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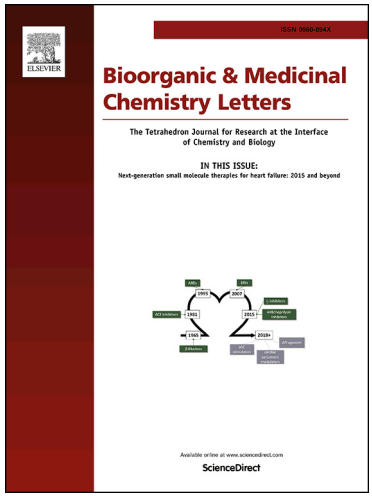

2-Substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*] imidazole-4,9-diones: design, synthesis and antiproliferative activity

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2-Substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*] imidazole-4,9-diones: design, synthesis and antiproliferative activity

Zhanxiong Liu[a], Zhenfeng Zhang[b], Wanbin Zhang[ab*], Deyue Yan[a*]

[a] *Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, and* [b]*School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, P. R. China*

Abstract: Thirty-six of novel compounds 2-substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones, bearing a *N*-(2-morpholinoethyl) group and a 2-substituted imidazole segment on a naphthoquinone skeleton, were designed, synthesized and tested as anticancer agents. Cytotoxicity was evaluated *in vitro* against three human cancer cell lines: human breast carcinoma cell line (MCF-7), human cervical carcinoma cell line (Hela), and human lung carcinoma cell line (A549); and one normal cell line: mouse fibroblast cell line (L929). Among them, the compound 2-(3-chloro-4-methoxyphenyl)-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4, 9-dione showed good antiproliferative activity against MCF-7, Hela and A549 (IC_{50} values are equal to 10.6 μM, 8.3 μM and 4.3 μM respectively) and low cytotoxicity to L929 (IC_{50} value is equal to 67.3 μM).

Keywords: naphthoquinone; imidazole; 2-morpholinoethyl; antiproliferative; anticancer agent.

[*]Corresponding authors. *E-mail addresses*: wanbin@sjtu.edu.cn (W. Zhang); dyyan@sjtu.edu.cn (D. Yan)

The quinone structural motif, especially naphthoquinone, is a very important framework and pharmacophore in a number of chemotherapy agents, such as Doxorubicin, Mitoxantrone and Alkannin, as well as in other biologically active natural products.[1] Around ten years ago, 2-morpholino ethylamino-substituted naphthoquinones[2] were designed rationally as inhibitors for the dual specificity protein hosphatase CDC25, which is considered to be a potential target for anticancer agents (Figure 1).[3-6] The above mentioned CDC25 inhibitors showed good antiproliferative activity *in vitro*. However, no experimental data was reported concerning their selectivity, which in addition to their activity is also a key criterion for anticancer agents. Recently, a 2-substituted imidazole segment has been appended to the naphthoquinone framework and the resulting compounds have shown increased selectivity[7] while retaining high activity.[7-11] Inspired by these previous works, new types of molecules, 2-substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones, possessing both a *N*-(morpholinoethyl) group and a 2-substituted imidazole segment on a naphthoquinone skeleton, were developed with the aim of achieving high antiproliferative activity as well as good selectivity. To the best of our knowledge, such species have not been reported thus far.

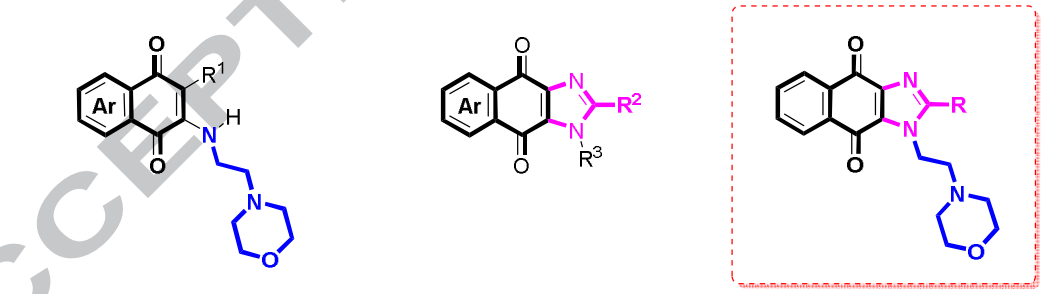
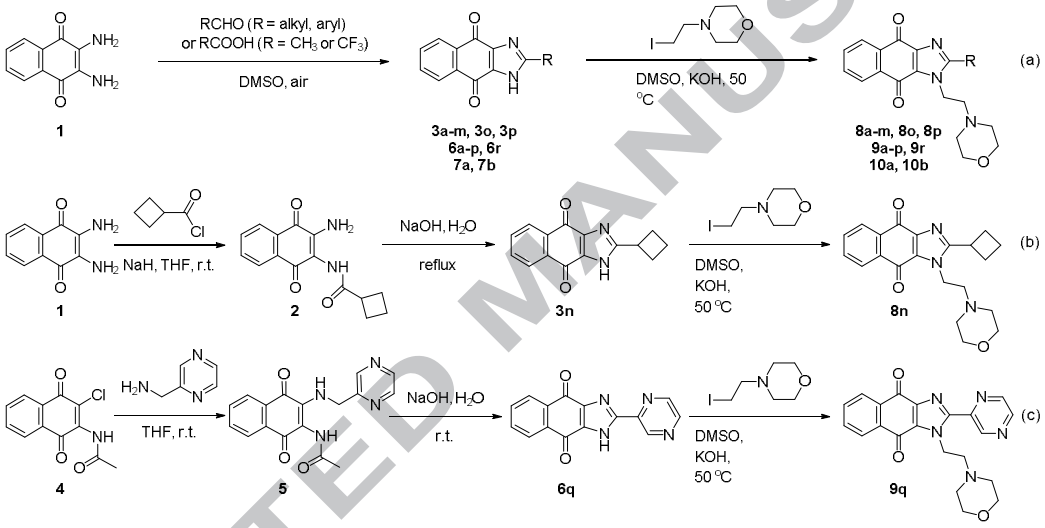


Figure 1. The design of new anticancer agents.

Most of the target compounds, 2-substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones, were prepared in two steps (Scheme 1, eq. a). In the first step, the 2-substuted-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones were obtained after a one-step

imidazole ring formation from **1**[12] and the corresponding aldehydes or acids under an atmosphere of air.[13-15] In the second step, the desired compounds were obtained via the substitution of 2-substuted-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones with 4-(2-iodoethyl)morpholine. The compounds **8n** and **9q** were obtained by other methods differing in the imidazole ring formation step. Compound **8n** was produced from **1** and cyclobutanecarbonyl chloride via amidation, imidazole ring formation and substitution (eq. b), while **9q** was prepared from the known compound **4**[6] and pyrazin-2-ylmethanamine via amination, imidazole ring formation and substitution (eq. c).



Scheme 1. Synthetic routes for the target compounds.

The desired products bearing 2-alkyl, 2-alkenyl, and 2-aryl substituents on the imidazole ring were obtained in good yields (Figure 2). It should be noted that the 2-alkyl group of **8k** and **8l** are present in the natural material 3-(4-isopropylphenyl)isobutyraldehyde and the medical intermediate 5-methylfuran-2-propionaldehyde. The diverse aryl residues at the 2-position of **9c-q** were inspired by the listed anticancer drugs Gefitinib, Tasigna, Sorafenib and Lapatinib.

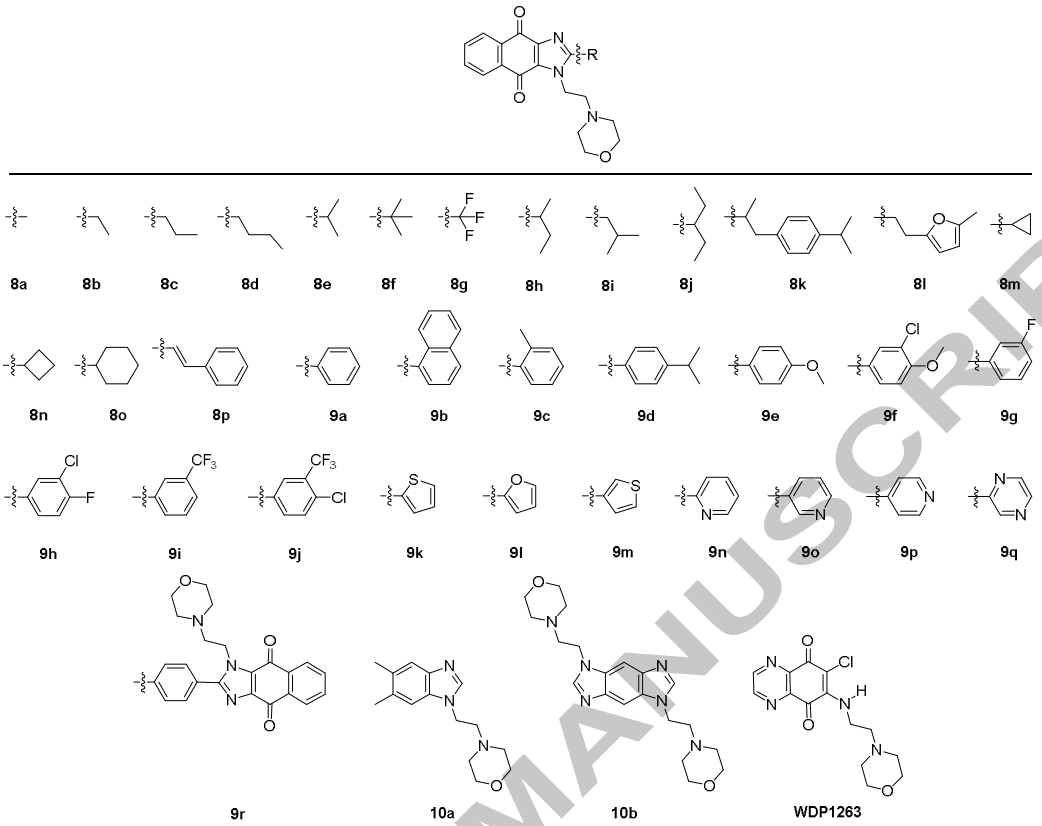
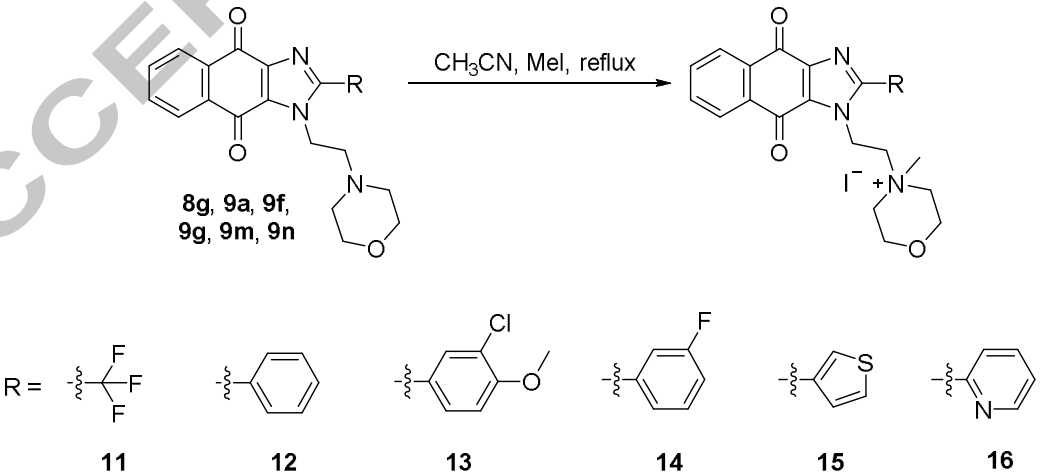


Figure 2. The tested compounds bearing an *N*-(2-morpholinoethyl) group.

In order to study the effects of the morpholino group, a number of additional compounds **11-16** were synthesized via methylation of **8g**, **9a**, **9f**, **9g**, **9m**, and **9n** (Scheme 2).



Scheme 2. Synthesis of methylated compounds **11-16**.

The structures of the target compounds were confirmed by the single crystal

diffraction of **3f** (CCDC 1827317), **9q** (CCDC 1827318) and **15** (CCDC 1827319) (Figure 3). These data are important for mechanistic biological studies.

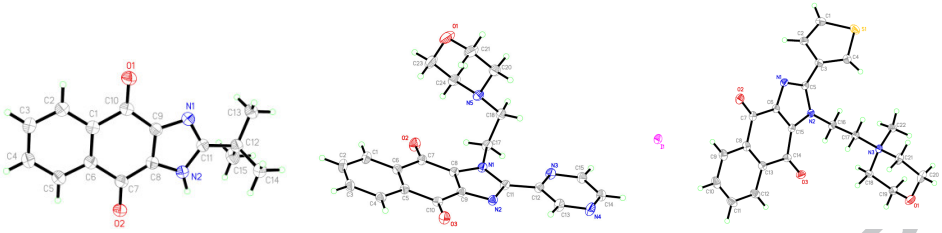


Figure 3. Molecular structure of **3f**, **9q** and **15** and hydrogen atom labelling scheme.

According to the modified method that our group previously reported,[16-18] an MTT assay was utilized to evaluate the antiproliferative activities and selectivity of the target compounds against MCF-7, Hela, A549, and L929 cells (Table 1). For the species bearing alkyl groups at the 2-position, most of these exhibited approximately 100 μM IC_{50} values for the four tested cell lines MCF-7, Hela, A549 and L929 (entries 1-15). **8f** provided moderate selectivity between cancer cell A549 and normal cell L929 (entry 6). Compound **8g** possessing an electron-withdrawing CF_3 group at the 2-position showed better antiproliferative activity than the others, but unsatisfactory selectivity (entry 7). Another compound **8m** bearing a 2-substituted cyclopropyl group also showed moderate antiproliferative activity (entry 13). Compound **8p** bearing a more electronegative 2-alkenyl substituent gave increased activity (entry 16). Most of the 2-aryl-substituted compounds **9a-r** displayed IC_{50} values below 100 μM (entries 17-34). Among them, **9c** and **9m** displayed good antiproliferative activity to A549 and a low cytotoxicity to L929 (entries 19 and 29). With regards to **9f**, an excellent balance was maintained between cytotoxicity and selectivity. Against MCF-7, Hela and A549, the IC_{50} values were 10.6 μM, 8.3 μM, 4.3 μM respectively, while very low toxicity was observed for the non-neoplastic cell L929 with an IC_{50} of 67.3 μM (entry 22). The non-quinone compounds **10a** and **10b** were also tested and showed almost no cytotoxicity to all the cells (entries 35 and 36). The positive control, WDP1263,[5] showed a good tolerance to both cancer cells and normal cell (entry 37), while, doxorubicin hydrochloride, showed good anticancer activity but low selectivity (entry 37).

Table 1 Cytotoxicity of 2-substituted-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4,9-diones.

Entry	No.	Cell Type (IC_{50}[a], μM)

		MCF- 7	Hela	A549	L929
1	8a	>200	>200	105	118
2	8b	179	144	84.9	106
3	8c	95.9	95.1	37.4	80.3
4	8d	113	166	107	>200
5	8e	123	125	32.5	79.8
6	8f	150	88.2	21.8	86.0
7	8g	17.9	15.1	15.9	16.2
8	8h	123	>200	175	100
9	8i	>200	180	25.9	157
10	8j	108	95.9	57.3	79.4
11	8k	110	122	>200	>200
12	8l	166	88.2	72.8	96.2
13	8m	64.7	61.2	36.0	40.0
14	8n	105	99.3	73.0	84.9
15	8o	>200	>200	76.4	80.3
16	8p	58.3	82.1	76.9	120
17	9a	70.1	61.4	24.5	40.6
18	9b	30.1	19.2	16.6	41.0
19	9c	116	121	23.6	80.2
20	9d	106	99.8	70.0	71.9
21	9e	86.9	112	84.5	101
22	9f	10.6	8.3	4.3	67.3

23	9g	106	133	87	131
24	9h	48.4	52.5	31.2	44.3
25	9i	134	185	33.3	72.5
26	9j	140	119	45.2	111
27	9k	137	173	91.9	177
28	9l	169	191	147	175
29	9m	115	136	20.9	122
30	9n	37.7	105	32.2	118
31	9o	167	109	37.8	44.6
32	9p	140	153	120	116
33	9q	53.5	79.1	78.3	49.6
34	9r	39.1	79.2	63.4	121
35	10a	181	>200	112	187
36	10b	>200	>200	>200	>200
37	WDP1263	>200	>200	158	149
37	Doxorubicin[b]	4.6	1.4	8.5	7.5

[a]IC_{50} values are defined as the concentrations corresponding to 50% growth inhibition for 72 h by MTT assay which were calculated from two or three independent experiments. [b]Doxorubicin hydrochloride.

Table 2 outlines the antiproliferative activity of methylated compounds **11**-**16**. Unfortunately, the antiproliferative activity of these compounds decreased tremendously compared to their corresponding starting materials (entries 1-6). The above experimental evidence illustrates the importance of the *N*-(2-morpholinoethyl) group.

Table 2. Cytotoxicity (IC_{50}) of methylated compounds **11-16**.

Entry	No.	Cell Type (IC_{50}[a], μM)			
		MCF- 7	Hela	A549	L929
1	11	139	116	93.5	>200
2	12	184	>200	>200	>200
3	13	>200	97.5	174	>200
4	14	166	166	166	>200
5	15	133	160	115	133
6	16	141	106	141	106

[a]IC_{50} values are defined as the concentrations corresponding to 50% growth inhibition for 72 h.

In summary, thirty-six of novel biological compounds bearing a *N*-(2-morpholinoethyl) group, a 2-substituted imidazole segment, and a naphthoquinone skeleton were designed, prepared, and evaluated as anticancer agents. Among them, 2-(3-chloro-4-methoxyphenyl)-1-(2-morpholinoethyl)-1*H*-naphtho[2,3-*d*]imidazole-4, 9-dione (**9f**, Figure 4) showed the most promising activity to MCF-7, Hela and A549, as well as a low toxicity to normal cell line L929. The primary research revealed that all three parts of the molecule, the *N*-(2-morpholinoethyl) group, imidazole segment, and naphthoquinone skeleton are necessary for good activity and selectivity. Further studies concerning the biological mechanism and the development of other new naphthoquinone imidazole structure-based derivatives are ongoing.

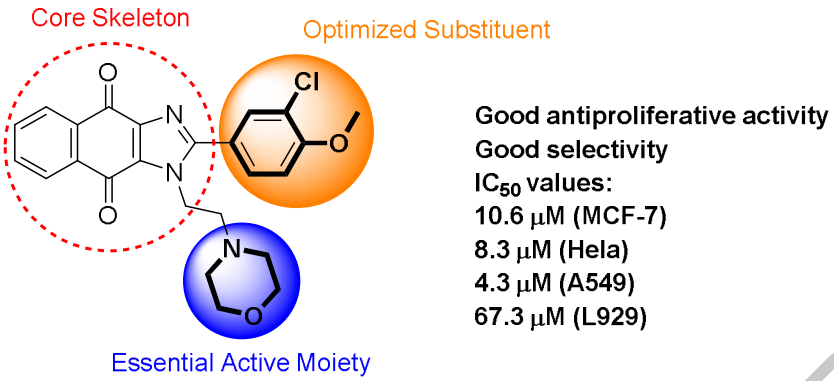


Figure 4. The optimized active compound **9f**.

Acknowledgments

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Graphical Abstract

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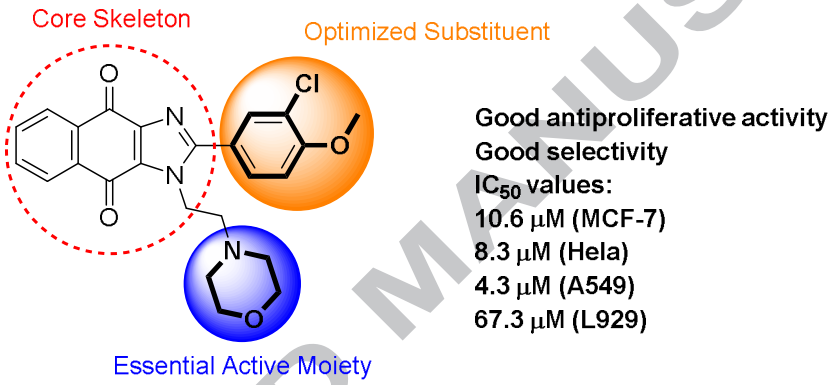

Highlights

1. 2-Substituted-1-(2-morpholinoethyl) naphthoquinone imidazoles as antitumor drugs.
2. Good cytotoxicity was observed against MCF-7, Hela and A549 cell lines.
3. Good selectivity was observed against L929 cell line.