Caspase-3 inhibition curves in the presence of different concentrations of the fluorogenic substrate.

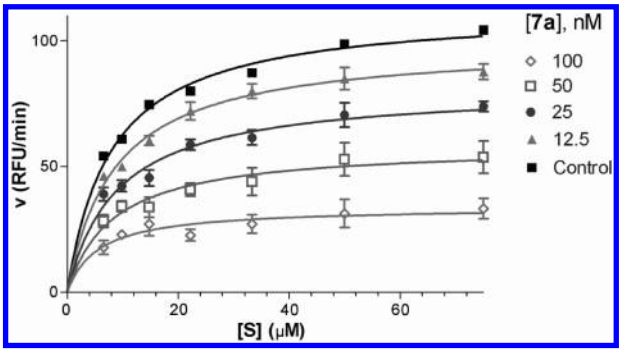


Figure 2. Studies on mechanism of recombinant human caspase-3 inhibition by **7a**.

after dilution, the measured activity of the sample was practically identical to that of inhibition in control samples, which were preincubated at normal concentrations of the compound and enzyme.

The plots also demonstrate the noncompetitive character of inhibition because the inhibitor decreases V_{max} without effecting the apparent K_m (Figure 2). The same conclusion can be made from an analysis of Lineweaver−Burk plots (see Supporting Information). The dissociation constant K_m for Ac-DEVD-AMC calculated from these plots was estimated to be 6 ± 2 μM at 95% confidence intervals. The selectivity of **7a** was estimated in the reactions with a panel of active recombinant human caspases-1−9 and the specific substrates for each individual enzyme. Compound **7a** was the most potent against caspase-3, but the level of selectivity versus the remaining caspases was modest.

To assess the antiapoptotic activity of **7a** in a cell-based assay, we measured the viability of human Jurkat T cells treated with 10 μM staurosporin. As a positive control, we used a known cell-permeable apoptosis inhibitor Z-VAD-FMK. Compound **7a** demonstrated a higher level of protection than the peptide inhibitor, as can be seen from the cell viability values (Figure 3). These data also suggest, though indirectly, good cell permeability of **7a**.

In summary, we synthesized a series of novel nonpeptide caspase-3 inhibitors based on a 1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline molecular scaffold. The inhibitory activity depends on the nature of the substituent in position 4 of this heterocyclic system. Compounds **7a** and **14a** with 4-methyl and 4-phenyl substituents are the most potent compounds (IC_{50} = 23 and 27 nM, respectively). Our primary data suggest noncompetitive and reversible character of caspase-3

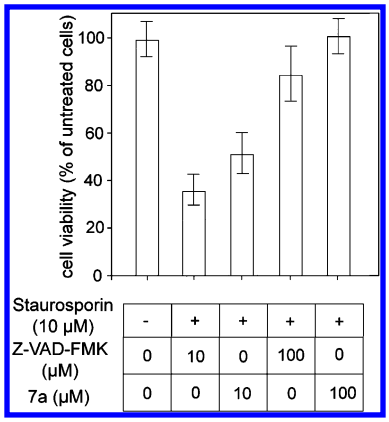


Figure 3. Protective effect of **7a** in a model of staurosporin-induced apoptosis in human Jurkat T cells.

inhibition. The selected compound **7a** showed moderate selectivity against caspase-3 and marked antiapoptotic activity in a cell-based assay. Further SAR exploration studies are continuing.

Acknowledgment. The authors thank Caroline T. Williams (Department of Analytical Chemistry, ChemDiv, Inc.) for NMR and LCMS data. The authors also thank the colleagues of the Scripps Center for Mass Spectrometry (La Jolla, CA) for HRMS data.

Supporting Information Available: Synthesis procedures and analytical data for intermediates and final products (^{1}H NMR, ^{13}C NMR, HRMS), experimental procedures for the biological tests, inhibition kinetics, and selectivity data for **7a**, and data on the effect of 4-substituents on the caspase-3 inhibitory activity. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Denault, J.-B.; Salvesen, G. S. Caspases: keys in the ignition of cell death. *Chem. Rev*. **2002**, *102*, 4489−4499. (b) Stennicke, H. R.; Ryan, C. A.; Salvesen, G. S. Reprieval from execution: the molecular basis of caspase inhibition. *Trends Biochem. Sci.* **2002**, *27*, 94−101. (c) O'Brien, T.; Lee, D. Prospects for caspase inhibitors. *Mini-Rev. Med. Chem*. **2004**, *4*, 153−165. (d) Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu. Rev. Biochem*. **1999**, *68*, 383−424.

(2) (a) Porter, A. G.; Janicke, R. U. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. **1999**, *6*, 99−104. (b) Nuttall, M. E.; Lee, D.; McLaughlin, B.; Erhardt, J. A. Selective inhibitors of apoptotic caspases: implications for novel therapeutic strategies. *Drug Discovery Today* **2001**, *6*, 85−91.

(3) Chapman, J.; Magee, W.; Stukenbrok, H.; Beckius, G.; Milici, A.; Tracey, W. A novel nonpeptidic caspase-3/7 inhibitor, (*S*)-(+)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin reduces myocardial ischemic injury. *Eur. J. Pharmacol*. **2002**, *456*, 59−68.

(4) Scott, C. W.; Sobotka-Briner, C.; Wilkins, D. E.; Jacobs, R. T.; Folmer, J. J.; Frazee, W. J.; Bhat, R. V.; Ghanekar, S. V.; Aharony, D. Novel small molecule inhibitors of caspase-3 block cellular and biochemical features of apoptosis. *J. Pharmacol. Exp. Ther*. **2003**, *304*, 433−440.

(5) Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nuttall, M. E.; Nadeau, D. P.; Kikly, K.; Winkler, J. D.; Sung, C. M.; Ryan, M. D.; Levy, M. A.; Keller, P. M.; DeWolf, W. E., Jr. Potent and selective nonpeptide inhibitors of caspases 3 and 7. *J. Med. Chem*. **2001**, *44*, 2015−2026.

(6) Ganjian, I.; Khorshidi, M.; Lalezari, I. Synthesis and cytotoxic activity of 2-dialkylaminoalkyl-1,3-dihydropyrrolo[3,4-*c*]quinoline-1,3-diones and 6-(2-dimethylaminoethyl)-1*H*-dibenz[*c*,*e*]-azepine-5,7-dione. *J. Heterocycl. Chem*. **1991**, *28*, 1173−1175.

(7) Shaw, K.; Yuan, J. Certain pyrroloquinolinones; a new class of GABA brain receptor ligands. U.S. Patent 5,604,235, 1994; *Chem. Abst*. **1994**, *120*, 106976j.

(8) (a) Ivachtchenko, A. V.; Kobak, V. V.; Il'yin, A. P.; Trifilenkov, A. S.; Busel, A. A. New scaffolds for combinatorial synthesis. II. 6-Sulfamoylquinolinecarboxylic acids. *J. Comb. Chem*. **2003**, *5*, 645−652. (b) Ivachtchenko, A.; Khvat, A.; Kysil, V.; Maliartchuk, S.; Tkachenko, S.; Okun, I. New potent heterocyclic caspase-3 and apoptosis inhibitor. *Drugs Future* **2004**, *29* (Suppl. A), 191. (c) Ivachtchenko, A. V.; Kobak, V. V.; Kisel, V. M.; Kuzovkova, Y. A.; Il'in, A. P.; Kravchenko, D. V.; Tkachenko, S. E.; Khvat, A. V.; Okun, I. M. Quinoline-carboxylic acids, their derivatives, targeted library, pharmaceutical compositions and methods of their preparation and use. WO 2004/078731, 2004; PCT RU 2004/000081, 2004.

(9) (a) Manske, R. H. The chemistry of quinolines. *Chem. Rev*. **1942**, *30*, 113−144. (b) Bergstrom, F. W. Heterocyclic nitrogen compounds. Part IIA. Hexacyclic compounds: pyridine, quinoline, and isoquinoline. *Chem. Rev*. **1944**, *35*, 77−277.

(10) Campaigne, E.; Hutchinson, J. H. Some *N*-substituted 2-amino- and 2-methylquinoline-3,4-dicarboximides. *J. Heterocycl. Chem*. **1970**, *7*, 655−659.

(11) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev*. **1995**, *95*, 2457−2483.

JM048987T