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Discovery of (naphthalen-4-yl)(phenyl)methanones and *N*-methyl-*N*-phenylnaphthalen-1-amines as new apoptosis inducers using a cell- and caspase-based HTS assay

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ABSTRACT

We report the discovery of a series of (naphthalen-4-yl)(phenyl)methanones as potent inducers of apoptosis using our proprietary cell- and caspase-based ASAP HTS assay. Through SAR studies, a group of *N*-methyl-*N*-phenylnaphthalen-1-amines also were identified as potent inducers of apoptosis. (1-(Dimethylamino)naphthalen-4-yl)(4-(dimethylamino)phenyl)methanone (**2a**), one of the most potent analogs, had EC_{50} values of 37, 49 and 44 nM in T47D, HCT116 and SNU398 cells, respectively. Compound **2a** also was highly active in a growth inhibition assay with an GI_{50} value of 34 nM in T47D cells. Functionally, compound **2a** arrested HCT116 cells in G_2/M followed by induction of apoptosis and inhibited tubulin polymerization.

Apoptosis plays an important role in promoting tissue homeostasis by eliminating damaged or excessive cells.[1] Defects in apoptosis signaling pathways could result in uncontrolled tumor cell growth as well as resistance to cancer treatment.[2] Therefore restoration of normal apoptosis or promotion of apoptosis could lead to cancer cell death as well as increase the response to chemotherapeutics.[3] In addition, it is known that many chemotherapeutics kill cancer cells through the induction of apoptosis.[4]

We therefore have been interested in the discovery and development of apoptosis inducers as potential anticancer agents and have reported the discovery of several novel series of apoptosis inducers, including 4-aryl-4*H*-chromenes (**1a**),[5] gambogic acid (**1b**),[6] 3-aryl-5-aryl-1,2,4-oxadiazoles (**1c**),[7] 4-anilino-2-(2-pyridyl)pyrimidines (**1d**),[8] *N*-phenyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-amines (**1e**)[9] and 4-anilinoquinazolines (**1f**)[10] (Chart 1), using our cell-based Anticancer Screening Apoptosis Program (ASAP) HTS assay.[11,12] Herein we report the discovery of (naphthalen-4-yl)(phenyl)methanones such as (1-(dimethylamino)naphthalen-4-yl)(4-(dimethylamino)phenyl)methanone (**2a**) as potent apoptosis inducers using our HTS assay, and SAR study of **2a** which led to the discovery of a group of *N*-methyl-*N*-phenylnaphthalen-1-amines such as N^1-(4-methoxyphenyl)-N^1,N^4,N^4-trimethylnaphthalene-1,4-diamine (**4a**) as potent apoptosis inducers.

Substituted (naphthalen-4-yl)(phenyl)methanones **2a**,[13,14] **2b**[15] and **2h** were obtained from ChemDiv and Asinex, and their struc-

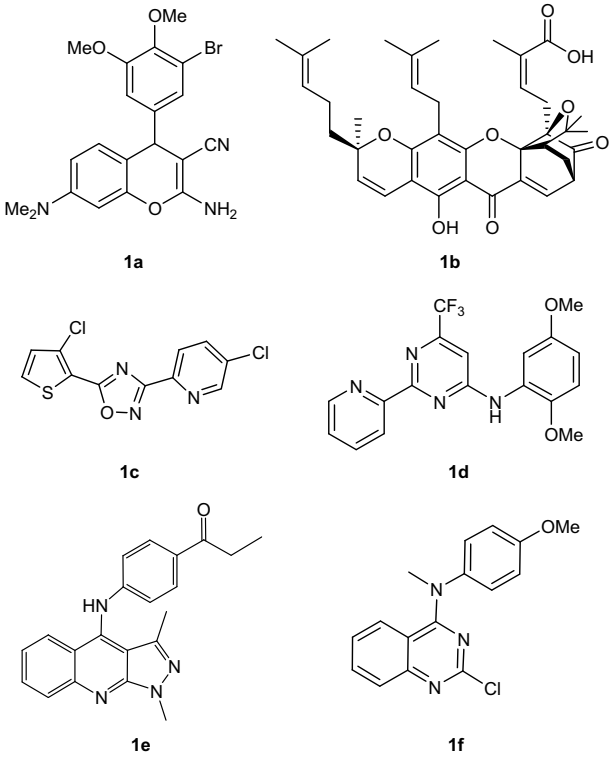

Chart 1.

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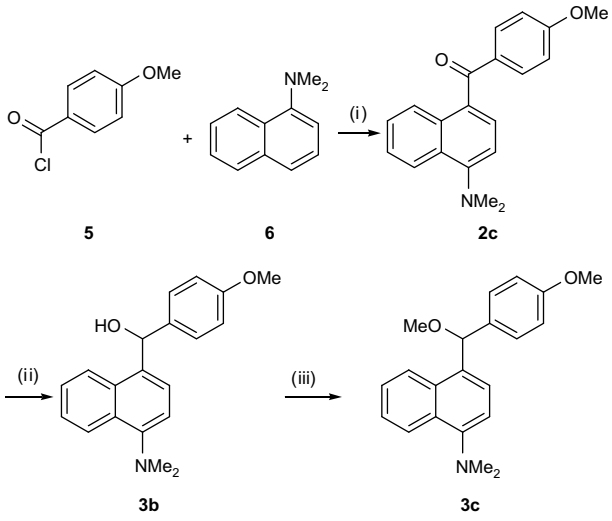
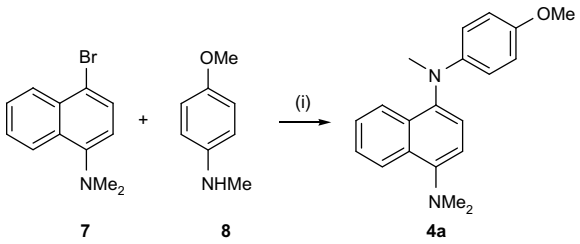
tures were confirmed by ^{1}H NMR and MS. Other substituted (naphthalen-4-yl)(phenyl)methanones were prepared as shown in Scheme 1 according to reported procedure.[13,14] For example, reaction of 4-methoxybenzoyl chloride (**5**) with 1-dimethylaminonaphthalene (**6**) in 1,1,2,2-tetrachloroethane and $AlCl_3$ produced (1-(dimethylamino)naphthalen-4-yl)(4-methoxyphenyl)methanone (**2c**). The ketone group in **2c** was reduced by $NaBH_4$ to give (1-(dimethylamino)naphthalen-4-yl)(4-methoxyphenyl)methanol (**3b**). Compound **3a**[13,14] was prepared similarly from reduction of **2a**. Reaction of **3b** with MeI in the presence of NaH produced (1-(dimethylamino)naphthalen-4-yl)(4-methoxyphenyl)methoxymethane (**3c**).

N-Methyl-*N*-phenylnaphthalen-1-amines (**4a**–**4d**) were prepared as shown in Scheme 2 according to the reported Buchwald–Hartwig procedure.[16] For example, reaction of 4-bromo-1-dimethylaminonaphthalene (**7**) with 4-methoxy-*N*-methylaniline (**8**) in the presence of $Pd(OAc)_2$/*t*-NaOBu/P(*t*-butyl)$_3$ in toluene produced N^1-(4-methoxyphenyl)-N^1,N^4,N^4-trimethylnaphthalene-1,4-diamine (**4a**).



Scheme 1. Reagents: (i) $AlCl_3$; (ii) $NaBH_4$; (iii) MeI, NaH.

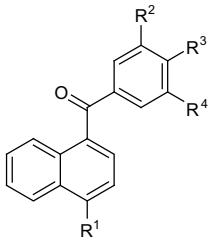


Scheme 2. Reagent: (i) $Pd(OAc)_2$/*t*-NaOBu/P(*t*-butyl)$_3$/toluene.

The apoptosis-inducing activity of substituted (naphthalen-4-yl)(phenyl)methanones and related compounds was measured using our cell- and caspase-based HTS assay[12] in human breast cancer cells T47D, human colorectal carcinoma cells HCT116 and hepatocellular carcinoma cancer SNU398 cells and the results were summarized in Table 1. Starting from **2a**, which had a potency of 37 nM in T47D cells, we first explored the replacement of one of the dimethylamino groups by a methoxy group. Compound **2b**, with the 1-dimethylamino group in the naphthalene ring replaced by a methoxy group, was about 20-fold less potent than **2a**, indicating that the 1-dimethylamino group is important for apoptosis-inducing activity. Compound **2c**, with the 4-dimethylamino group in the benzene ring replaced by a methoxy group, was only slightly less potent than **2a**, suggesting modification in the benzene ring might be more tolerated. By maintaining the 1-dimethylamino group in the naphthalene ring, we explored other substituents in the benzene ring. The 3-methoxy analog **2d** and 3,5-dimethoxy analog **2e** were about 20-fold less active than **2c**, indicating that a substituent at the 4-position might be important for apoptotic activity. The 3,4-dimethoxy analog **2f** and 3,4,5-trimethoxy analog **2g** were about 5-fold more potent than **2d**, confirming the importance of a substituent at the 4-position. The 4-nitro analog **2h** was inactive up to 10 μM, which was >10-fold less active than that of 2b, indicating that an electron-withdrawing group is not preferred.

To reduce the rigidity of the molecules and hopefully to improve the solubility profile of these compounds, we explored the

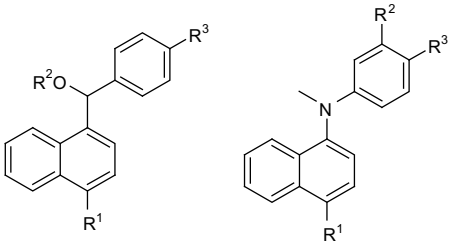
Table 1
SAR of (naphthalen-4-yl)(phenyl)methanones in the caspase activation assay



Entry	R^1	R^2	R^3	R^4	EC_{50}[a] (μM)		
					T47D	HCT116	SNU398
2a	NMe_2	H	NMe_2	H	0.037 ± 0.004	0.049 ± 0.009	0.044 ± 0.002
2b	OMe	H	NMe_2	H	0.81 ± 0.06	1.2 ± 0.1	0.98 ± 0.07
2c	NMe_2	H	OMe	H	0.072 ± 0.007	0.086 ± 0.019	0.074 ± 0.004
2d	NMe_2	OMe	H	H	2.6 ± 0.1	5.1 ± 0.2	3.5 ± 0.4
2e	NMe_2	OMe	H	OMe	1.3 ± 0.1	1.9 ± 0.4	1.5 ± 0.1
2f	NMe_2	OMe	OMe	H	0.47 ± 0.07	0.65 ± 0.03	0.56 ± 0.07
2g	NMe_2	OMe	OMe	OMe	0.42 ± 0.05	1.2 ± 0.04	0.57 ± 0.06
2h	OMe	H	NO_2	H	>10	>10	>10

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

Table 2
SAR of (naphthalen-4-yl)(phenyl)methanols and *N*-methyl-*N*-phenylnaphthalen-1-amines in the caspase activation assay



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Entry	R^1	R^2	R^3	EC_{50}[a] (μM)		
				T47D	HCT116	SNU398
3a	NMe_2	H	NMe_2	0.38 ± 0.01	0.49 ± 0.06	0.52 ± 0.04
3b	NMe_2	H	OMe	0.14 ± 0.01	0.26 ± 0.05	0.17 ± 0.02
3c	NMe_2	Me	OMe	0.59 ± 0.01	0.62 ± 0.01	0.76 ± 0.09
4a	NMe_2	H	OMe	0.51 ± 0.05	0.61 ± 0.02	0.72 ± 0.09
4b	NMe_2	OMe	H	4.4 ± 0.5	4.6 ± 0.5	4.7 ± 0.2
4c	NO_2	H	OMe	0.24 ± 0.02	0.28 ± 0.02	0.33 ± 0.09
4d	NH_2	H	OMe	12 ± 1.5	11 ± 0.3	5.3 ± 0.1

[a] Data are the mean of three or more experiments and are reported as mean ± standard error of the mean (SEM).

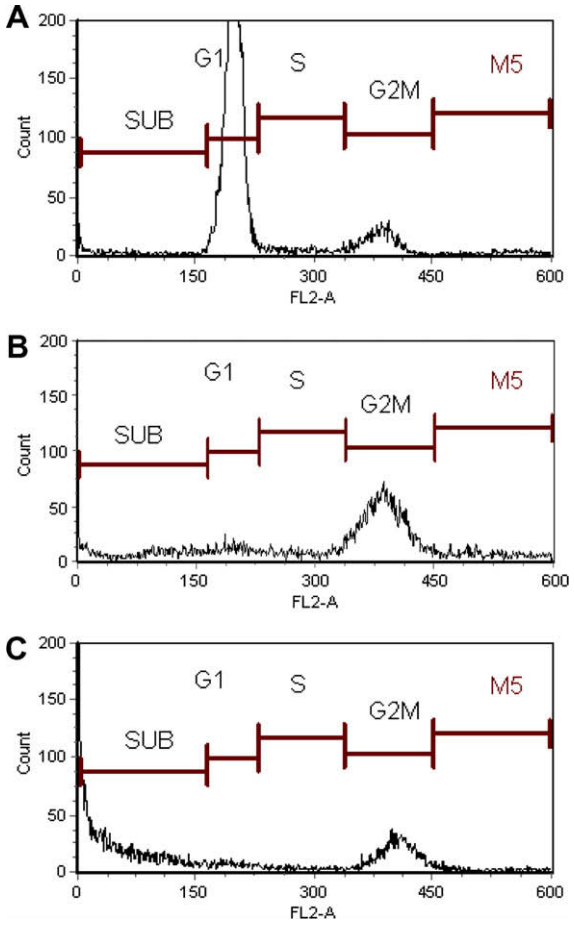
Table 3
Growth inhibition activity of (naphthalen-4-yl)(phenyl)methanones and related compounds

Entry	GI_{50}[a] (μM)		
	T47D	HCT116	SNU398
2a	0.034 ± 0.005	0.068 ± 0.017	0.027 ± 0.008
2c	0.039 ± 0.008	0.10 ± 0.035	0.027 ± 0.009
3b	0.23 ± 0.07	0.49 ± 0.10	0.17 ± 0.01
4a	0.26 ± 0.17	0.32 ± 0.11	0.15 ± 0.02

[a] Data are the mean of three experiments and are reported as mean ± standard error of the mean (SEM).

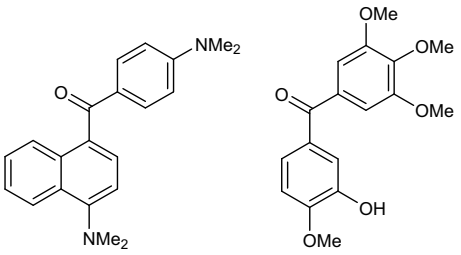
reduction of the ketone group into a hydroxy group (Table 2). Compound **3a** was about 10-fold less active than **2a**, while **3b** was about 2-fold less active than **2c**, indicating that converting the ketone group into a hydroxy group is more tolerated for the methoxy analog **2c** than the dimethylamino analog **2a**. Conversion of the hydroxy group in **3b** into the methoxy group in **3c** also was well tolerated with <3-fold reduction in potency.

Although the more flexible methanol group could be used to replace the rigid ketone group, it created a chiral center. To remove the chiral center and simplify the structure, we explored the replacement of the methanol group with a non-chiral methylamino group. The basic nitrogen could also improve the solubility



Entry	Sub	G_1	S	G_2/M
1A	4	78	5	12
1B	13	7	9	70
1C	53	5	5	37

Figure 1. Drug-induced apoptosis in HCT116 cells as measured by flow cytometric analysis. The *x*-axis is the fluorescence intensity and the *y*-axis is the number of cells with that fluorescence intensity. (A) Control cells showing most of the cells in G_1 phase (78%) of the cell cycle. (B) Cells treated with 100 nM of compound **2a** for 24 h showing most of the cells arrested in G_2/M phase (70%). (C) Cells treated with 100 nM of compound **2a** for 48 h showing a progression from G_2/M to cells with sub-diploid DNA content (53%), which are apoptotic cells with fragmented nuclei.



2a **Phenstatin**

Chart 2.

profile of these compounds. Compounds **4a** was about 3-fold less active than **3b**, indicating that a methylamino group can replace the methanol group in **3b**. The 3-methoxy analog **4b** was about 9-fold less active than the 4-methoxy analog **4a**, indicating that similar to the observed SAR for the ketones, a substituent at the 4-position is important for activity. Interestingly, the 1′-nitro analog **4c** was about 2-fold more active than **4a**. The 1′-amino analog **4d** was about 20-fold less active than the 1′-dimethylamino analog **4a**, suggesting a preference for hydrophobic group at the 1′-position.

Overall, the apoptosis-inducing activities of these compounds in human breast cancer T47D cells was similar to that observed in human colon cancer HCT116 cells and hepatocellular carcinoma cancer SNU398 cells (Tables 1 and 2). Compound **2a** and **2c**, two of the most active analogs in T47D cells, also were the most active ones in HCT116 and SNU398 cells, suggesting that compound **2a** and related analogs most probably will be broadly active against many cancer cell lines.

Selected compounds were assayed in a traditional growth inhibition (GI_{50}) assay to confirm that the active compounds in the caspase induction assay also 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[12] and the data were summarized in Table 3. Compound **2a** had GI_{50} values of 34, 68 and 27 nM in T47D, HCT116 and SNU398 cells, respectively. Compound **2c** was about as active as **2a**, and compounds **3b** and **4a** were less active than **2a** and **2c**, which were similar to what was observed in the caspase activation assay. These data confirmed that the cell-based caspase activation HTS assay is not only useful for the identification of inducers of apoptosis, but also for subsequent optimization and SAR studies.

The apoptosis-inducing activity of the potent analog **2a** was also characterized by cell cycle analysis. HCT116 cells were treated with 100 nM of compound **2a** for 24 or 48 h at 37 °C, then stained with propidium iodide and analyzed by flow cytometry (Fig. 1). An increase in G_2/M population (from 12% to 70%) was observed after 24 h treatment with **2a**, together with an increased apoptotic sub-G_1 population (from 4% to 13%). Sub-G_1 population was increased to 53% after 48 h treatment with **2a**, indicating that many cells were apoptotic. These data indicated that compound **2a** arrested cancer cells in G_2/M followed by induction of apoptosis.

Based on the characteristics of broad activity against different cancer cell lines and G_2/M arrest preceding apoptosis, as well as the structural similarity between **2a** and phenstatin (Chart 2), a well known potent inhibitor of tubulin polymerization,[17] we suspected that **2a** and related analogs might be tubulin inhibitors. In a tubulin polymerization assay,[18] the ketone compound **2a** as well as the methylamine compound **4a** were found to inhibit tubulin polymerization with an IC_{50} value of 500 nM, suggesting inhibition of tubulin polymerization as the main mechanism of action for these compounds.

In conclusion, we have identified a series of substituted (naphthalen-4-yl)(phenyl)methanones as potent apoptosis inducers. Compound **2a** was found to arrest cancer cells in G_2/M and to inhibit tubulin polymerization, which most probably is its main mechanism of action for inhibiting cell proliferation and inducing apoptosis. Through SAR studies, a group of *N*-methyl-*N*-phenylnaphthalen-1-amines also was identified as potent apoptosis inducers. Compound **2a** and **2c** were highly active in the caspase activation assay with EC_{50} values in T47D, HCT116 and SNU398 cells of 37–49 nM and 72–86 nM, respectively. Compound **2a** also was highly active in the growth inhibition assay with a GI_{50} value of 34 nM in T47D cells.

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