Bioorganic & Medicinal Chemistry Letters 30 (2020) 127508

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of triaryl compounds as novel 20S proteasome inhibitors

Yajun Yang[a], Ke Wang[a], Bo Wu[a], Ying Yang[a], Fangfang Lai[b], Xiaoguang Chen[b], Zhiyan Xiao[a,*]

[a] Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

[b] The State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

ARTICLE INFO

Keywords:
Non-covalent
Proteasome inhibitors
Triaryl compounds

ABSTRACT

Thirty novel triaryl compounds were designed and synthesized based on the known proteasome inhibitor PI-1840. Most of them showed significant inhibition against the β5c subunit of human 20S proteasome, and five of them exhibited IC_{50} values at the sub-micromolar level, which were comparable to or even more potent than PI-1840. The most active two (**1c** and **1d**) showed IC_{50} values of 0.12 and 0.18 μM against the β5c subunit, respectively, while they displayed no obvious inhibition against the β2c, β1c and β5i subunits. Molecular docking provided informative clues for the subunit selectivity. The potent and subunit selective proteasome inhibitors identified herein represent new chemical templates for further molecular optimization.

In 2004, the Nobel Prize in Chemistry was awarded to Aaron Ciechanover, Avram Hershko, and Irwin Rose for their discovery of ubiquitin mediated protein degradation by the ubiquitin–proteasome system (UPS).[1] 26S proteasome is the main proteolytic component of the UPS, a proteolytic system precisely controlling the cellular protein homeostasis.[2–4] 26S proteasome is a highly conserved protease complex, which is consisted of the 19S regulatory particles and the 20S proteasome catalytic particle.[5–8] Three different catalytic subunits, namely β5, β2 and β1, are presented in 20S proteasome and are responsible for chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L) proteolytic activities, respectively.[9,10] The *N*-terminal Thr1 of the three catalytic subunits serves as the nucleophile to start the cleavage of corresponding peptide bonds.[11] It was reported that as compared to the β1 and β2 subunits, the β5 subunit plays a more important role in the degradation of tumor suppressor proteins, and a much more significant degradation was observed by inhibiting the β5 subunit.[10] Therefore, considerable research interests have been devoted to the development of proteasome inhibitors with CT-L inhibitory activity as cancer therapy.[12]

Three 20S proteasome inhibitors, Bortezomib (Velcade), carfilzomib (Kyprolis) and Ixazomib (Ninlaro), have been approved for the treatment of relapsed or refractory multiple myeloma (MM) in 2003, 2012 and 2015, respectively.[13] However, as covalent proteasome inhibitors, they act by forming covalent bonds with Thr1 of the catalytic subunits, which leads to poor specificity and thus severe side effects.[14–16] Non-covalent proteasome inhibitors are expected to alleviate the aforementioned drawbacks related to covalent proteasome inhibitors, and thereby have drawn more and more research interests recently.[17,18] In particular, non-covalent proteasome inhibitors with non-peptide skeletons are assumed to possess improved pharmacokinetic and safety profiles as compared to those peptidic covalent inhibitors currently available in clinical uses.[19,20]

PI-1833 is a non-covalent proteasome inhibitor initially discovered via high-throughput screening.[21] The oxadiazole-isopropylamide scaffold presented in PI-1833 was new for proteasome inhibitors, and further structural optimization of PI-1833 led to the discovery of PI-1840 (Fig. 1), a potent non-covalent CT-L selective inhibitor with in vivo antitumor activity against solid tumors.[21,22] Inspired by the novel scaffold and promising pharmacological profile of PI-1840, we attempted to explore the structure–activity relationship of this compound class as non-covalent 20S proteasome inhibitors. As illustrated in Fig. 1, the structure of PI-1840 is characterized by a unique oxadiazole-isopropylamide scaffold with three aryl moieties presenting in molecular areas A, B and C. To identify more potent and subunit selective proteasome inhibitors, two series of compounds were designed accordingly. In series 1 (**1a-1l**), the oxadiazole-isopropylamide core was maintained, while the aryl moieties in areas A and C were varied. Virtually, chemical manipulations in area A include substitution of the *n*-butyl on the phenyl and incorporation of benzoheterocyclic rings (Ar^1) to replace the phenoxy fragment. While in area C, the pyridinyl

* Corresponding author.
E-mail address: xiaoz@imm.ac.cn (Z. Xiao).

https://doi.org/10.1016/j.bmcl.2020.127508
Received 25 April 2020; Received in revised form 14 August 2020; Accepted 17 August 2020
Available online 24 August 2020
0960-894X/ © 2020 Published by Elsevier Ltd.

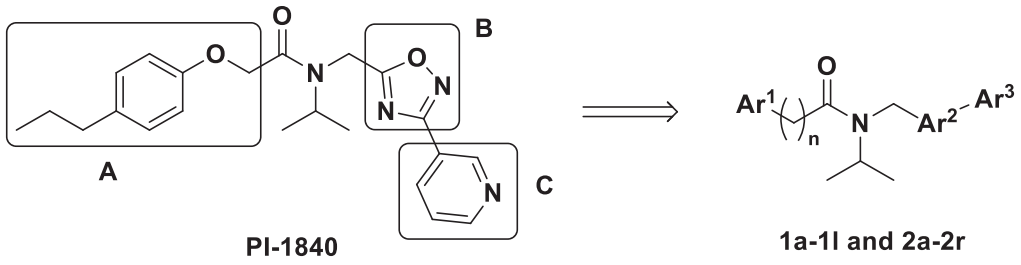
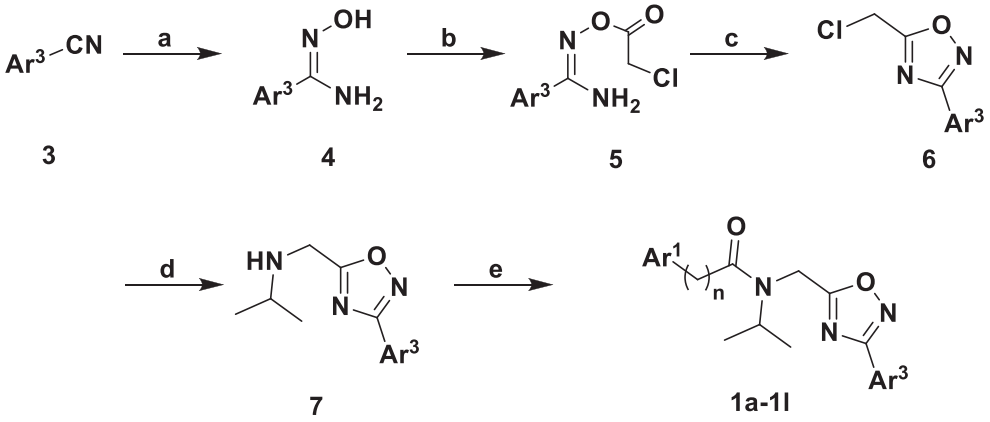


Fig. 1. Molecular design based on PI-1840.



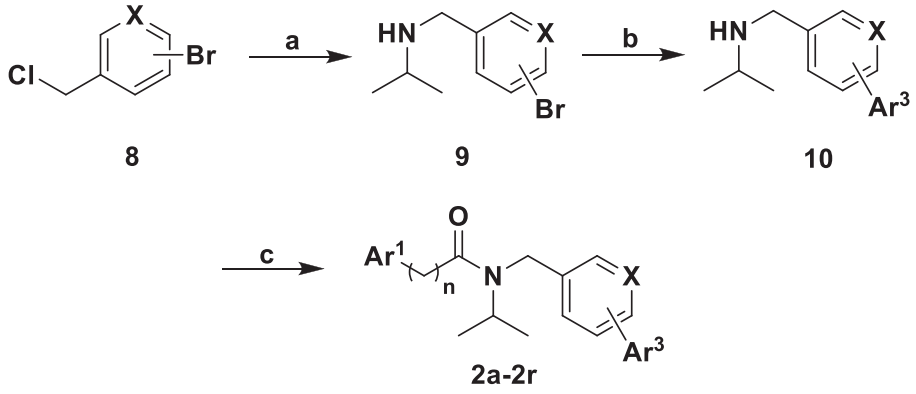
Scheme 1. Synthetic route for **1a-1l**. Reagents and conditions: (a) Na_2CO_3, hydroxylamine hydrochloride, H_2O, 70 °C, 14 h, 88%-98.8%; (b) chloroacetyl chloride, acetone, rt, 30 min, 77%-95%; (c) toluene, reflux, 2 h, 83%-98%; (d) isopropylamine, K_2CO_3, CH_3CN, reflux, 30 min, 88%-91%; (e) 1) $Ar^1(CH_2)_nCOOH$, $(COCl)_2$, toluene, reflux, 3 h; 2) Et_3N, THF, rt, 15 min, 70%-91%.

was changed to other aromatic rings (Ar^3). The structural variations in areas A and C were designed to extend the SAR presented previously, [21] and moieties with different bulky hindrance and number of heteroatoms were investigated. Besides areas A and C, the oxadiazole ring in area B was also altered to phenyl or pyridinyl (Ar^2) in series 2 (**2a-2r**) to further expand the chemical diversity of this compound class.

Two different synthetic routes were adopted to obtain the target compounds. As shown in Scheme 1, compounds **1a-1l** were synthesized from commercially available nitriles **3**.[21] Briefly, the hydroxyamidines **4** were prepared by reacting the nitriles **3** with hydroxylamine hydrochloride, and were subsequently treated with chloroacetyl chloride to yield compounds **5**. Cyclization of compounds **5** in reflux toluene afforded oxadiazoles **6**. The latter reacted with isopropylamine in reflux acetonitrile to give compounds **7**, which were further acylated to provide target compounds **1a-1l**. Alternatively, compounds **2a-2r** were obtained by following the synthetic route shown in Scheme 2. Substituted[23] benzyl chlorides **8** were reacted with isopropylamine in reflux acetonitrile to provide compounds **9**, which were subsequently reacted with aromatic boric acids to yield compounds **10**. Compounds **10** were further reacted with acyl chlorides to give target compounds **2a-2r**. The structures of compounds **1a-1l** and **2a-2r** are listed in Table 1. Similar to PI-1840 derivatives,[21] atropisomers of compounds **1a-1l** and **2a-2r** were also observed in ^{1}H NMR spectroscopy, which was attributed to the hindered rotation of the amide bond. Compound **1c** was taken as an example and subjected to variable-temperature ^{1}H NMR. The signals for the minor component in the spectrum measured at 25 °C almost disappeared in that measured at 80 °C, which confirmed the existence of atropisomers.

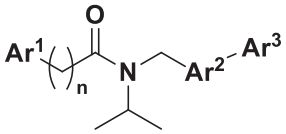
All the synthesized compounds were evaluated for their inhibitory activities against the CT-L activity of human 20S proteasome and PI-1840 was used as a reference compound. As shown in Table 1, most of the compounds showed apparent inhibition against the CT-L activity of human 20S proteasome, and thirteen of them exhibited IC_{50} values at micromolar or sub-micromolar level. The five most active compounds (**1b**, **1c**, **1d**, **1i** and **1j**) were comparable to or even more potent than the template PI-1840.

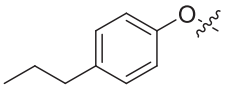
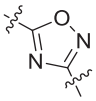
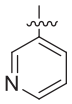
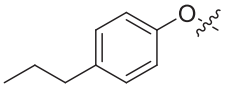
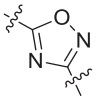
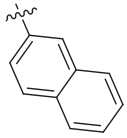
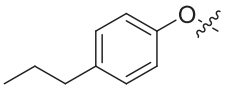
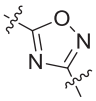
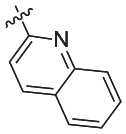
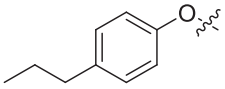
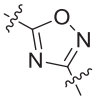
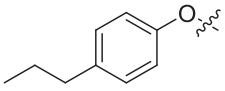
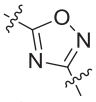
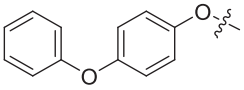
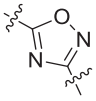
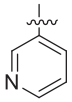
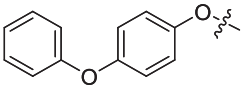
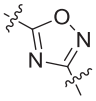
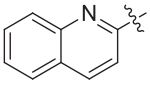
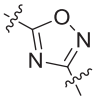
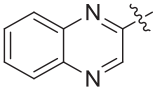
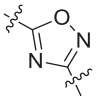
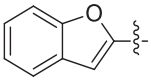
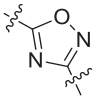
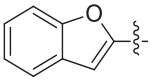
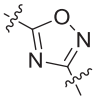
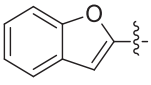
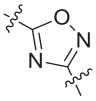
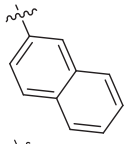
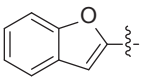
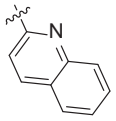
The oxadiazole-isopropylamide core and the phenyl-containing moiety in area A were initially maintained, and only the terminal



Scheme 2. Synthetic route for compounds **2a-2r**. Reagents and conditions: (a) isopropylamine, K_2CO_3, CH_3CN, reflux, 30 min; (b) $Ar^3B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 aq, toluene, ethanol, reflux under nitrogen protection, 85%-93% for a and b; (c) 1) $Ar^1(CH_2)_nCOOH$, $(COCl)_2$, toluene, reflux, 3 h; 2) Et_3N, THF, rt, 15 min, 64%-97%.

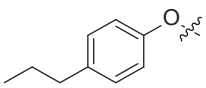
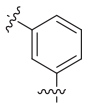
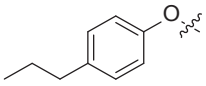
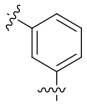
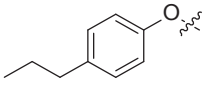
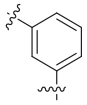
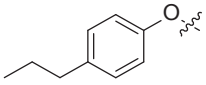
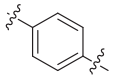
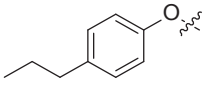
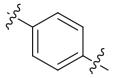
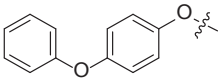
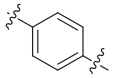
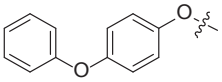
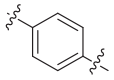
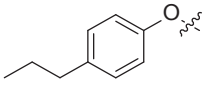
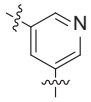
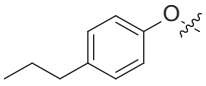
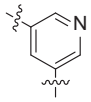
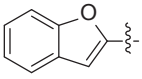
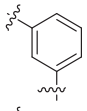
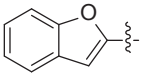
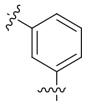
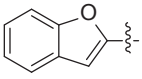
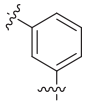
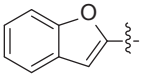
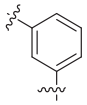
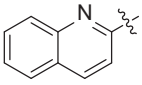
Table 1
Inhibition against the CT-L activity of human 20S proteasome by compounds **1a-1 l** and **2a-2r.**



Compd	Ar[1]	n	Ar[2]	Ar[3]	IC_{50}(μM)[1]
PI-1840[2]		1			0.63 ± 0.15
1a		1			4.31 ± 0.48
1b		1			0.88 ± 0.05
1c		1			0.12 ± 0.04
1d		1			0.18 ± 0.05
1e		1			1.61 ± 0.16
1f		1			14.7 ± 0.45
1g		0			NA[3]
1h		0			NA
1i		0			0.61 ± 0.09
1j		0			0.39 ± 0.09
1k		0			NA
1l		0			NA
2a		1			1.08 ± 0.05

(*continued on next page*)

Table 1 (*continued*)

Compd	Ar[1]	n	Ar[2]	Ar[3]	IC_{50}(μM)[1]
2b		1			2.27 ± 0.06
2c		1			10.7 ± 0.62
2d		1			9.45 ± 0.52
2e		1			1.14 ± 0.37
2f		1			33.5 ± 0.68
2g		1			1.37 ± 0.50
2h		1			NA
2i		1			NA
2j		1			NA
2k		0			20.2 ± 0.05
2l		0			NA
2m		0			47.3 ± 0.45
2n		0			NA
2o		0			61.6 ± 0.73
2p		0			3.36 ± 0.17
2q		0			NA
2r		0			NA

[1] The IC_{50} values are calculated from two independent measures.

[2] The IC_{50} value for PI-1840 was 27 nM in Ref. 21. However, purified 20S rabbit proteasome was used for the measurement therein. Similar discrepancy was previously observed by us.[24]

[3] NA (not active) represents a percentage inhibition less than 30% under the concentration of 10 μM.

Table 2
Inhibitory activities of selected compounds against CT-L, *T*-L and C-L activities of human 20S proteasome.

Compd	IC_{50} (μM)[1]		
	CT-L	T-L	C-L
1c	0.12 ± 0.04	> 50	> 50
1d	0.18 ± 0.05	> 50	> 50
2a	1.08 ± 0.05	> 50	> 50
PI-1840	0.63 ± 0.15	> 50	> 50

[1] The IC_{50} values are calculated from two independent measures.

pyridinyl was varied (compounds **1a-1d**). It looks like that the Ar^3 moiety in area C is tolerable to structural variations, while heteroaromatic groups seem to be favored (**1a** *vs* **1b**, **1c** and **1d**), especially those with a hetero-atom vicinal to the oxadiazole ring (PI-1840 *vs* **1b**, **1c** and **1d**). The *n*-butyl on the phenyl in area A of PI-1840 was then replaced with phenoxyl group (**1e**), which caused a slight decrease in the inhibition against the CT-L activity. However, the effects of such a substitution on inhibitory activities appeared to vary with different Ar^3 moieties. When Ar^3 changed to furyl (**1c**), substitution of *n*-butyl with phenoxyl (**1f**) resulted in a significant decline in the inhibitory activity. When benzoheterocyclic rings were incorporated in area A (**1 g-1l**), the inhibitory activities were generally weaker. Again, the discrepancy in activity loss for compounds with different Ar^3 was observed. The replacement of area A in PT-1840 with 2-benzofuryl only led to marginal decrease in the inhibitory activity when five-membered furyl or thienyl presented at Ar^3 (**1c**, **1d** *vs* **1i, 1j**), while for compounds with naphthyl or 2-quinolyl at Ar^3, a dramatic drop in activity was detected (**1a, 1b** *vs* **1k, 1l**). The different extent in activity loss for compounds with different Ar^3 implicates the involvement of alternative binding poses, which might be sensitive to the Ar^3 substitution.

When the oxadiazole ring in PI-1840 was replaced by *meta*- (**2a**) or *para*-phenyl (**2e**), only a slight decrease in the inhibitory activity was observed. Further substitution of the 3-pyridinyl at Ar^3 with other aromatic rings, the inhibitory activity diminished in various extent (**2b-2d** and **2f**). Compounds with *meta*- or *para*-phenyl introduced at Ar^2 (**2a-2h** and **2k-2n**) were generally less active than their analogs with an oxadiazole ring at this area. The substitution of the oxadiazole ring in PI-1840 with a pyridinyl (**2i**) was evidently unfavored, and the adverse effect of a pyridinyl at Ar^2 was further supported by the activity data of compounds **2j**, **2q** and **2r**. Interestingly, although **2e** and **2g** were comparably active, the presence of a five-membered thienyl at Ar^3 obviously made the compound more sensitive to the substitution of *n*-butyl with phenoxyl and results in significant loss of activity (**2f** *vs* **2h**), which is consistent with data obtained for series 1.

Compounds (**1c**, **1d** and **2a**) with the highest potency against CT-L activity in each series were further evaluated for their effects on T-L and C-L activities of human 20S proteasome. PI-1840 was again taken as a reference compound. As illustrated in Table 2, all the four compounds showed no significant inhibition against the T-L and CT-L activities.

The constitutive proteasome (cCP) and the immunoproteasome (iCP) are two different types of eukaryotic proteasomes. The former is expressed in all eukaryotic cells, whereas the latter is mainly expressed in cells of hematopoietic origin. PI-1840 was selective for β5c over β5i,[22] and consistently, compounds **1c** and **1d** showed no significant inhibition against the β5i subunit (with percentage inhibition of 7.3% and 25.3% at 10 μM, respectively).

Molecular docking of compound **lc** was performed to explore the molecular basis for the selective inhibition against different 20S proteasome subunits. As shown in Fig. 2, compound **lc** forms hydrogen bonds with residues Thr1 and Thr21 in the active site of the β5c subunit. Additional hydrophobic interactions with Ala20, Met45-Ala49, Gly129 and Tyr169 of the β5c subunit were also observed (Fig. 2**A**). For

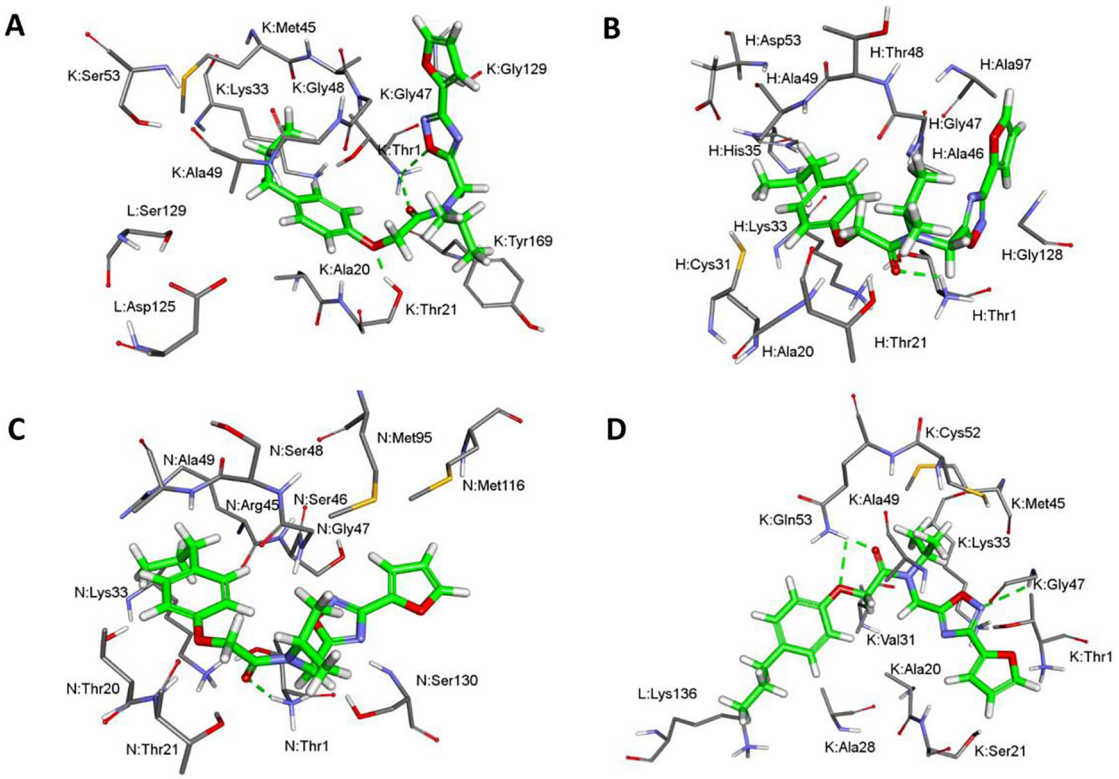


Fig. 2. The interaction modes of compound **lc** with (**A**) β5c, (**B**) β2c, (**C**) β1c and (**D**) β5i subunits as proposed by molecular docking. For cCP and iCP, the structures of 5LF3 and 6E5B were applied respectively.

Table 3
In vitro antitumor activity of selected compounds against MDA-MB-468.

Compd	IC_{50}(μM)[1]	Compd	IC_{50}(μM)[1]
1b	35.0 ± 0.06	**1j**	> 40
1c	38.8 ± 0.67	**2a**	19.3 ± 2.16
1d	27.0 ± 2.95	**2b**	30.2 ± 0.21
1e	6.8 ± 1.22	**2 g**	> 40
1i	> 40	**2p**	31.9 ± 0.63
PI-1840	7.6 ± 0.85		

[1] The IC_{50} values are calculated from two independent measures.

β2c and β1c subunits, only one hydrogen bond was detected between compound **1c** and Thr1, while interactions with the residues Asp53 (for β2c) or Arg45 (for β1c), which were believed to be critical for effective inhibition,[25] were not observed (Fig. 2**B and 2C**). To fit the active site of the β5i subunit, compound **lc** adopted a pose different from that in the active site of β5c subunit. Although hydrogen bonds are monitored between compound **lc** and residues Gly47 and Gln53, the crucial hydrogen bonds with Thr1 and Ser21 were absent (Fig. 2**D**). Molecular docking revealed the interaction modes of compound **1c** with different subunits, and partially explained the β5c selectivity of compound **lc**. However, the structural features of this compound class responsible for the subunit selectivity is still ambiguous.

PI-1840 was previously reported to inhibit the proliferation of human breast cancer cell line MDA-MB-468.[21] To further compare their biological activities of the target compounds and PI-1840, ten compounds were tested in parallel to PI-1840 against MDA-MB-468 cells using the SRB assay. As shown in Table 3, seven compounds showed apparent inhibition against the cancer cells, and compound **1e** exhibited comparable activity to PI-1840.[22]

It was observed from Tables 1 and 3 that the inhibition against CT-L activity is inconsistent with the cytotoxicity of the selected compounds and activities at the molecular level were generally more potent than those at the cellular level. Such observations imply the involvement of poor cellular permeability and/or the presence of alternative mechanisms.

In summary, thirty compounds were designed based on the known non-covalent 20S proteasome inhibitor PI-1840. With structural variations on different molecular areas of PI-1840, preliminary structure–activity relationships of this compound class as proteasome inhibitors were drawn and novel structures with IC_{50} values at the sub-micromolar level against the CT-L activity of human 20S proteasome were obtained. Compounds with β5c selectivity and cellular activity were also identified, which are worth of further optimization. However, the inconsistency between activities at molecular and cellular levels suggests poor cellular permeability or alternative mechanisms are involved.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by National Natural Science Foundation of China (21672263) and CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-I2M-3-009).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127508.

References

1. Rentsch A, Landsberg D, Brodmann T, et al. *Angew. Chem. Int. Ed.* 2013;52:5450–5488.
2. Finley D. *Annu. Rev. Biochem.* 2009;78:477–513.
3. Murata S, Yashiroda H, Tanaka K. *Nat. Rev. Mol. Cell Biol.* 2009;10:104–115.
4. Gallastegui N, Groll M. *Trends Biochem. Sci.* 2010;35:634–642.
5. Santoro AM, Cunsolo A, D'Urso A, et al. *Chem. Sci.* 2016;7:1286–1297.
6. Gräwert MA, Groll M. *Chem. Commun.* 2012;48:1364–1378.
7. Nandi D, Tahiliani P, Kumar A, et al. *J. Biosci.* 2006;31:137–155.
8. Thibaudeau TA, Smith DM. *Pharmacol. Rev.* 2019;71:170–197.
9. Borissenko L, Groll M. *Chem. Rev.* 2007;107:687–717.
10. Philipp MC, Craig MC. *ACS Cent. Sci.* 2017;3:830–838.
11. Groll M, Biochim HR. *Biophys. Acta, MolCell Res.* 2004;1695:33–44.
12. Genin E, Reboud-Ravaux M, Vidal J. *Curr. Top Med. Chem.* 2010;10:232–256.
13. Sherman DJ, Li J. *Molecules.* 2020;25:671.
14. Perfetti V, Palladini G, Brunetti L, et al. *Eur. J. Gastroenterol. Hepatol.* 2007;19:599–601.
15. Enrico O, Gabriele B, Nadia C, et al. *Bri. J. Daematol.* 2007;138:396–397.
16. Villarrubia B, Betlloch I, Mataix J, et al. *Bri. J. Dermatol.* 2007;156:784–785.
17. Marechal X, Pujol A, Richy N, et al. *Eur. J. Med. Chem.* 2012;52:322–327.
18. Huber EM, Groll M. *Angew. Chem. Int. Ed.* 2012;51:8708–8720.
19. Guedes RA, Aniceto N, Andrade MAP, et al. *Int. J. Mol. Sci.* 2019;20:5326.
20. Gallastegui N, Beck P, Arciniega M, et al. *Angew Chem. Int. Ed.* 2012;51:247–249.
21. Ozcan S, Kazi A, Marsilio F, et al. *J. Med. Chem.* 2013;56:3783–3805.
22. Kazi A, Ozcan S, Tecleab A, et al. *J. Bio. Chem.* 2014;289:11906–11915.
23. Li Z, Gelbaum C, Fisk JS, et al. *J. Org. Chem.* 2016;81:8520–8529.
24. Xu K, Xiao Z, Tang YB, et al. *Bioorg. Med. Chem. Lett.* 2012;22:2772–2774.
25. Schrader J, Henneberg F, Mata RA, et al. *Science.* 2016;353:594–598.