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Design and synthesis of a novel class of furan-based molecules as potential 20S proteasome inhibitors

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Abstract—A novel class of furan-based compounds as potential 20S proteasome inhibitors have been designed and synthesized, among which nine compounds are peptide derivatives and six molecules are statine peptidomimetics. The C-terminal furanyl moiety was introduced to target molecules as furan-based amino acids. All the compounds were obtained steadily with moderate to high yield. Compound **12** was a selective moderate potent proteasome peptidomimetic inhibitor. It inhibited HepG2 and HL-60 proliferation effectively.

The 26S proteasome identified more than 20 years ago[1,2] is an unusually high molecular weight (about 700 kDa) multicatalytic protein complex.[3] The proteasome is composed of the 20S catalytic core that has a cylindrical shape with α and β subunits forming four stacking rings,[4–6] and two 19S regulatory caps which recognize protein substrates labeled with multiple ubiquitin chains[7] and facilitate the removal of the ubiquitin molecules from the substrate, and promote the unfolding of the substrate protein as it enters the central catalytic core.[8,9] Three major proteolytic activities of proteasome can be distinguished as trypsin-like (T-L), chymotrypsin-like (CT-L), and peptidyl-glutamyl peptide hydrolase (PGPH) activities which cleave peptide bonds on the carboxyl side of basic, hydro-phobic, and acidic amino acid residues, respectively.[10] The hydroxyl group on the side chain of Thr1 is the catalytic core of 20S proteasome and responsible for the catalytic cleavage of substrates through nucleophilic attack (addition-elimination mechanism).[11] Besides some essential physiological roles,[12] the proteasome is also responsible for the inappropriate or accelerated protein degradation that occurs as a result or cause of pathological conditions. One notable example is cancer, in which the unregulated proteasome-mediated degradation of cell cycle regulatory proteins[13–15] results in accelerated and uncontrolled mitosis, thereby promoting cancer growth and spread.[16] Inhibition of this enzymatic activity with β-subunit-specific proteasome inhibitors may provide an anti-tumor effect by inhibiting cell proliferation and angiogenesis, and selectively inducing apoptosis of tumor cells.[17,18]

To date, several classes of proteasome inhibitors have been described.[11,19–27] PS-341, a peptide boronic acid, was approved for cancer treatment for multiple myeloma patients by FDA of The United States In 2003.[28] However, many of these molecules cannot act as drug-like compounds due to their high toxicity, poor selectivity and difficulties of synthesis, as well as the lack of diversity of C-terminal groups. With the inspiration from the inhibition mechanism of wortmannine (Fig. 1a), a phosphatidylinositol-3 inhibitor, in which the acylfuran reacts with a nitrogen nucleophile by an addition-elimination process, producing a ring-opened Michael-adduct,[29] we choose the furanyl group as the C-terminal of our new proteasome inhibitors and dipeptide core structure **I** (Fig. 1b) as parent skeleton based on the amino acid residuals in the active site of 20S proteasome. In addition, the statine structure **II** (Fig. 1b) can exhibit a good recognition between the inhibitor and proteasome.[30] Furthermore, peptidomimetics are also more stable than peptide-based molecules in vivo and have improved transmembrane characteristics and

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Keywords: Proteasome inhibitor; Peptidomimetic; Furan; Synthesis; Antitumor.

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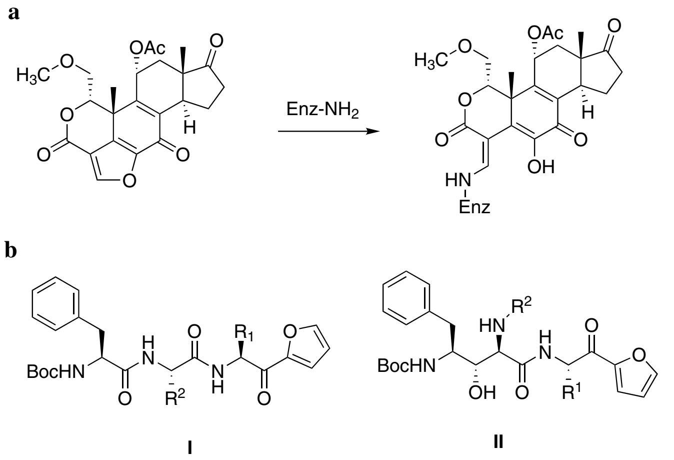
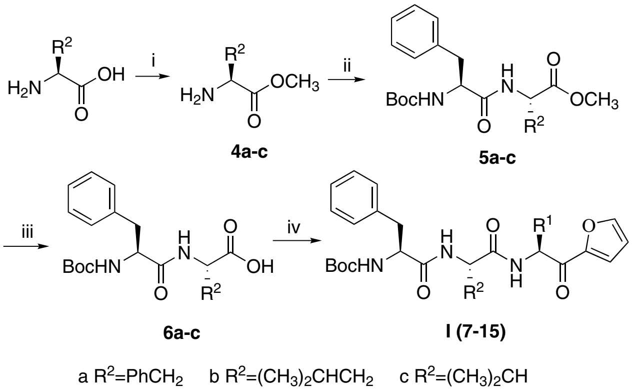


Figure 1. (a) The inhibitory mechanism of wortmannine. (b) The structures of two series of designed proteasome inhibitors.

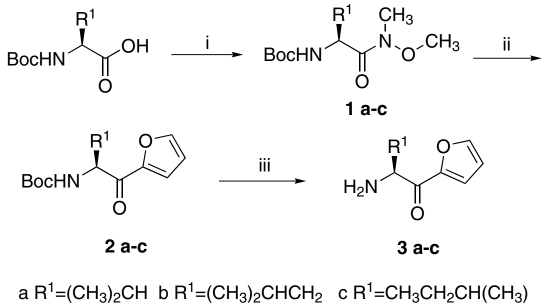
bioavailability.[19] Herein, we report the design and synthesis of two series of furan-based compounds **I** and **II** (Fig. 1b) as well as the activity evaluation of these novel classes of proteasome inhibitors.

The synthesis of the furan-based amino acids **3** began with the Boc-protected α-amino acid (Scheme 1). Weinreb amide **1** was obtained with published approach,[31] and then treated with *n*-BuLi and furan to give the Boc-protected furan-based amino acid **2** with high yield (92% for **2a**, 80% for **2b**, and 88% for **2c**). Based on the yields and TLC data, the favorable reaction temperature was −78 °C. Then, **2a–c** were deprotected with 50% TFA/CH_2Cl_2 to yield the intermediates **3a–c** which were neutralized in situ by *N*-methylmorpholine (NMM) and used for next step without further purification.

The synthetic route of the target series **I** is described in Scheme 2. The amino acids with hydrophobic side chain conducted methylation to give the methyl esters **4** which were further coupled with N-protected (*S*)-phenylalanine under typical peptide coupling conditions with the yields of 70–80% after recrystallization (ethyl acetate/petroleum ether). The obtained dipeptide esters **5** were hydrolyzed to give the acids **6**, followed by the coupling with **3** to form **7–15** (30–57% yields), which were characterized by ^1H NMR and MS. The results are summarized in Table 1.



Scheme 2. The synthesis of peptide-based inhibitors. Reagents and conditions: (i) $SOCl_2$, MeOH, rt, 5 h; (ii) Boc-Phe-OH, *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt), NMM, DMF, rt, 12 h; (iii) 1 N NaOH, THF, rt, 10 h; (iv) **3**, EDC·HCl, HOBt, NMM, DMF, rt, 12 h.

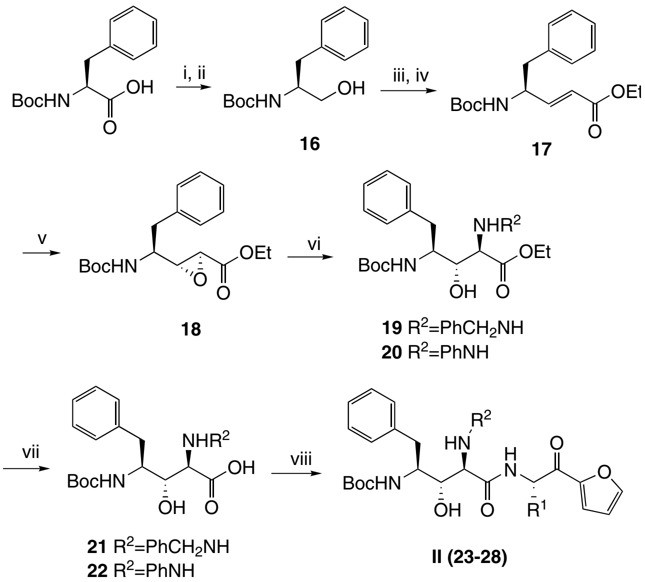


Scheme 1. The synthesis of furan-based amino acids. Reagents and conditions: (i) isobutyl chloroformate (IBCF), *N*-methylmorpholine (NMM), HCl·HN(OCH_3)CH_3, CH_2Cl_2, 0 °C, then rt, 5 h; (ii) *n*-BuLi, furan, THF, 0 °C, 30 min, then −78 °C, 2.5 h; (iii) 50% TFA (trifluoroacetic acid)/CH_2Cl_2, rt, 2 h.

The series **II** were synthesized with the approaches described in Scheme 3. Compound **16** was obtained from N-protected (*S*)-phenylalanine,[32] followed by the oxidation with Py·SO_3, the obtained aldehyde was transformed in Wittig reaction to the corresponding γ-amino-α,β-unsaturated ester **17**. The *trans* conformation of the double bond in **17** was proved by the coupling constant (J = 15.6 Hz) between the two hydrogens of alkene. Upon the treatment of γ-amino olefin **17** with *m*-chloroperbenzoic acid (*m*-CPBA), the glycidic ester **18** was obtained. During silica gel chromatography, about half of inputing **17** was recovered leading to the low yield of **18** (40%), but the conversion rate of **17** to **18** was rather high (91%). After the attack of phenylamine or benzylamine, a smooth ring-opening reaction took place to give the desired 2-phenylamino- or 2-benzylamino substituted statine derivatives **19** and **20** (87.5% and 60% yields, respectively) which were subjected to hydrolysis in the presence of THF and 1 N solution of sodium hydroxide followed by peptidyl bond formation under

Table 1. Yields and melting points of peptide-based compounds **7**–**15**

Compound	R^1	R^2	Yield (%)	Mp (°C)
7	$(CH_3)_2CH$	$(CH_3)_2CH$	39	196–197
8	$(CH_3)_2CH$	$(CH_3)_2CHCH_2$	43	165–166
9	$(CH_3)_2CH$	$PhCH_2$	30	170–172
10	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$	57	149–151
11	$(CH_3)_2CHCH_2$	$(CH_3)_2CHCH_2$	45	151–153
12	$(CH_3)_2CHCH_2$	$PhCH_2$	35	145–147
13	$CH_3CH_2CH(CH_3)$	$(CH_3)_2CH$	49	181–182
14	$CH_3CH_2CH(CH_3)$	$(CH_3)_2CHCH_2$	37	164–165
15	$CH_3CH_2CH(CH_3)$	$PhCH_2$	45	190–192



Scheme 3. The synthesis of statine-based inhibitors. Reagents and conditions: (i) CH_3I, $NaHCO_3$, DMF, rt, 5 h; (ii) $CaCl_2$, $NaBH_4$, EtOH/THF, 0 °C then rt, 5 h; (iii) $Py{\cdot}SO_3$, Et_3N, CH_2Cl_2/DMSO, −10 °C then rt, 30 min; (iv) $Ph_3PCHCOOEt$, toluene, 80 °C, 1 h; (v) *m*-CPBA, CH_2Cl_2, 5 days; (vi) benzylamine or phenylamine, 60 °C, 3 days; (vii) 1 N NaOH, THF, 10 h; (viii) **3**, EDC·HCl, HOBt, NMM, DMF, rt, 12 h.

common conditions. The yields of series **II** compounds **23**–**28** were 24–60%. The structures of **23**–**28** were confirmed by the ^1H NMR and MS data. Table 2 shows the results of series **II**.

The proteasome chymotryptic-like (ChT-L) activity inhibitory effects of compounds **7**–**15** and **23**–**28**, and also the trypsin-like (T-L), peptidyl-glutamyl peptide hydrolase (PGPH) activities of compound **12** were assayed in vitro. Due to the poor solubility of these compounds, 0.1% DMSO was used in the medium. The different peptidase activities of the proteasome were assayed using the following fluorogenic peptides: Suc-Leu-leu-Val-Tyr-AMC (Suc represents succinyl and AMC represents 7-amido-4-methylcoumarin) for chymotryptic-like (ChT-L) activity; Z-Ala-Arg-Arg-AMC (Z represents benzyloxycarbonyl) for trypsin-like activity; Z-Leu-Leu-Glu-βNA (βNA represents β-naphthylamide) for PGPH activity. MG132 (1 μM), an known inhibitor of proteasome ChT-L activity, and 0.1% DMSO were used as positive and solvent control. The results in Table 3 shows that only compound **12** had moderate potent inhibition activity ($IC_{50} = 7.85\ \mu M$). All the other compounds were not conspicuously bioactive for proteasome inhibition ($IC_{50} > 100\ \mu M$). Furthermore, compound **12** shows no inhibitory effect for T-L and PGPH subunit activity ($IC_{50} > 100\ \mu M$). Basing on these primary bioactive data, the cell growth inhibition effects of compound **12** on human hepatoma cell line (HepG2) and acute human myeloid leukemic cell line (HL-60) were further tested using a standard MTT or SRB-based colorimetric assay. IC_{50} values were 34.2 and 37.07 μM for HepG2 and HL-60, respectively.

The molecular modeling of inhibitor binding was done to compare the binding modes of MG132 and compound **12** to proteasome (Fig. 2). The distance between the furanyl group of **12** and the Thr1 amino group was 7.37 Å (Fig. 2b), which was very close to the distance from the aldehyde carbonyl group of MG132 to the Thr1 Oγ (6.03 Å, Fig. 2d). This demonstrated that the active centers of the two inhibitors entered the same pocket when they bound to proteasome. As shown in Figure 2a, the side-chain substitute groups of **12** fitted into the individual pocket of the 20S proteasome, among which the benzyl groups showed good recognition, and this gave a possible explanation to the fact that compound **12** showed inhibitory activity, and also demonstrated that the P1 and P2 substitutions of molecules

Table 2. Yields and melting points of statine peptidomimetics **23**–**28**

Compound	R^1	R^2	Yield (%)	Mp (°C)
23	$(CH_3)_2CH$	$PhCH_2$	37	147–148
24	$(CH_3)_2CHCH_2$	$PhCH_2$	60	77–78
25	$CH_3CH_2CH(CH_3)$	$PhCH_2$	30	67–69
26	$(CH_3)_2CH$	Ph	25	181–182
27	$(CH_3)_2CHCH_2$	Ph	26	206–208
28	$CH_3CH_2CH(CH_3)$	Ph	24	189–190

Table 3. Proteasome inhibition activities

Compound	IC_{50} (ChT-L)/μM
7	>100
8	>100
9	>100
10	>100
11	>100
12[a]	7.85
13	>100
14	>100
15	>100
23	>100
24	>100
25	>100
26	>100
27	>100
28	>100

[a] No inhibitory activity for T-L and PGPH subunit activity (IC_{50} > 100 μM).

were important for the potency of inhibitors. Neither valine nor isoleucine residue was suitable for the S1 pocket of β5 subunit, whereas the isobutyl group can interact with the Met45 well,[33] which partly contributed to the activity of **12**. The P2 substitution was once thought not important for the recognition between inhibitor and proteasome, but the activity loss of **10** and **11** compared to **12** showed that specific binding requirement at P2 cannot be disregarded in inhibitor design. Furthermore, the lack of acidic or basic residues in **12** led to the selectivity of CT-L activity of proteasome over the T-L or PGPH activity. According to the published literatures, statine isostere derivatives can act as good proteasome inhibitors, but the diminution of activity of **23**–**28** showed that the skeleton variation cannot be ignored when the inhibition mechanism changes (covalent vs noncovalent). Besides, the N-terminal *tert*-butoxycarbonyl group may be an unfavorable N-protecting group for the proteasome binding.

In conclusion, a new class of potential proteasome inhibitors with two different parent skeletons was designed and synthesized. The C-terminal furanyl substitution may contribute to the inhibition of the activity of 20S proteasome with unique mechanism. Except for the typical peptide coupling procedures which yielded the series of peptide-based target molecules, the synthetic routes of statine-based peptidomimetics and furan-based amino acids were also described in detail. All the reaction steps were demonstrated to be easily performed with moderate or high yield. The primary evaluation of proteasome inhibitory activity and tumor cell proliferation inhibition effect of these compounds showed that compound **12** was a selective and moderate potent proteasome ChT-L activity inhibitor, which inhibited HepG2 and HL-60 proliferation effectively.

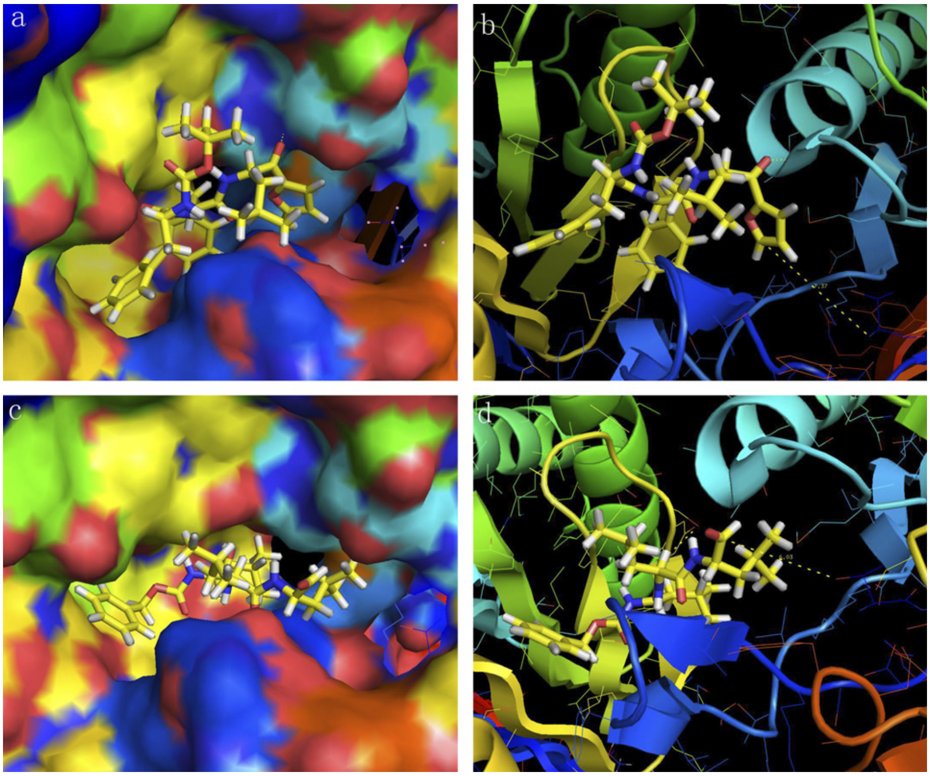


Figure 2. (a) Binding mode of compound **12**. (b) The distance between the 5′-C of furanyl group and amino group of proteasome Thr1. (c) Binding mode of MG-132. (d) The distance between the aldehyde carbonyl and proteasome Thr1 Oγ. The two inhibitors were docked into the β5 and β6 subunits of the bovine proteasome structure 1IRU using the AMBER_Parm99 force field in DOCK (UCSF, San Francisco, CA) as rendered in PyMOL (DeLano Scientific, South San Francisco, CA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.11.020.

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