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Short communication

Pyrrolo[3,4-c]quinoline-1,3-diones as potent caspase-3 inhibitors. Synthesis and SAR of 2-substituted 4-methyl-8-(morpholine-4-sulfonyl)-pyrrolo[3,4-*c*]quinoline-1,3-diones

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Abstract

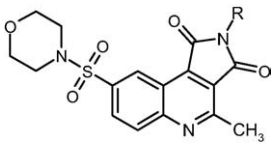
Synthesis, biological evaluation and structure–activity relationships for a series of 2-substituted 4-methyl-8-(morpholine-4-sulfonyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinolines are described. These compounds represent a new chemotype of nonpeptide small molecule inhibitors of caspase-3. Among the studied compounds, several potent inhibitors with IC_{50} in the range of 3–10 nM have been identified. The most active compound within this series, **7***{49}* and **7***{58}*, inhibited caspase-3 with IC_{50} = 3 nM.

Keywords: Caspase-3; Inhibitor; 1*H*-pyrrolo[3,4-*c*]quinoline; Heterocycles; Parallel synthesis; Libraries

1. Introduction

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]. Dysregulated apoptosis has been intensely studied in recent years and is believed to play a role in several diseases of therapeutic interest including cardiovascular, neurodegenerative, infectious and metabolic disorders [3,4]. Therefore, caspases are attractive targets for therapeutic intervention in these diseases because of the central role played by apoptosis in those conditions. For instance, inhibitors of caspase-3 were described as promising cardioprotectants [5,6] and neuroprotectants [7].

The most of potent caspase-3 inhibitors reported to-date have peptide or peptidomimetic nature [8–11]. However, such inhibitors often possess poor cell permeabilities, low metabolic stability and unfavorable physico-chemical profile [12]. To overcome this problem, research efforts were initiated to identify nonpeptide small molecule inhibitors. For example, several potent and selective inhibitors based on isatin [13,14] or quinazoline [12] molecular scaffolds were described. Recently, we reported the discovery of a novel class of potent small molecule inhibitors of caspase-3 [15]. In this paper, we describe synthesis, biological evaluation and structure–activity relationships for this series of novel nonpeptide small molecule inhibitors of caspase-3 based on 4-methyl-8-(morpholine-4-sulfonyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo-[3,4-*c*]quinoline scaffold (general structure **I**).



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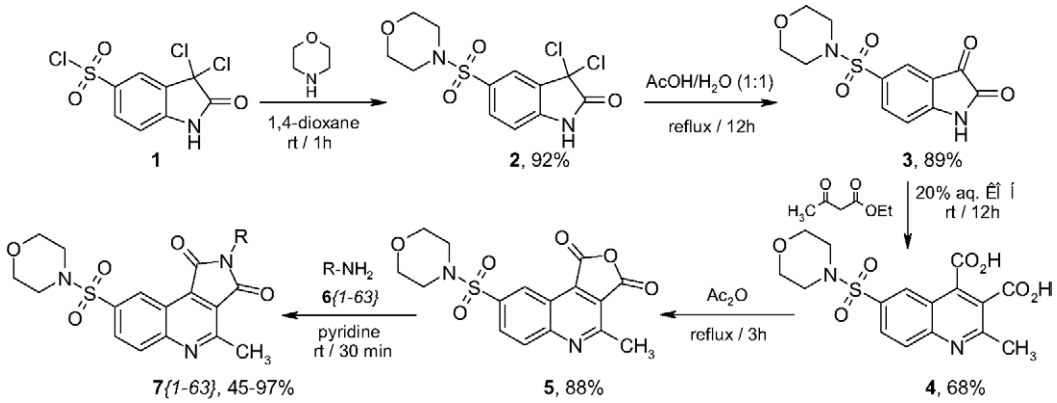


Scheme 1.

2. Chemistry

In this work, we used a synthetic method recently developed by us for the synthesis of pyrrolo[3,4-*c*]quinoline-1,3-diones [16] (Scheme 1). Initial chloride **1** was reacted with morpholine in 1,4-dioxane to give sulfonyl amide **2** in 92% yield. The latter was refluxed in AcOH/water (1:1 v/v) mixture to afford 8-sulfamoyl isatin **3** (yield 89%). Dicarboxylic acid **4** was prepared via a Pfitzinger reaction [17] of **3** with ethyl acetoacetate under strong alkali conditions (yield 68%). Acid **4** was then converted into furan-2,5-dione **5** upon the reaction with acetic anhydride (yield 88%). Reactions of anhydride **5** with a series of different primary amines **6***{1–63}* smoothly led to imides **7***{1–63}* in good to excellent yields (45–97%).

With respect to amine component, various aliphatic and aromatic primary amines **6***{1–63}*, such as substituted anilines and heteroarylamines, *O*-substituted hydroxylamines, *N*,*N*-disubstituted hydrazines, linear and branched aliphatic amines and nitrogen-containing compounds, were tolerated without any limitations. 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.

3. Results and discussion

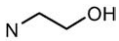
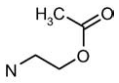
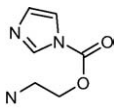
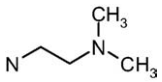
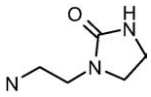
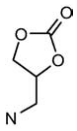
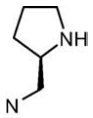
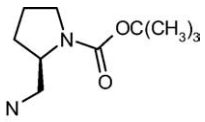
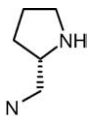
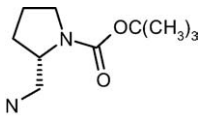
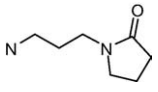
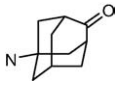
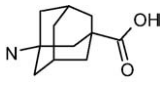
Compounds **7***{1–63}* have been tested on their ability to inhibit caspase-3 catalyzed proteolytic breakdown of its fluorogenic substrate. For all compounds that exhibited more than 50% inhibition at a concentration of 100 μM, the dose-dependent caspase-3 inhibition curves were registered and the IC_{50} values were calculated. The synthesized 2-substituted 4-methyl-8-(morpholine-4-sulfonyl)-pyrrolo[3,4-*c*]quinoline-1,3-diones **7***{1–63}* displayed high activity in this in vitro caspase-3 inhibition assay (Tables 1–4). The activity strongly depends on the nature of substituents in the position 2 of this heterocyclic system.

Thus, in a group of 2-alkyl substituted compounds **7***{1–16}* (Table 1), the activity changed by more than three orders of magnitude (from 20 to 5500 nM). The most active compound within this group, 2-(2-oxo-imidazolidin-1-yl)-ethyl derivative **7***{7}* has IC_{50} = 20 nM. The lowest IC_{50} value was observed for 2-dimethylaminoethyl derivative **7***{6}* (IC_{50} = 5500 nM). Interestingly, its close structural analogs with aminoalkyl substituents, such as **7***{7,9–12,14}*, displayed a relatively high level of activity against caspase-3 with IC_{50} in the range of 20–97 nM. Compounds **7***{15}* and **7***{16}* with bulky adamantyl substituents in the position 2 are less active (IC_{50} = 152 and 196 nM, respectively) than most of their spatially less hindered analogs. Given the observed difference in activity, it likely appears that these residues are accommodated in a sterically hindered region of the caspase-3 active site. Substituent's chirality did not significantly influence the inhibitory activity for two pairs of individual stereoisomers **7***{9}*–**7***{11}* and **7***{10}*–**7***{12}*. Recently, a dramatic difference in caspase-3 inhibitory activity was observed for individual stereoisomeric 5-sulfamoylisatins [13,14].

Compounds **7***{17–25}* with several different α-amino acid residues (in the form of alkyl esters) in the position 2 of the pyrrolo[3,4-*c*]quinoline-1,3-dione scaffold also possessed a high activity against caspase-3 with IC_{50} in the range of 11–85 nM (Table 2). The most active compounds within this group, derivatives of methyl esters of valine **7***{21}* and leucine **7***{22}*, inhibited caspase-3 with IC_{50} = 11 nM. Deprotection of carboxylate function resulted in almost 10-fold decrease in activity in the case of phenylalanine derivatives **7***{23}* and **7***{24}* (IC_{50} = 661 and 73 nM, respectively).

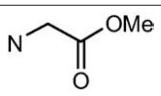
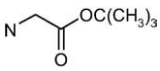
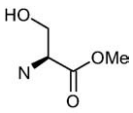
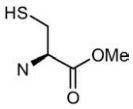
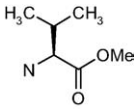
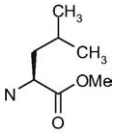
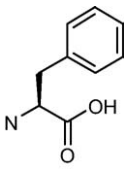
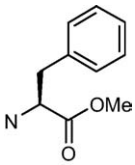
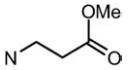
Strong caspase-3 inhibitory activity was also observed for compounds **7***{26–38}* with differently substituted phenyl fragments in the position 2 of the pyrrolo[3,4-*c*]quinoline-1,3-dione heterocyclic system. For this group, the activity ranged from 9 to 24 nM (Table 3); the most active compounds within this group, 4-hydroxyphenyl **7***{31}*, 4-fluoro-2-methylphenyl **7***{35}* and 2,6-difluorophenyl **7***{34}* derivatives, inhibited caspase-3 with IC_{50} = 8, 9 and 10 nM, respectively. The exception are compounds **7***{29}* and **7***{37}* with IC_{50} = 2300 and 406 nM, respectively. Interestingly, 2-morpholin-4-ylphenyl derivative **7***{38}* (IC_{50} = 20 nM) is 20-fold more potent inhibitor than its close structural analog **7***{37}* with 2-piperidin-1-ylphenyl moiety.

Table 1
Structures and in vitro caspase-3 inhibition activity for 2-alkyl substituted derivatives **7***{1–16}*

No.	N-R	IC_{50}, nM
7*{1}*		75
7*{2}*		44
7*{3}*		54
7*{4}*		23
7*{5}*		41
7*{6}*		5500
7*{7}*		20
7*{8}*		32
7*{9}*		97
7*{10}*		56
7*{11}*		58
7*{12}*		55
7*{13}*		53
7*{14}*		24
7*{15}*		152
7*{16}*		196

2-Heteroaryl substituted compounds **7***{39–59}* appeared to be the most potent caspase-3 inhibitors among the compounds studied in this work. Typically, the IC_{50} values for

Table 2
Structures and in vitro caspase-3 inhibition activity for α-amino acid substituted derivatives **7***{17–25}*

No.	N-R	IC_{50}, nM
7*{17}*		16
7*{18}*		30
7*{19}*		21
7*{20}*		85
7*{21}*		11
7*{22}*		11
7*{23}*		661
7*{24}*		73
7*{25}*		37

various substituted 2-heteroarylimides were in the range of 3–18 nM (Table 3). The most active compounds within this series have pyrazol-4-yl (structures **7***{49}*–**7***{51}*, IC_{50} in the range of 3–4 nM), 1-phenylpyrazol-5-yl (structures **7***{47}* and **7***{48}*, IC_{50} = 5 nM) and 4-pyridyl (structure **7***{58}*, IC_{50} = 3 nM) substituents. The polar functional groups present in the heterocyclic substituents might represent additional noncovalent binding sites and positively effect the affinity to the active site of the enzyme.

In this work, we also synthesized four compounds **7***{60–63}* which represent 2-alkyloxy (2-alkylamino) and 2-aryloxy (2-arylamino) derivatives of pyrrolo[3,4-*c*]quinoline-1,3-dione scaffold. Activity within this small group changed by more than three orders of magnitude with IC_{50} values in the range of 15–2980 nM. In the studied cases, the inhibitory potential was highly dependent on the nature of 2-substituent,

Table 3
Structures and in vitro caspase-3 inhibition activity for 2-aryl **7***{26–38}* and 2-heteroaryl **7***{39–59}* substituted compounds

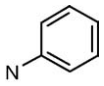
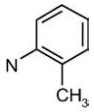
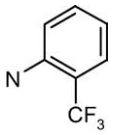
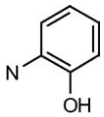
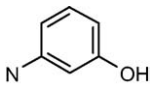
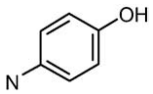
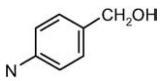
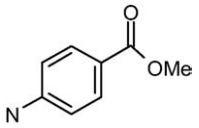
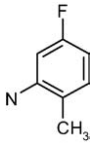
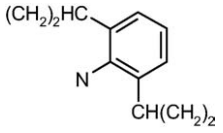
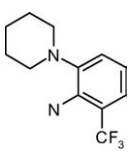
No.	N-R	IC_{50}, nM
7*{26}*		19
7*{27}*		15
7*{28}*		24
7*{29}*		2300
7*{30}*		24
7*{31}*		8
7*{32}*		22
7*{33}*		20
7*{34}*		10
7*{35}*		9
7*{36}*		22
7*{37}*		406

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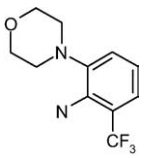
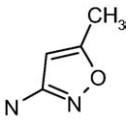
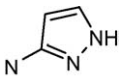
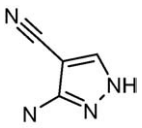
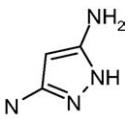
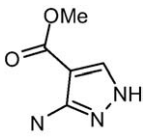
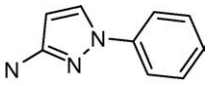
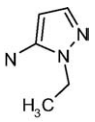
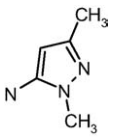
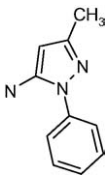
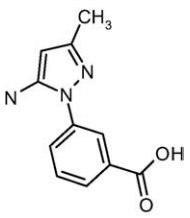
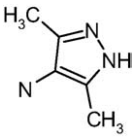
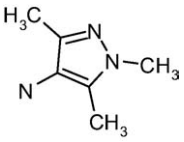
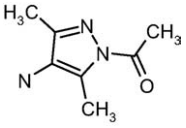
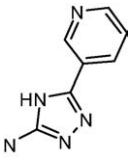
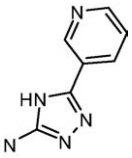
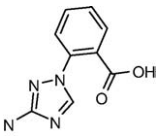
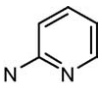
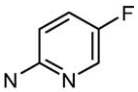
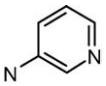
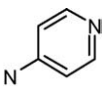
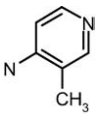
Table 3
(continued)

No.	N-R	IC_{50}, nM
7*{38}*		20
7*{39}*		35
7*{40}*		17
7*{41}*		13
7*{42}*		14
7*{43}*		8
7*{44}*		9
7*{45}*		6
7*{46}*		6
7*{47}*		5
7*{48}*		5

(continued on next page)

Table 3
(continued)

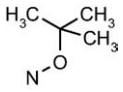
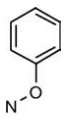
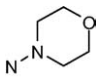
No.	N-R	IC_{50}, nM
7*{49}*		3
7*{50}*		4
7*{51}*		4
7*{52}*		14
7*{53}*		11
7*{54}*		12
7*{55}*		18
7*{56}*		14
7*{57}*		13
7*{58}*		3
7*{59}*		82

rather than on the nature of atom (*O* or *N*) linked to the imide nitrogen.

4. Conclusions

In summary, here we have described synthesis and activity of a novel class of potent caspase-3 inhibitors based on 4-methyl-8-(morpholine-4-sulfonyl)-1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]quinoline molecular scaffold. Caspase-3 inhibitory activity of the synthesized compounds is highly dependent on the nature of 2-substituents on the core scaffold. In general, 2-heteroaryl substituted compounds showed an increased inhibitory potency as compared to other chemical series synthesized in this work. Structures **7***{49}* and **7***{58}* are the lead compounds with potent inhibitory activity (IC_{50} = 3 nM). Evaluation against other caspases involved in apoptosis, as well as further SAR studies, are continuing and will be reported in due course.

Table 4
Structures and in vitro caspase-3 inhibition activity for substituted 2-hydroxy- and 2-amino-4-methyl-8-(morpholine-4-sulfonyl)-pyrrolo[3,4-*c*]quinoline-1,3-diones **7***{60–63}*

No.	N-R	IC_{50}, nM
7*{60}*		15
7*{61}*		404
7*{62}*		18
7*{63}*		2980

5. Experimental

5.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-300 spectrometers (300, 13 MHz for ^{1}H NMR and 75, 46 MHz for ^{13}C NMR) in DMSO-d_6 using TMS as an internal standard. LCMS spectra were recorded with PE SCIEX API 150EX liquid chromatograph equipped with a UV detector (λ_{max} 215 and 254 nm) and using a C_{18} column (100 × 4 mm). Elution started with water and ended with acetonitrile/water (95:5, v/v) and used a linear gradient at a flow rate of 0.15 ml/min and an analysis cycle time of 25 min. High resolution mass-spectra have been recorded using electrospray ionization time-of-flight reflectron experiments (ESI-TOF) on Agilent ESI-TOF mass spectrometer. Samples were electrosprayed into the TOF reflectron analyzer at an ESI voltage of 4000 V and a flow rate of 200 μl/min.

5.1.1. Starting materials and intermediates

All solvents and reagents were obtained from Acros Organics, Aldrich or ChemDiv and used without purification. 3,3-Dichloro-2-oxoindoline-5-sulfonyl chloride **1** was prepared from isatin using a previously described approach [16].

*5.1.1.1. 3,3-Dichloro-5-(morpholin-4-ylsulfonyl)-1,3-dihydro-2H-indol-2-one (**2**).* A solution of morpholine (8.7 g, 0.1 mol) in 1,4-dioxane (50 ml) was slowly added to a stirred solution

of chloride (**1**) (15 g, 0.05 mmol) in 1,4-dioxane (150 ml) at 5 °C. The reaction mixture was stirred at 5 °C for 1 h, then ice-cold water (200 ml) was slowly added. The formed precipitate was filtered off, washed with ice-cold water and dried by lyophilization to give 16.1 g (92%) of (**2**). ^{1}H NMR: δ = 11.88 (s, 1H, exch. with D_2O), 7.92 (d, *Jm* = 1.8 Hz, 1H), 7.80 (dd, *Jo* = 8.4 Hz, *Jm* = 1.8 Hz), 7.23 (d, *Jo* = 8.4 Hz, 1H), 3.64 (m, 4H), 2.89 (m, 4H); ^{13}C NMR: δ = 169.02, 143.58, 132.70, 129.60, 129.47, 123.90, 112.24, 73.82, 65.23, 45.82; LCMS m/z 352 (M + 1); HRMS: m/z [M + H$^+$] calcd for $C_{12}H_{12}Cl_2N_2O_4S$: 351.2097; found: 351.2099.

*5.1.1.2. 5-(Morpholin-4-ylsulfonyl)-1H-indole-2,3-dione (**3**).* Morpholide (**2**) (15.8 g, 0.045 mol) was added to a solution of AcOH (150 ml) in water (150 ml). The mixture was stirred under reflux overnight, concentrated in vacuo to one third of original volume, then ice-cold water (200 ml) was slowly added. The formed precipitate was filtered off, washed with water and dried to give 11.9 g (89%) of (**3**). ^{1}H NMR: δ = 11.49 (s, 1H, exch D_2O), 7.91 (dd, *J* = 8.2 Hz, *J* = 2.1 HZ, 1H), 7.69 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 1H), 3.63 (m, 4H), 2.88 (m, 4H); ^{13}C NMR: δ = 159.45, 153.92, 137.10, 128.26, 123.46, 118.25, 112.79, 65.21, 45.85; HRMS: m/z [M + H$^+$] calcd for $C_{12}H_{12}N_2O_5S$: 297.0540; found: 297.0552.

*5.1.1.3. 2-Methyl-6-(morpholin-4-ylsulfonyl)-3,4-quinolinedicarboxylic acid (**4**).* KOH (20 g) was added to a suspension of (**3**) (13.33 g, 45 mmol) in water (100 ml) in nitrogen atmosphere. Ethyl acetoacetate (16 ml, 126 mmol) was added and the reaction mixture was stirred for 12 h. The mixture was cooled to 0 °C, then conc. HCl (40 ml) was slowly added. The formed precipitate was filtered off, washed with water and dried in vacuo at 70 °C to give 32.6 g (68%) of (**4**). LCMS (ELSD and UV_{254} nm detectors), m/z 381 (M + 1).

*5.1.1.4. 4-Methyl-8-(morpholin-4-ylsulfonyl)-furo[3,4-c]quinoline-1,3-dione (**5**).* A mixture of (**4**) (1.3 g, 3.43 mmol) and acetic anhydride (15 ml, 158.68 mmol) was heated to reflux until all of the initial (**4**) was dissolved. The obtained solution was cooled to 0 °C and kept at this temperature for 4 h. The formed precipitate was filtered off, washed with ether and hexane, and dried in vacuo at 60 °C to give 1.1 g (88%) of (**5**). LCMS (ELSD and UV_{254} nm detectors), *m/z* 361 ($M^{\bullet+}$ – 1).

*5.1.2. General procedure for preparation of 2-substituted 4-methyl-8-(morpholin-4-ylsulfonyl)-1,3-dihydro-pyrrolo[3,4-c]quinoline-1,3-diones **7**{1–63}*

Amine (**6***{1–63}*) (60 mmol) was added to a solution of (**5**) (21.7 g, 60 mmol) in pyridine (50 ml) at r.t. and the resulting mixture was stirred for 30 min. Acetic anhydride (50 ml) was added and the mixture was heated to reflux for 3 h. The reaction mixture was evaporated to dryness in vacuo, the residue was washed with isopropanol and dried to give the corresponding imide (**7***{1–63}*) in 45–97% yield.

5.1.3. Selected data

*5.1.3.1. Methyl (2S)-4-methyl-2-[4-methyl-8-(morpholin-4-ylsulfonyl)-1,3-dioxo-1,3-dihydro-2H-pyrrolo[3,4-c]quinolin-2-yl]pentanoate **7**{22}.* This compound was obtained in 56% yield. ^{1}H NMR: δ = 0.88–0.93 (m, 6H), 1.55–1.66 (m, 1H), 1.86-1.95 (m, 1H), 2.12-2.21 (m, 1H), 3.00 (s, 3H), 3.02 (m, 4H), 3.65 (m, 3H), 3.67 (s, 3H), 8.20 (dd, J_o = 9.2 Hz, J_m = 1.8 Hz, 1H), 8.36 (d, J_o = 9.2 Hz, 1H), 9.01 (d, J_m = 1.8 Hz, 1H); ^{13}C NMR: δ = 167.9, 167.5, 167.2, 158.4, 152.2, 137.9, 137.4, 125.7, 123.2, 119.7, 66.1, 54.7, 54.1, 47.2, 44.3, 34.9, 24.0, 22.5, 22.1 ppm; LC MS m/z 524 (M + 1).LC MS m/z 490 (M + 1).

*5.1.3.2. Methyl (2S)-2-[4-methyl-8-(morpholin-4-ylsulfonyl)-1,3-dioxo-1,3-dihydro-2H-pyrrolo[3,4-c]quinolin-2-yl]-3-phenylpropanoate **7**{24}.* This compound was obtained in 76% yield. ^{1}H NMR: δ = 2.93 (s, 3H), 3.01 (m, 4H), 3.50–3.80 (m, 6H), 5.29–5.33 (m, 1H), 7.05–7.35 (m,5H), 8.19 (dd, J_o = 9.2 Hz, J_m = 1.8 Hz, 1H), 8.34 (d, J_o = 9.2 Hz, 1H), 8.93 (d, J_m = 2.0 Hz, 1H); ^{13}C NMR: δ = 169.7, 167.5, 167.4, 158.7, 152.4, 137.7, 137.1, 136.1, 131.6, 130.6, 129.8, 129.3, 127.6, 125.9, 123.5, 120.0, 66.3, 54.1, 53.8, 46.7, 34.9, 22.9 ppm; LC MS m/z 524 (M + 1).

*5.1.3.3. 2-(2,6-Dicyclopropylphenyl)-4-methyl-8-(morpholin-4-ylsulfonyl)-1H-pyrrolo[3,4-c]quinoline-1,3(2H)-dione **7**{36}.* This compound was obtained in 84% yield. ^{1}H NMR: δ = 1.06–1.2 (m, 12H,), 2.83–2.86 (m, 2H, CH), 3.02–3.15 (m, 4H, CH_2), 3.00 (s, 3H, CH_3), 3.63 (s, 3H, CH3), 3.62–3.69 (m, 3H, CH_2), 7.34 (d, *J* = 7.6 Hz, 2H), 7.49 (t, 1H, ArH), 8.21 (dd, J_o = 9.2 Hz, J_m = 2.0 Hz, 1H, ArH), 8.37 (d, J_o = 9.2 Hz, 1H, ArH), 9.02 (d, J_m = 2.0 Hz, 1H, ArH); ^{13}C NMR: δ = 168.6, 164.1, 152.0, 146.1, 137.2, 136.4, 135.7, 133.8, 131.4, 130.1, 128.7, 127.1, 126.7, 123.1, 120.7, 109.6, 68.3, 54.9, 47.3, 23.4, 16.4, 5.9 ppm; LC MS m/z 522 (M + 1).

*5.1.3.4. Methyl 3-[4-methyl-8-(morpholin-4-ylsulfonyl)-1,3-dioxo-1,3-dihydro-2H-pyrrolo[3,4-c]quinolin-2-yl]-1H-pyrazole-4-carboxylate **7**{43}.* This compound was obtained in 51% yield. ^{1}H NMR: δ = 2.98-3.00 (m, 4H, CH_2), 3.00 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 3.62 (m, 3H, CH_2), 8.21 (dd, J_o = 9.2 Hz, J_m = 2.0 Hz, 1H, ArH), 8.38 (d, J_o = 9.2 Hz, 1H, ArH), 8.57 (s, 1H, ArH), 9.01 (d, J_m = 2.0 Hz, 1H, ArH), 13.93 (br. s., 1H); ^{13}C NMR: δ = 167.1, 162.7, 159.2, 152.5, 140.8, 137.8, 136.2, 135.5, 131.8, 130.8, 126.1, 124.4, 120.4, 109.7, 66.3, 52.4, 46.8, 23.2 ppm; LC MS m/z 486 (M + 1).

*5.1.3.5. 2-(3,5-Dimethyl-1H-pyrazol-4-yl)-4-methyl-8-(morpholin-4-ylsulfonyl)-1H-pyrrolo[3,4-c]quinoline-1,3(2H)-dione **7**{49}.* This compound was obtained in 89% yield. ^{1}H NMR: δ = 2.05 (s, 6H, $2CH_3$), 2.98–3.01 (m, 4H, CH_2), 2.99 (s, 3H, CH_3), 3.62 (m, 2H, CH_2), 8.17 (dd, J_o = 9.2 Hz, J_m = 2.0 Hz, 1H, ArH), 8.35 (d, J_o = 9.2 Hz, 1H, ArH), 9.04 (d, J_m = 2.0 Hz, 1H, ArH), 12.58 (br. s., 1H); LC

MS m/z 456 (M + 1); ^{13}C NMR: δ = 168.3, 163.9, 152.1, 140.9, 137.4, 136.2, 135.5, 131.4, 130.2, 127.2, 123.2, 120.4, 109.4, 66.7, 52.1, 47.9, 23.6, 14.1 ppm; HRMS: m/z [M + H$^+$] calcd for $C_{21}H_{21}N_5O_5S$: 456.1336; found: 456.1337.

5.1.3.6. 4-Methyl-8-(morpholin-4-ylsulfonyl)-2-(1,3,5-trimethyl-1H-pyrazol-4-yl)-1H-pyrrolo[3,4-c]quinoline-1,3(2H)-dione ***7****{50}*. This compound was obtained in 85% yield. ^{1}H NMR: δ = 1.99 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.98 (m, 4H), 2.99 (s, 3H), 3.62 (m, 3H), 3.71 (s, 3H), 8.17 (dd, J_o = 9.2 Hz, J_m = 2.0 Hz, 1H, ArH), 8.35 (d, J_o = 9.2 Hz, 1H, ArH), 9.04 (d, J_m = 2.0 Hz, 1H, ArH); LC MS m/z 470 (M + 1); ^{13}C NMR: δ = 167.0, 162.6, 152.9, 141.0, 137.4, 136.0, 135.7, 130.9, 130.5, 126.5, 124.3, 120.4, 109.7, 66.3, 52.4, 46.8, 37.2, 23.2, 13.1 ppm; HRMS: m/z [M + H$^+$] calcd for $C_{22}H_{23}N_5O_5S$: 470.1493; found: 470.1491.

5.1.3.7. 4-Methyl-8-(morpholin-4-ylsulfonyl)-2-(4-pyridinyl)-1H-pyrrolo[3,4-c]quinoline-1,3(2H)-dione ***7****{58}*. ^{1}H NMR: δ = 9.10 (d, 1H, J_m = 1.8 Hz), 8.80 (d, 2H, J = 4.6 Hz), 8.41 (d, 1H, J_o = 8.8 Hz), 8.23 (dd, 1H, J_o = 8.8 Hz, J_m = 1.8 Hz), 7.63 (d, 2H, J = 4.6 Hz), 3.65 (m, 4H), 3.05 (s, 3H), 3.02 (m, 4H); ^{13}C NMR: δ = 168.3, 163.9, 152.1, 148.3, 145.8, 137.4, 136.2, 135.5, 131.4, 130.2, 127.2, 123.2, 120.4, 111.1, 109.4, 66.7, 52.1, 47.9, 23.6 ppm; HRMS: m/z [M + H$^+$] calcd for $C_{21}H_{18}N_4O_5S$: 439.1071; found: 439.1074.

5.1.4. Caspase-3 inhibition assay

Compounds ***7****{1–63}* 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 manufacturer's protocol [18] using VICTOR^{2}V (PerkinElmer) 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 of active compounds displayed dose–response curves with a Hill slopes close to unity, which indicates a high probability of the compounds being real inhibitors and not promiscuous ones.

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