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Design and synthesis of naphthoquinone derivatives as antiproliferative agents and 20S proteasome inhibitors

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ABSTRACT

Fourteen naphthoquinone derivatives (**1**–**14**) were designed based on a putative proteasome inhibitor **PI-083**. These compounds were synthesized and evaluated against A549, DU145, KB, and KBvin tumor cell lines. Six compounds (**2**, **4**, **8**, **9**, **10**, and **13**) showed antiproliferative activities comparable to that of **PI-083**. Among them, compound **8** was confirmed as a 20S proteasome inhibitor in both in vitro and cell-based assays. These findings endorse further optimization efforts based on this structural phenotype to develop potential anticancer drug candidates.

The ubiquitin-proteasome system (UPS) precisely regulates cellular proteolysis via enzymatic cascades that involve the distinct steps of ubiquitylation, recognition, and degradation of protein substrates. Since protein homeostasis is integral for sustaining normal cell functions, targeting components of the UPS pathway has been regarded as a feasible strategy for the treatment of various pathological conditions.[1]

As the main proteolytic component of the UPS pathway, the 26S proteasome consists of one 20S catalytic core and two 19S regulatory particles. The cylinder-shaped complex of 20S proteasome is formed by four stacked heptameric ring structures, and is responsible for the proteolytic activities. In eukaryotes, three major proteolytic activities are associated with three different β-subunits: chymotrypsin-like (β5), trypsin-like (β2), and caspase-like (β1) activities.[2]

Inhibitors of the 20S proteasome have been extensively explored as potential anti-tumor, anti-inflammatory, anti-viral, and immunosuppressive agents.[2] With the approval of the first 20S proteasome inhibitor, bortezomib, by the US FDA, proteasome has become a validated target for the development of new cancer therapy.[3] However, the problems associated with bortezomib, including severe side effects, acquired drug resistance, and unsatisfactory pharmacokinetic profiles, encourage a continued search for novel proteasome inhibitors.[4–6]

PI-083 (Fig. 1a) is a non-peptidic proteasome inhibitor reported recently. As compared to bortezomib, **PI-083** exhibited broader antitumor activity, and more notably, it was selective for malignant over normal cells either in vitro or in vivo.[7,8] Interestingly, the naphthoquinone scaffold, in particular 2-amino-1,4-napthquinone, is a structural motif widely present in natural and synthetic compounds with biological significance.[9–11] Therefore, **PI-083** and its naphthoquinone scaffold represent interesting structural phenotypes for the identification of novel antiproliferative agents and 20S proteasome inhibitors. We report herein the design,

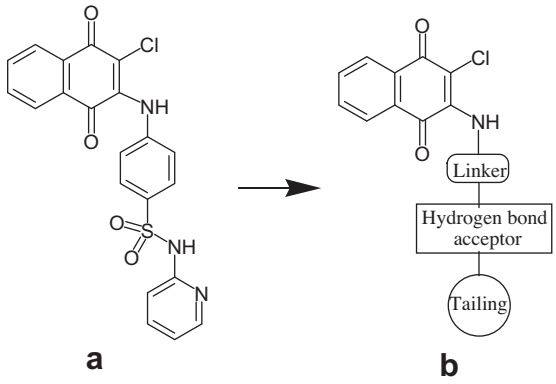

Figure 1. Structure of **PI-083** (a) and general formula of target compounds (b).

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synthesis, and biological evaluation of a series of 1,4-napthquinone derivatives.

Previous docking and structure–activity relationship (SAR) studies[8] suggested a hypothetical pharmacophore for 1,4-napthquinone proteasome inhibitors. The 1,4-napthquinone scaffold is assumed to be crucial for proteasome inhibition, and its two oxygen atoms form hydrogen bonds with Thr21 and Gly47 of 20S proteasome, respectively. Similarly, a hydrogen bond was observed between the sulfonamide group in **PI-083** and Asp114 in the β6 subunit of 20S proteasome, which implies that future structural design of new inhibitors should incorporate a hydrogen bond acceptor in this molecular area. Accordingly, 1,4-napthquinone derivatives with the general formula illustrated in Figure 1b were designed and synthesized, and structural variations on linker, hydrogen bonding acceptor, and tailing group were introduced to allow sufficient SAR study.

As shown in Scheme 1, compound **1** was designed to investigate the effect of substituting a carbonyl group for the sulfonyl group in **PI-083**, and the pyridyl group was also replaced with different tailing groups (compounds **2**–**4**) to explore the effect of this molecular region on antiproliferative activity. Compounds **5**–**7** were introduced according to the reversed amide strategy. Compounds (**8**–**11**) with diverse substituents bearing hydrogen bond acceptors, were intended to further examine SAR in this molecular area. In compounds **12** and **13**, the phenyl linker was replaced with an aliphatic linker to expand the SAR exploration and improve solubility. Compound **14** contains a weak hydrogen bond acceptor, the sulfur atom in a thienyl group, to verify the necessity for hydrogen bonding interaction.

All synthesized compounds were tested for their inhibitory activities against the growth of A549, DU145, KB, and KBvin cell lines.[12] Six compounds (**2**, **4**, **8**–**10**, and **13**) showed antiproliferative activities comparable or superior to that of the phenotype structure **PI-083** (Table 1), and the most active compound (**10**) exhibited significant inhibition against all four tested tumor cell lines, especially KBvin cells. As expected, these compounds were generally effective against KBvin cell growth, since they presumably act through a molecular mechanism other than inhibiting tubulin polymerization. Preliminary SAR on the antitumor activity of these 1,4-napthquinone derivatives could also be deduced from the data listed in Table 1. Replacing the sulfonamide group in **PI-083** with an amide group decreased the antitumor activity (**1**, **5** vs **PI-083**). The structure of the tailing group could have a considerable effect on the activity, and incorporation of additional hydrogen bond forming atoms in this group might be beneficial for the antitumor activity (**2**, **4** vs **1**, **3**). However, the activity of compound **8** (R = *p*-nitrophenyl) also indicated that a tailing group might be dispensable. In addition, an ether extended with a bulky and hydrophobic tailing group provided a favorable effect on the activity (e.g. compound **10**, R = *p*-phenoxyphenyl). The results for compound **13** implied that an appropriately substituted aliphatic linkage could also be accommodated with retention of activity.

Table 1
Inhibitory activities of selected compounds against tumor cell growth[a]

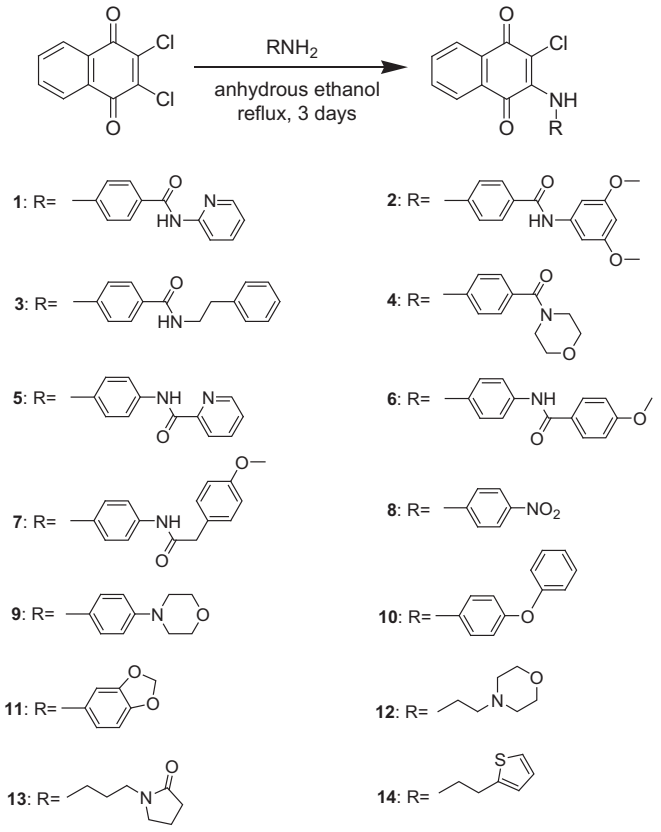
Compounds	IC_{50} (μM)			
	A549	DU145	KB	KBvin
PI-083	12.19 ± 1.09	10.82 ± 1.36	9.57 ± 3.16	11.66 ± 3.93
2	9.64 ± 3.37	10.46 ± 0.99	10.43 ± 2.89	11.26 ± 0.84
4	12.45 ± 0.71	13.68 ± 1.79	13.99 ± 3.05	14.49 ± 2.39
8	23.18 ± 3.71	18.28 ± 2.16	16.55 ± 2.25	18.34 ± 2.98
9	18.76 ± 4.64	15.83 ± 1.93	12.88 ± 0.62	14.23 ± 1.71
10	2.90 ± 0.27	4.55 ± 2.21	8.12 ± 1.20	1.30 ± 0.53
13	20.40 ± 3.70	18.12 ± 2.40	16.17 ± 4.66	19.14 ± 4.72

[a] The remaining compounds did not show significant inhibition at 10 μg/mL.

The six most active compounds were further examined for their proteasome inhibition activity. There are generally two types of assays for proteasome inhibition, in vitro and cell-based assays. These assays vary in their applicability, and inconsistent results between in vitro and cell-based assays were observed previously.[13] Therefore, it is necessary to verify the underlying mechanism of proteasome inhibition with both in vitro and cell-based assays, so as to identify those active in both assays.

A cell-based assay was first performed with HeLa-GFP cells[14], and lactacystin was tested in parallel as a positive control. Surprisingly, at the tested concentration of 10 μg/mL, only compound **8** showed noticeable activity, while **PI-083** and the remaining five compounds did not exhibit any evident activity. The lack of activity in a cell-based assay could be attributed to poor cellular permeability or susceptibility to cellular metabolism of this compound class. The discrepancy between antitumor activity and cell-based proteasome inhibitory activity also implicates that a molecular mechanism other than proteasome inhibition may be involved.

Compound **8** was further evaluated with the in vitro assay[14] for inhibition against the chymotrypsin-like activity of 20S proteasome, which is considered as a primary hallmark for proteasome



Scheme 1. Synthesis of compounds **1**–**14**.

Table 2
Inhibition of the chymotrypsin-like activity of 20S proteasome

Compounds	**8**	**PI-083**	Lactacystin
IC_{50}[a] (μM)	3.65	18.56[b]	10.09

[a] The IC_{50} values are for inhibition against the chymotrypsin-like activity of 20S proteasome, which are the averages from two independent experiments with purified 20S human proteasome.

[b] The IC_{50} value for **PI-083** was 1.0 μM in Ref. 8 However, purified 20S rabbit proteasome was used for the measurement therein.

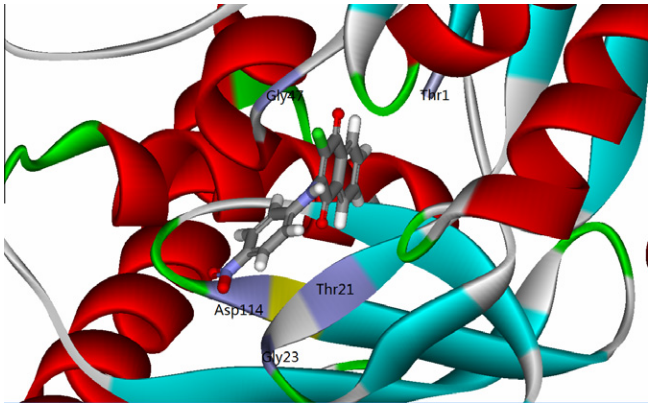


Figure 2. Interaction mode of compound **8** proposed by the docking study.

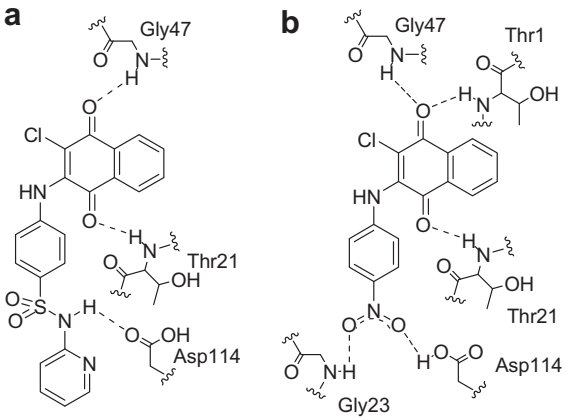


Figure 3. Schematic representation of interactions between **PI-083** and 20S proteasome (a), and between compound **8** and 20S proteasome (b).

inhibition. Notably, compound **8** was more potent than both lactacystin and **PI-083** (Table 2) in this assay.

A docking study of compound **8** with the 20S proteasome suggested that the compound situated between the β5 and β6 subunits with a binding position similar to that of **PI-083** (Fig. 2). Hydrogen bonds between compound **8** and residues Gly 47, Thr21, and Asp114 were also observed, as in the **PI-083–**proteasome complex. Remarkably, as compared to **PI-083**, compound **8** formed two additional hydrogen bonding interaction with residues Thr1 and Gly23 of 20S proteasome, which could at least partially explain its strong potency (Fig. 3).

In summary, molecular design based on a putative proteasome inhibitor **PI-083** has led to the synthesis and evaluation of novel naphthoquinone derivatives as antiproliferative agents and 20S proteasome inhibitors. Six compounds showed significant antiproliferative activities, and one of them (compound **8**) was identified as a potent proteasome inhibitor by both in vitro and cell-based assays. Further structural optimization following this molecular design is ongoing.

Acknowledgment

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Supplementary data

All target compounds were characterized by melting point, ^{1}H NMR, and mass spectra analyses. Supplementary data on general preparation procedures and spectroscopic data of the target compounds are available online. Biological assays were performed by following experimental protocols in the cited references without modification.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.086.

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