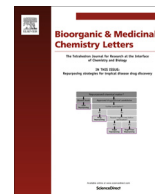




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Development of novel proteasome inhibitors based on phthalazinone scaffold

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ABSTRACT

In this study we designed a series of proteasome inhibitors using pyridazinone as initial scaffold, and extended the structure with rational design by computer aided drug design (CADD). Two different synthetic routes were explored and the biological evaluation of the phthalazinone derivatives was investigated. Most importantly, electron positive triphenylphosphine group was first introduced in the structure of proteasome inhibitors and potent inhibition was achieved. As **6c** was the most potent inhibitor of proteasome, we examined the structure–activity relationship (SAR) of **6c** analogs.

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The ubiquitin proteasome pathway (UPP) is essential for intracellular protein degradation in eukaryotic cells and is involved in many vital cellular processes such as signal transduction, apoptosis, cell cycling and antigen processing for appropriate immune responses.¹ The 26S proteasome is known as a large multicatalytic protein complex, consisting of two 19S regulatory particles and one 20S catalytic core. The active sites of the 20S proteasome are located on the β_1 , β_2 and β_5 subunits with caspase-like (C-L), trypsin-like (T-L), and chymotrypsin-like (ChT-L) activities, respectively. The proteasome is a validated drug target for treatment of cancer, and three proteasome inhibitors bortezomib, carfilzomib, and marizomib have recently been approved by FDA for the treatment of multiple myeloma. Besides, several second-generation proteasome inhibitors are currently in clinical trials.^{2,3}

In terms of the molecular structure or scaffold, current proteasome inhibitors are mainly based on peptides and peptidomimetics, which mimic the substrates binding to threonine of β_5 subunit. For instance, we previously reported a series of furan-based proteasome β_5 subunit inhibitors, which are simply di- or tri-peptide derivatives.^{4,5} However, many novel proteasome inhibitors have been developed in the past two decades and various small-molecule proteasome inhibitors with different scaffolds have been investigated.^{6–12}

Pyridazinone has been proved as a superior pharmacophore for building a broad range of chemicals with versatile functions.^{13–29}

When the C4 and C5 of pyridazinone are conjugated with a benzene ring, the π conjugation is extended and the new structure is formed as phthalazinone. Phthalazinone is another important pharmacophore contained in several drugs including Olaparib (Fig. 1a), the first poly ADP-ribose polymerase (PARP) inhibitor approved to treat advanced ovarian cancer in women with defective BRCA genes.³⁰ However, neither pyridazinone, nor phthalazinone has ever been reported in designing proteasome inhibitors.

Our group is devoted to discover proteasome inhibitors with novel scaffolds. In this study, we designed a series of proteasome inhibitors using pyridazinone as initial scaffold and extended the structure through rational design. Two different synthetic routes were explored and the biological evaluation of the phthalazinone derivatives was investigated. Most importantly, positively charged triphenylphosphine group was first introduced into a proteasome inhibitor and potent activity has been achieved.

Based on our previous results, we proposed that three important positions of the pyridazinone scaffold could be modified and the stepwise extension strategy was shown in Figure 1b. In order to occupy the small S2 pocket of the proteasome β_5 site, we introduced a benzene ring conjugated to the C4 and C5 positions of pyridazinone yielding phthalazinone. Then we introduced a *N,N*-diethylaniline group targeting S1 pocket, and a bromobutyl group extending to S3 pocket. Thus the compound **5a** was first designed and the corresponding docking model showed that **5a** was able to fully occupy all three pockets. Unfortunately, compound **5a** did not show any observable proteasome inhibition (Table 1). We speculated that perhaps the S3 pocket may have low affinity to

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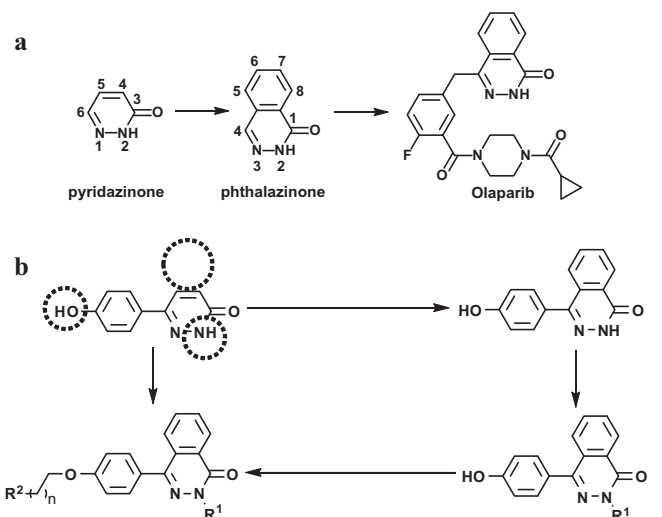


Figure 1. (a) Phthalazinone structure in drug. (b) The design of phthalazinone derivatives by stepwise extension of the pyridazinone scaffold.

the bromobutyl chain. Thus, we considered to substitute it with triphenylphosphine group, which is much more bulky, to bind to the wider S3 pocket. As we expected, the biochemical evaluation result showed that the new compound **6a** exhibited significant enhancement of proteasome inhibition with a single modification (Fig. 2).

To further optimize our design, we synthesized a series of phthalazinone **6a** derivatives, and evaluated their proteasome inhibition activity. As shown in Figure 3, we designed **7a**, **7b** and **7c** by eliminating the extended moieties of phthalazinone scaffold. As we expected, when the phthalazinone scaffold was replaced by phenyl or pyridazinone, no activity has been observed, which proved the phthalazinone structure is irreplaceable. Moreover, **7c** showed no activity that confirmed the necessary modification of phenol group by alkyl chain. Furthermore, we investigated the substituted position and length of alkyl chain. When the alkyl was linked to the *meta*-phenol of **7d**, the activity diminished due to the altered trend of alkyl chain toward the S3 pocket. In the case of **7e**, the length of alkyl chain was prolonged while the activity maintained.

With the results above, we summarized the first round SAR information. It is confirmed that both the phthalazinone and positively charged triphenylphosphine group are irreplaceable. The substituted position of alkyl on the phenol group is also crucial for the proteasome inhibition activity but the length is not important.

In the second round optimization, a series of phthalazinone derivatives were designed and synthesized with some common features including (1) retention of the phthalazinone and triphenylphosphine groups; (2) modification of 2-NH of phthalazinone by various aromatic and aliphatic groups; (3) fixation of –OH on the *para*-position of the phenyl group; (4) installation of the alkyl on the –OH group is *n*-butyl.

Depending on the different N-substitution of the phthalazinone, two synthetic routes were carried out.^{33–37} In one case, the aromatic group was introduced to the 2-NH of phthalazinone by copper(I) catalyzed C–N bond coupling reaction. As shown in Scheme 1, compound **6** can be synthesized efficiently by 5 steps. The starting material 2,3-dihydrophthalazine-1,4-dione **1** was refluxed with POBr₃ for 24 h. After cooling, the white precipitation can be filtered and subsequently refluxed in acetic acid for 2 h. After these two steps, a white product 4-bromophthalazin-1(2H)-one **2** can be filtered out for next step without purification. Suzuki coupling reaction was employed to prepare **3** with 4-hydrox-

ylphenylboronic acid. Compound **4** can be obtained by copper(I) catalyzed C–N bond coupling reaction between **3** and various aromatic halides. Next, compound **5** was easily obtained by reflux of **4** and 1,4-dibromobutane in acetonitrile. Finally, **6** was prepared by nucleophilic substitution of **5** by different nucleophiles such as triphenylphosphine. The total yields were ranging from 18% to 30%.

In another case, different aliphatic groups were introduced to the 2-NH of phthalazinone. Thus the synthetic route was altered.

Table 1
Structure and proteasome ChT-L inhibitory activity by enzyme assays^a

Compd	R ¹	R ²	Inhib. [%] ^b	IC ₅₀ [μM]
5a		–Br	NI ^c	ND ^d
6a		–PPh ₃ ⁺	99.1 ± 0.1	10.25 ± 0.45
6b		–PPh ₃ ⁺	93.2 ± 0.3	17.63 ± 0.56
6c		–PPh ₃ ⁺	99.5 ± 0.6	9.88 ± 0.21
6d		–PPh ₃ ⁺	94.3 ± 2.0	14.23 ± 0.15
6e		–PPh ₃ ⁺	98.7 ± 0.2	13.76 ± 0.22
6f		–P(Cy) ₃ ⁺	98.5 ± 1.6	13.34 ± 0.08
6g			8.6 ± 0.4	ND
6h			5.8 ± 0.6	ND
6i			10.3 ± 0.9	ND
6j		–PPh ₂ ⁺	19.6 ± 2.1	ND
6k			5.1 ± 2.0	ND
6l			8.0 ± 1.3	ND
6m			13.2 ± 2.6	ND
8a	–Me	–PPh ₃ ⁺	NI	ND
8b	–Et	–PPh ₃ ⁺	NI	ND
8c	<i>n</i> -Propyl	–PPh ₃ ⁺	12.1 ± 1.4	ND
8d	<i>n</i> -Butyl	–PPh ₃ ⁺	35.8 ± 1.1	ND
8e		–PPh ₃ ⁺	52.4 ± 0.4	26.05 ± 0.11
8f		–PPh ₃ ⁺	48.2 ± 2.8	23.05 ± 0.27
8g		–PPh ₃ ⁺	70.5 ± 0.7	21.54 ± 0.09
8h		–PPh ₃ ⁺	84.9 ± 0.5	16.14 ± 0.29
8i		–PPh ₃ ⁺	80.9 ± 2.4	11.86 ± 0.17
MG-132 ^e	–	–	–	37.0 ± 3.2 nM

^a Values represent the mean ± SD of three independent experiments, each based on four biological replicates.

^b Percent inhibition at 25 μg mL^{–1}.

^c NI: no inhibition.

^d ND: not determined.

^e *N*-(Benzyloxycarbonyl)-leucinyll-leucinyll-leucinal: a potent proteasome inhibitor, used as positive control.

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