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Discovery of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. 2: Structure–activity relationships of the 4-, 5-, 6-, 7- and 8-positions

William Kemnitzer, Jared Kuemmerle, Songchun Jiang, Nilantha Sirisoma, Shailaja Kasibhatla, Candace Crogan-Grundy, Ben Tseng, John Drewe, Sui Xiong Cai *

EpiCept Corporation, Inc. 6650 Nancy Ridge Drive, San Diego, CA 92121, USA

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ABSTRACT

As a continuation of our efforts to discover and develop the apoptosis inducing 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines as potential anticancer agents, we explored substitutions at the 4-, 5-, 6-, 7- and 8-positions of pyrrolo[1,2-*a*]quinoline. SAR studies showed that substitution at the 6-position by a small group such as Cl resulted in potent compounds. Substitutions at the 5- and 8-positions were tolerated while substitutions at the 4- and 7-position led to inactive compounds. Several compounds, including **2c**, **3a**, **3b** and **3f**, were found to be highly active against human breast cancer cells T47D with EC_{50} values of 0.053–0.080 μM, but much less active against human colon cancer cells HCT116 and hepatocellular carcinoma cancer cells SNU398 in the caspase activation assay. Compound **3f** also was found to be highly active with a GI_{50} value of 0.018 μM against T47D cells in a growth inhibition assay.

Apoptosis is a highly regulated process of cellular suicide and plays a key role for the maintenance of cell homeostasis.[1] It is well known that abnormal or excessive inhibition of apoptosis could result in uncontrolled tumor cell growth as well as tumor resistance to chemotherapeutic treatment.[2] The pathways of apoptosis involve a cascade of sequentially activated initiator and effector caspases.[3] Caspase-3 is the main executioner in apoptosis and its activation leads to the cleavage of many protein substrates and cell death.[4] Since many chemotherapeutics are known to induce apoptosis in cancer cells,[5] promotion or activation of apoptosis via targeting apoptosis regulators has been suggested as a promising strategy for anticancer drug discovery.[6]

Utilizing our proprietary fluorescent caspase-3 substrates,[7] we have developed a cell- and caspase-based HTS assays called Anticancer Screening Apoptosis Program (ASAP) for the discovery of apoptosis inducers as potential anticancer agents.[8] Applying this assay, we have reported the discovery of several series of novel apoptosis inducers including 4-aryl-4*H*-chromenes (**1a**),[9] gambogic acid (**1b**),[10] 3-aryl-5-aryl-1,2,4-oxadiazoles (**1c**),[11] 4-anilino-2-(2-pyridyl)pyrimidines (**1d**)[12] and 4-anilinoquinazolines (**1e**)[13] (Chart 1), as well as the identification of the molecular targets for gambogic acid[14] and 3-aryl-5-aryl-1,2,4-oxadiazoles.[15] Recently, we have reported the discovery of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines (**1f**) as a potent apoptosis inducer. Structure–activity relationship (SAR) study of the 1-benzoyl and 3-cyano groups showed that substitution at the 4-position of the 1-benzoyl group was important for activity, and replacing the 3-cyano by an ester or ketone group led to inactive compounds.[16] Herein we wish to report the synthesis and SAR study of the 4-, 5-, 6-, 7- and 8-positions of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines as potent apoptosis inducers.

1-Benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines **2a–2e**, **2h–2j, 3a–3b** and **3e–3f** were synthesized in two steps from reaction of the corresponding 2-bromoacetylphenones (**4**) with substituted quinolines (**5**) to give the quinolinium salts (**6**), followed by cyclization with acrylonitrile in the presence of an oxidant, such as tetrapyridinecobalt(II) dichromate (TPCD, $Co(II)Py_4(HCrO_4)_2$) or manganese(IV) oxide (MnO_2) and a base, as reported previously (Scheme 1).[16–18] Compounds **2f** and **2g** were prepared from reaction of 1-benzoyl-3-cyano-6-hydroxypyrrolo[1,2-*a*]-quinoline (**2e**) with *N*-(2-chloro-ethyl)-morpholine and 2-chloro-*N*,*N*-dimethylethanamine in the presence of potassium carbonate and potassium iodide, respectively (Scheme 2). Compounds **3c** was made from reaction of 6-chloro-3-cyano-1-(4-fluorobenzoyl)-pyrrolo[1,2-*a*]-quinoline (**3a**) with imidazole in the presence of potassium carbonate (Scheme 3). Amino compounds **3d** and **3g** were synthesized from reduction of the corresponding nitro compounds

* Corresponding author. Tel.: +1 858 202 4006; fax: +1 858 202 4000.
E-mail address: scai@epicept.com (S.X. Cai).

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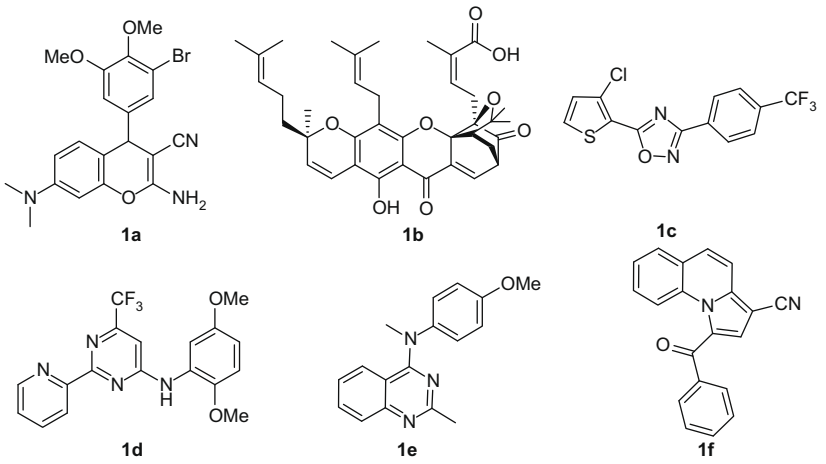
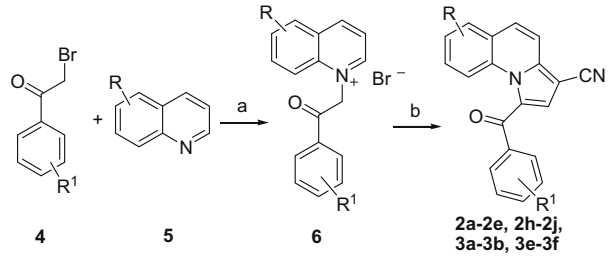
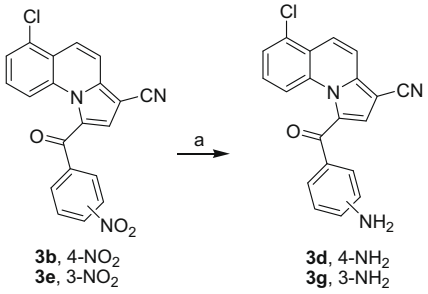


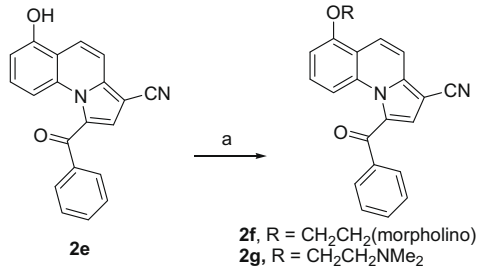
Chart 1.



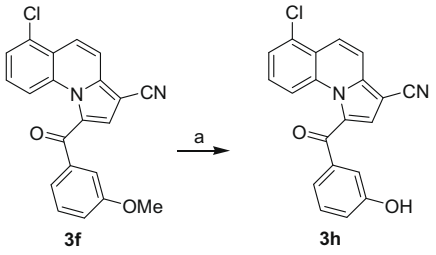
Scheme 1. Reagents and conditions: (a) CH_3CN, 85 °C, 4–6 h; (b) acrylonitrile, TPCD, DMF, Na_2CO_3, 95 °C, 5 h or acrylonitrile, MnO_2, DMF, Et_3N, 95 °C, 5 h.



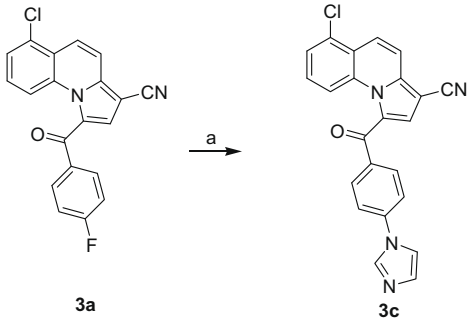
Scheme 4. Reagents and conditions: (a) $SnCl_2$, EtOH, 90 °C, 7 h.



Scheme 2. Reagents and conditions: (a) RCl, acetone, K_2CO_3, KI, 60 °C, 2.5 days.



Scheme 5. Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 °C, then 10 °C, 1.5 h.

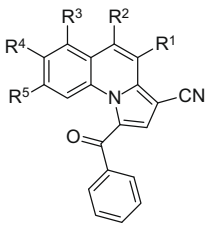


Scheme 3. Reagents and conditions: (a) imidazole, DMF, K_2CO_3, 160 °C, 20 h.

3b and **3e** using tin(II) chloride dihydrate (Scheme 4). The hydroxyl compound **3h** was prepared from demethylation of **3f** using boron tribromide (Scheme 5).

The apoptosis inducing activity of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines was measured by our proprietary cell- and caspase-based HTS assay as described previously[19] in human breast cancer cells T47D, human colon cancer cells HCT116 and hepatocellular carcinoma cancer cells SNU398, and the results are summarized in Tables 1 and 2. The original hit **1f** was a potent compound against T47D cells and less active in HCT116 and SNU398 cells and the SAR of the 1-benzoyl and 3-cyano groups has been reported.[16] Here we explored further the SAR of **1f** by maintaining the 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinoline structure, and introducing various substitutions at the 4-, 5-, 6-, 7- and 8-positions of the pyrrolo[1,2-*a*]quinoline ring. Caspase activation data in Table 1 showed that compared with **1f**, introduction of a methyl group at the 4-position (**2a**) led to >70-fold reduction in activity. A methyl group at the 5-position (**2b**) was somewhat tolerated with about sevenfold reduction in activity against T47D cells, and became nonselective with similar activity in all the three cell lines. The 6-chloro analog **2c** was highly active against T47D cells, and with lower activity in HCT116 and SNU398 cells, indicat-

Table 1
SAR of 4-, 5-, 6-, 7- and 8-positions of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines in the caspase activation assay



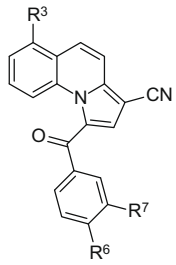
Entry	R^1	R^2	R^3	R^4	R^5	EC_{50} (μM)[a]		
						T47D	HCT116	SNU398
1f[b]	H	H	H	H	H	0.078 ± 0.002	5.5 ± 0.069	5.2 ± 0.13
2a	Me	H	H	H	H	5.8 ± 0.2	>10	>10
2b	H	Me	H	H	H	0.52 ± 0.09	0.31 ± 0.01	0.22 ± 0.02
2c	H	H	Cl	H	H	0.083 ± 0.018	1.9 ± 0.02	>10
2d	H	H	NO_2	H	H	>10	>10	>10
2e	H	H	OH	H	H	>10	>10	>10
2f	H	H	OR[c]	H	H	>10	>10	>10
2g	H	H	OR[d]	H	H	2.8 ± 0.3	6.0 ± 0.4	4.9 ± 0.1
2h	H	H	H	Me	H	>10	>10	>10
2i	H	H	H	Cl	H	>10	>10	>10
2j	H	H	H	H	Me	0.28 ± 0.03	>10	5.0 ± 0.1

[a] Cells were treated with the test compounds for 48 h, data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] Data from Ref. 16.
[c] R = CH_2CH_2(morpholino).
[d] R = $CH_2CH_2NMe_2$.

ing that a small group at the 6-position maintains both the potency and selectivity of **1f**. Interestingly, the 6-nitro analog **2d** and 6-hydroxy analog **2e** were not active in all the three cell lines up to 10 μM. Compounds designed to improve aqueous solubility properties, were either inactive in all three cell lines (2-morpholinoethoxy**, 2f)** or significantly less active (2-dimethylaminoethoxy**, 2g),** indicating that the 6-position might be size limited. Moving to the 7-position, introduction of a methyl or chloro group (**2h** and **2i**) led to inactive compounds, indicating substitution at this position is not tolerated. The 8-methyl analog **2j** retained the selective activity of **1f** but was threefold less active against T47D cells, indicating that a small substitution at the 8-position is tolerated.

We have found previously that substitution at the 1-benzoyl group was important for activity and selectivity of **1f** and related compounds.[16] To determine whether a chloro group at the 6-position changes the SAR of the 1-benzoyl group, we prepared a group of compounds with substitutions at the 3- and 4-positions of the benzoyl group of the 6-chloro analog **2c** (Table 2). Table 2 shows that compounds with a small group, such as fluoro (**3a**) or nitro group (**3b**) at the 4-position of the benzoyl group, were highly active against T47D cells, and maintained good selectivity being inactive in HCT116 and SNU398 cells. Analogs with either a relatively large group imidazole (**3c**) or hydrophilic amino group (**3d**) at the 4-position were 2–3-folds less active than **2c**, and lost some selectivity against HCT116 and SNU398 cells. The 3-nitro (**3e**), 3-amino (**3g**) and 3-hydroxy (**3h**) analogs were 2–3-folds less active than **2c**, while the 3-methoxy analog **3f** was about as active as **2c** with similar selectivity profiles. Overall, compounds **3a–3h** were all less active in HCT116 and SNU398 cells, showing that all the compounds with a chloro group at 6-position of the pyrrolo[1,2-*a*]quinoline ring have selectivity for T47D cells versus other cells. For comparison, two well known tubulin interacting anticancer agents vinblastine and paclitaxel were found to be active in all the three cell lines (Table 2), suggesting that the selective compounds may interact with different molecular targets from that of vinblastine and paclitaxel.

Table 2
SAR of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines in the caspase activation assay



Entry	R^3	R^6	R^7	EC_{50} (μM)[a]		
				T47D	HCT116	SNU398
3a	Cl	F	H	0.053 ± 0.017	>10	>10
3b	Cl	NO_2	H	0.062 ± 0.010	>10	>10
3c	Cl		H	0.29 ± 0.01	1.9 ± 0.4	1.4 ± 0.1
3d	Cl	NH_2	H	0.15 ± 0.02	3.8 ± 0.3	1.4 ± 0.1
3e	Cl	H	NO_2	0.15 ± 0.01	>10	>10
3f	Cl	H	OMe	0.080 ± 0.003	3.7 ± 1.2	1.5 ± 0.1
3g	Cl	H	NH_2	0.21 ± 0.05	5.6 ± 0.6	5.0 ± 0.1
3h	Cl	H	OH	0.28 ± 0.06	2.7 ± 1.2	2.6 ± 0.1
7a[b]	H	F	H	0.036 ± 0.011	1.2 ± 0.059	0.88 ± 0.21
7b[b]	H	NO_2	H	2.8 ± 0.17	> 10	> 10
7c[b]	H		H	0.034 ± 0.001	0.074 ± 0.011	0.039 ± 0.001
7d[b]	H	NH_2	H	0.17 ± 0.022	0.24 ± 0.044	0.20 ± 0.036
7e[b]	H	H	OMe	0.17 ± 0.014	1.7 ± 0.42	1.6 ± 0.51
Vinblastine	NA[c]	NA	NA	0.032 ± 0.006	0.036 ± 0.009	0.026 ± 0.008
Paclitaxel	NA	NA	NA	0.037 ± 0.003	0.018 ± 0.001	0.009 ± 0.001

[a] Cells were treated with the test compounds for 48 h, data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] Data from Ref. 16.
[c] NA, not applied.

Table 2 also includes data from several substituted analogs of **1f** for direct comparison.[16] Compound **7a** and the corresponding 6-chloro analog **3a** had similar activity profiles with high activity against T47D cells and low activity in HCT116 and SNU398 cells. Compounds **7e** and **3f** also had similar activity profiles, with high activity against T47D cells and good selectivity. In contrast, the nitro substituted compound **7b** is >40 fold less active than **3b**, indicating that these two series of compounds do not have the exact same SAR. Interestingly, compound **3c** was >8-, 25- and 35-fold less active than **7c** against T47D, HCT116 and SNU398 cells, respectively. These data indicated that an imidazolyl group at the 4-position of the benzoyl group, which is preferred when there is no substitution at the 4–8 positions, is not preferred with a chloro group at the 6-position of the pyrrolo[1,2-*a*]quinoline ring. Similarly, compound **3d** was selective against T47D cells, while compound **7d** were broadly active against all three cell lines. These data showed that a chloro group at the 6-position of the pyrroloquinoline did change the SAR of the 1-benzoyl group.

Selected compounds were also tested by the traditional cell growth inhibition assay (GI_{50}) to confirm that the active compounds can inhibit tumor cell growth. The growth inhibition assays in T47D, HCT116 and SNU398 cells were run in a 96-well microtiter plate as described previously.[9] Compound **3f** was found to be highly active with a GI_{50} value of 0.018 μM against T47D cells, and much less active in HCT116 and SNU398 cells, with GI_{50} values of 8.6 and 4.8 μM, respectively (Table 3). Compounds **3h** also was

Table 3
Inhibition of cell growth of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines

Entry	GI_{50} (μM)[a]		
	T47D	HCT116	SNU398
1f[b]	0.070 ± 0.015	5.8 ± 0.13	2.0 ± 0.50
3f	0.018 ± 0.001	8.6 ± 1.6	4.8 ± 0.2
3h	0.16 ± 0.05	5.9 ± 0.2	2.6± 0.4
7c[b]	0.041 ± 0.008	0.065 ± 0.015	0.059 ± 0.009
Vinblastine	0.007 ± 0.001	0.010 ± 0.003	0.005 ± 0.001
Paclitaxel	0.026 ± 0.003	0.060 ± 0.007	0.061 ± 0.005

[a] Cells were treated with the test compounds for 48 h, data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).
[b] Data from Ref. 16.

more active against T47D cells than HCT116 and SNU398 cells, indicating that these 6-chloro substituted analogs maintain the selectivity of the original hit **1f**. In comparison, analog **7c** as well as the reference compounds vinblastine and paclitaxel, which were active in all three cell lines in the caspase activation assay, also were broadly active in the growth inhibition assay, indicating a good correlation between caspase activation assay and growth inhibition assay as has been observed for other series of apoptosis inducers.[8]

We have previously found that compound **1f**, which was active against breast cancer cells but much less active against several other cancer cell lines, arrested cells in G_2/M followed by apoptosis as characterized by cell cycle analysis, and was not active in the tubulin polymerization assay.[16] Compounds **2c**, **3a** and **3h,** which were selective against T47D cells, also were found to arrest T47D cells in G_2/M and induce apoptosis as assayed by cell cycle analysis,[9] and were inactive in a tubulin polymerization assay[20] up to 50 μM. These data indicate that similar to **1f**, compound **2c** and related analogs are not tubulin inhibitors, and the mechanism of action and molecular target for apoptosis induction remains to be determined. In comparison, the nonselective compounds **2b**, **7c** and **7d** all inhibited tubulin polymerization, with IC_{50} values of less than 5 μM.

In conclusion, we have explored the SAR of the 4-, 5-, 6-, 7- and 8-positions of the apoptosis inducing 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines. It was found that substitution at the 4- and 7-positions by a small group such as methyl or chloro led to >70-folds drop in activity against T47D cells compared to the corresponding non-substituted analog **1f**. Substitution at the 5- and 8-positions by a methyl group were somewhat tolerated with 3–7-folds reduction in activity. Substitution at the 6-position by a small chloro group resulted in compound **2c** that maintained the high activity and selectivity of **1f**. A group of compounds with substitutions at the 3- and 4-positions of the benzoyl group of **2c** were prepared and found to maintain the high activity and selectivity of **2c**. Through SAR studies of the 4- to 8-positions of 1-benzoyl-3-cyanopyrrolo[1,2-*a*]quinolines, several potent compounds such as **2c**, **3a**, **3b** and **3f** were identified, with low nanomolar potency against T47D cells in the caspase activation assay.

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