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Evaluation of 4-phenylamino-substituted naphthalene-1,2-diones as tubulin polymerization inhibitors

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ABSTRACT

A series of 4-phenylamino-substituted naphthalene-1,2-dione derivatives were prepared and evaluated as effective antiproliferative agents. MTT assays showed that the compounds with a methyl group on the nitrogen linker exhibited potent antiproliferative activities against human cancer cells. The mechanistic study revealed that these compounds could induce mitochondrial depolarization, which resulted in intracellular ROS production, and they also acted as tubulin polymerization inhibitors. Moreover, the typical compound could arrest A549 cells in the G2/M phase, resulting in cellular apoptosis and induced mitotic arrest in A549 cells through disrupting microtubule dynamics.

Malignant tumors, commonly known as cancer, are a major disease that is the second most serious threat to human life and health after cardiovascular disease, and cancer has become the world's leading "life killer." It is estimated that by 2020, the number of deaths worldwide may double due to cancer, and approximately 84 million people will die from cancer in the next 10 years.[1,2] With the rapid increase in cancers worldwide, the existing drugs are far from meeting the needs. Therefore, in the past several decades, many anticancer agents aimed at various targets have been developed.[3–6] Among them, tubulin polymerization or depolymerization inhibitors have attracted much attention, as tubulin-microtubule systems play a critical role for a wide range of cellular processes and represent a prominent cancer drug target. The use of taxane and vinca alkaloids to treat a variety of human cancers in the past decades are such an example.[7] At the same time, new tubulin inhibitors are also being developed. For example, MPC-6827, a quinazoline amino-substituted derivative, could effectively inhibit the polymerization of tubulin in vitro and disrupt the formation of microtubules in a variety of cancer cell lines, and has reached phase II for the treatment of recurrent glioblastoma.[8–11] Since cancer is prone to drug resistance, from the view point of both the academic and applied science realms, the discovery and development of highly effective and low toxicity candidate drug molecules for the treatment of cancer is still important.

Reactive oxygen species (ROS) are closely related to tumorigenesis and treatment. In cancer cells, growth and proliferation are encouraged under the condition of a modest rise in the intracellular ROS. On the other hand, apoptosis is also induced at higher levels of ROS. Small molecules that modulate antioxidant levels and/or enhance intracellular ROS could disturb the cellular oxidative environment and induce cell death. Thus, the utilization of ROS-inducing small molecules to target cancer has been considered as a potential strategy. The enhancement of intracellular ROS could be achieved by endogenous antioxidant inhibition, thus modulating the functions of proteins responsible for maintaining redox homeostasis in the presence of small molecule ROS.[12] Quinones are one of the major sources of ROS in cells, and many quinone derivatives, such as mitomycin C,[13] geldanamycin,[14] mitoxantrone,[15] and eoxynyboquinone,[16] have been used as anticancer agents. Recently, Potter et al. reported that β-lapachones are potent, reversible, and selective inhibitors of human liver carboxylesterase (CEs) with K_i values in the nanomolar range, which are ubiquitous enzymes that are responsible for the metabolism of xenobiotics including drugs such as the anticancer drugs irinotecan and oseltamivir.[17] Inspired by the structure and anticancer activity of compound MPC-6827, we proposed that 4-phenylamino-substituted naphthalene-1,2-diones should possess the antiproliferative activity against cancer cells. To further promote the application of these compounds in the treatment of cancer, and to investigate the structure-activity relationships, herein, we reported the evaluation of a series 4-phenylamino-substituted naphthalene-1,2-diones as antiproliferative agents as well as their mechanism of action.

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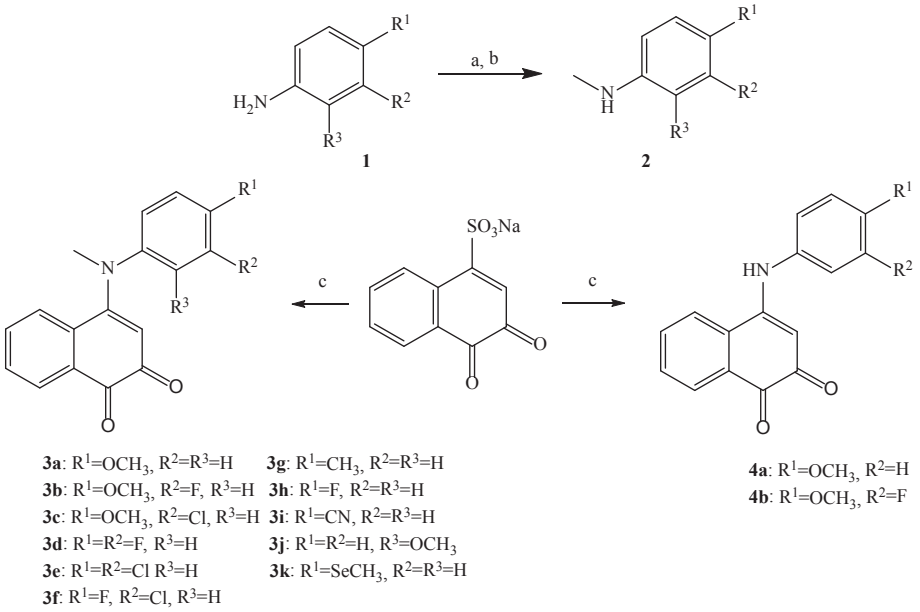

Scheme 1. The synthesis of 4-phenylamino-substituted naphthalene-1,2-diones. Reagents and conditions Reagents and conditions: (a) Na, MeOH, $(CH_2O)_n$. (b) $NaBH_4$, 60 °C. (c) **1** or **2**, H_2O, rt.

Compounds **3a**, **3g**, **3h** and **4a** were previously synthesized and evaluated as selective inhibitors of human liver CEs.[17] To study their activity of anti-tubulin aggregation, we prepared **3a–k** and **4a–b** (Scheme 1) according the method described in the same literatures.[17,18] Briefly, aniline derivatives reacted with paraformaldehyde in the presence of sodium, methanol and sodium borohydride to afford N-methyl aniline derivatives. The commercial sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonic acid reacted with the N-methyl aniline derivatives gained above or other aniline derivatives to afford target compounds **3** or **4** in water under mild conditions.

To evaluate the antiproliferative activities of the 4-(phenylamino) naphthalene-1,2-dione derivatives, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed toward five human cancer cell lines (A549, nonsmall cell lung carcinoma; HeLa, human epithelial cervical cancer cell line; HepG2, human hepatoma carcinoma cells; RKO and HCT-116, human colon cancer cell lines), and the results are listed in Table 1. Among the tested compounds, **3a** (4-((4-methoxyphenyl)(methyl)amino)naphthalene-1,2-dione) and **3b** exhibited potent antiproliferative activities with IC_{50} values ranging from 0.091 to 0.471 μM. The substitutes on the ortho position of the methoxy group, especially larger groups, seem unfavorable for the activity; **3c**, with Cl at the R_2 position, gave relatively weak activity (0.684–1.441 μM). Compounds **3d**, **3e** and **3f** containing halogen groups exhibited weak activities, which suggested that the methoxy at the 4-position of the phenylamino moiety is necessary. Compound **3g**, with methyl group at the 4-position of the phenylamino moiety exhibited good activity with 1.091–2.352 μM of the IC_{50} values. However, cyano group at the 4-position provided very poor activity (**3i**: > 10 μM of the IC_{50} values for five cancer cell lines). The position of methoxy at the phenylamino moiety is also important to the activity; **3j**, with methoxy group at the 2-position, exhibited 5.924–6.853 μM of the IC_{50} values, which was much weaker than its isomer **3a**. We previously reported the synthesis and evaluation of selenium-containing 4-anilinoquinazoline derivatives as novel antimitotic agents. Among them, the optimal compound, N,2-dimethyl-N-(4-(methylselanyl)phenyl)quinazolin-4-amine, exhibited IC_{50} values of 2–9 nM against six human cancer cell lines including A549 and Hct116.[19] However, compound **3g**, with $SeCH_3$ at the 4-position of the phenylamino moiety, provided moderate activities, with the IC_{50} values ranging from 1.005 to 3.515 μM. To examine the effect of methyl group on the linker nitrogen,

Table 1
Antiproliferative activities (μM) of **3a–3k** and **4a–4c** against five human cancer cell lines.[a,b]

	A549	HELA	HEPG2	RKO	HCT116
3a	0.097 ± 0.004	0.091 ± 0.001	0.096 ± 0.001	0.116 ± 0.010	0.471 ± 0.025
3b	0.093 ± 0.019	0.092 ± 0.014	0.101 ± 0.008	0.332 ± 0.022	0.106 ± 0.012
3c	0.684 ± 0.015	0.864 ± 0.044	0.785 ± 0.015	0.784 ± 0.059	1.441 ± 0.011
3d	2.154 ± 0.036	4.081 ± 0.324	6.546 ± 0.063	4.641 ± 0.158	6.229 ± 0.327
3e	4.933 ± 0.351	4.059 ± 0.036	5.091 ± 0. 358	2.208 ± 0.321	3.851 ± 0.253
3f	1.036 ± 0.063	1.985 ± 0.095	2.468 ± 0.117	1.447 ± 0.021	8.4245 ± 0.215
3g	1.091 ± 0.011	1.230 ± 0.012	1.295 ± 0.012	1.371 ± 0.015	2.352 ± 0.012
3h	1.63 ± 0.021	1.59 ± 0.027	1.89 ± 0.025	1.713 ± 0.031	1. 915 ± 0.023
3i	> 10	> 10	> 10	> 10	> 10
3j	6.463 ± 0.035	6.910 ± 0.032	6.751 ± 0.037	5.924 ± 0.032	6.853 ± 0.042
3k	1.003 ± 0.081	1.661 ± 0.142	3.515 ± 0.092	2.572 ± 0.157	2.242 ± 0.106
4a	0.159 ± 0.071	0.416 ± 0.056	0.776 ± 0.041	2.490 ± 0.114	2.496 ± 0.098
4b	6.632 ± 0.072	4.905 ± 0.0188	3.967 ± 0.039	8.407 ± 0.603	8.591 ± 0.548
Colchicine	0.023 ± 0.003	0.051 ± 0.006	0.046 ± 0.005	0.043 ± 0.006	0.039 ± 0.005
MPC6827	0.005 ± 0.001	0.007 ± 0.002	0.006 ± 0.002	0.005 ± 0.001	0.009 ± 0.002

[a] Cell lines were treated with the compounds for 48 h. The cell viability was measured by the MTT assay as described in the Experimental Section.
[b] IC_{50} values are indicated as the mean ± SD (standard error) of at least three independent experiments.