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Peptidyl prolyl isomerase Pin1-inhibitory activity of D-glutamic and D-aspartic acid derivatives bearing a cyclic aliphatic amine moiety

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ABSTRACT

Pin1 is a peptidyl prolyl isomerase that specifically catalyzes *cis–trans* isomerization of phosphorylated Thr/Ser-Pro peptide bonds in substrate proteins and peptides. Pin1 is involved in many important cellular processes, including cancer progression, so it is a potential target of cancer therapy. We designed and synthesized a novel series of Pin1 inhibitors based on a glutamic acid or aspartic acid scaffold bearing an aromatic moiety to provide a hydrophobic surface and a cyclic aliphatic amine moiety with affinity for the proline-binding site of Pin1. Glutamic acid derivatives bearing cycloalkylamino and phenylthiazole groups showed potent Pin1-inhibitory activity comparable with that of known inhibitor VER-1. The results indicate that steric interaction of the cyclic alkyl amine moiety with binding site residues plays a key role in enhancing Pin1-inhibitory activity.

Pin1 (protein interacting with never in mitosis A-1) is a member of the peptidyl prolyl isomerase (PPIase) family, and specifically catalyzes *cis–trans* isomerization of phosphorylated Thr-Pro or phosphoryl Ser-Pro peptide bonds in its substrate proteins and peptides. There are three subfamilies of PPIase: cyclophilins (Cyps), FK506-binding proteins (FKBPs), and purvulins. Pin1 is a member of the purvulin family and is the only enzyme that catalyzes isomerization of phosphorylated substrates in humans.[1,2] Pin1 is reported to be involved in regulation of kinase signaling processes by mediating change in the ratio of *cis*-/*trans*-conformers of phosphorylated proteins.[3] For example, signal transduction pathways involving cyclin-dependent kinases and MAP kinases, as well as cell cycle controllers, are known to be regulated by Pin1 activity.[4] Substrates of Pin1 includes cancer-related signaling proteins such as cyclin D1, NF-kappaB, and p53.[5–8] Furthermore, Pin1 is overexpressed in various types of cancer cells, including prostate cancer, rectal cancer, hepatic cancer, and esophageal cancer.[2] It was also reported that the prognosis of prostate cancer is related to the expression level of Pin1 in the cancer cells.[9] Based on these reports, Pin1 may be a new therapeutic target for these cancers. Pin1 is also involved in the pathogenesis of Alzheimer's disease by isomerizing phosphorylated tau proteins, resulting in a reduction of tau-dependent fibril formation. Thus, Pin1 catalyzes a unique reaction,

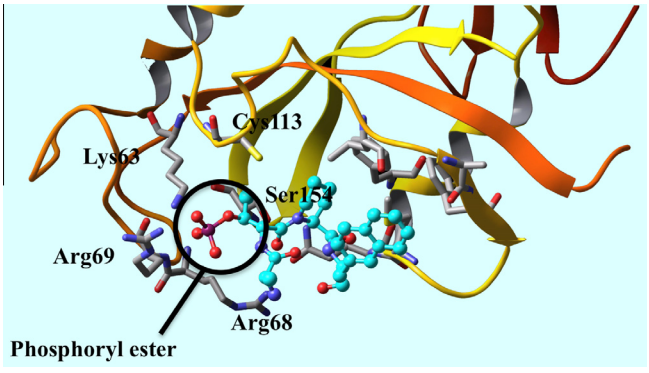

Figure 1. Structure of Pin1 catalytic domain with D-peptide (PDB 2ITK).

and it is also considered to contribute to the temporal regulation of protein phosphorylation, acting like a 'molecular timer'.[10] The catalytic domain of Pin1 enzyme, containing the cation-recognition site, consists of Lys63, Arg68, and Arg69, which serve to stabilize the phosphoryl moiety of the substrate peptide via electrostatic effect[11] (Fig. 1).

Several Pin1 inhibitors (**1**–**7**) have been reported to date, as illustrated in Figure 2.[11–19] Very recently, it was reported that all-trans retinoic acid (ATRA) (**8**) is also an inhibitor of Pin1.[19] Among these inhibitors, compound **4** has a very high affinity for the Pin1 catalytic

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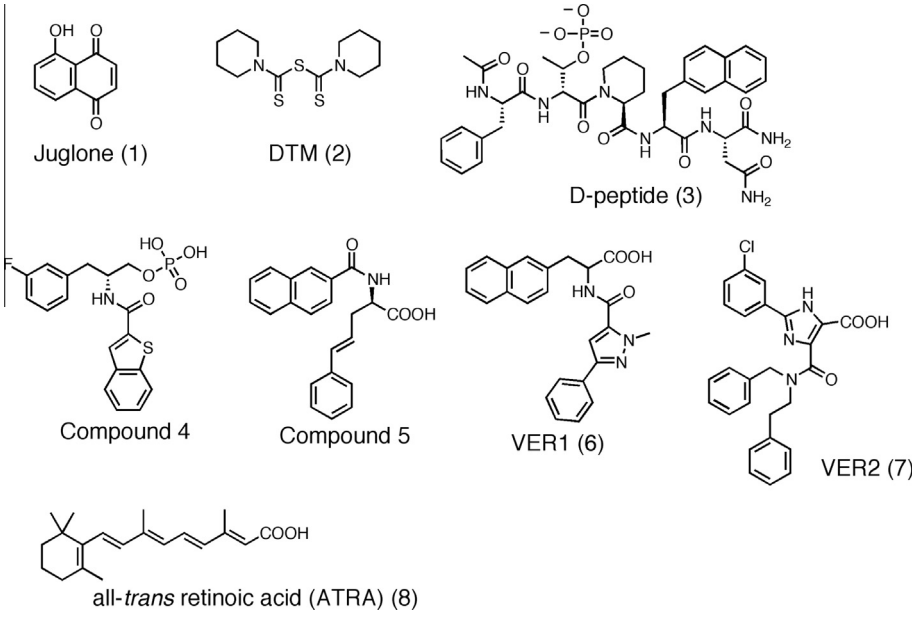


Figure 2. Previously reported Pin1 inhibitors.

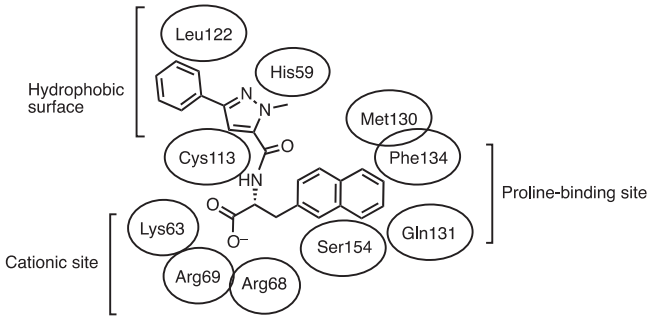


Figure 3. Schematic illustration of the interaction of Pin1 catalytic site and VER1.

site, but is not applicable to cell-based systems due to its high hydrophilicity arising from the presence of the phosphonate group. On the other hand, inhibitors bearing a carboxyl group, instead of a phosphonate group, such as VER1 (**6**) and VER2 (**7**) are applicable to cells, and show cell growth-inhibitory activity.

Structural analysis of VER1–Pin1 complexes has shown that VER1 binds to Pin1 mainly through three interactions: ionic interaction of the carboxylate group of VER1 with the cationic pocket in the Pin1 catalytic site, hydrophobic interaction of the aromatic group of VER 1 with the proline-binding pocket of Pin1, and interaction of another aryl group of VER1 with the hydrophobic surface of the Pin1 catalytic site (Figs. 3 and S1).

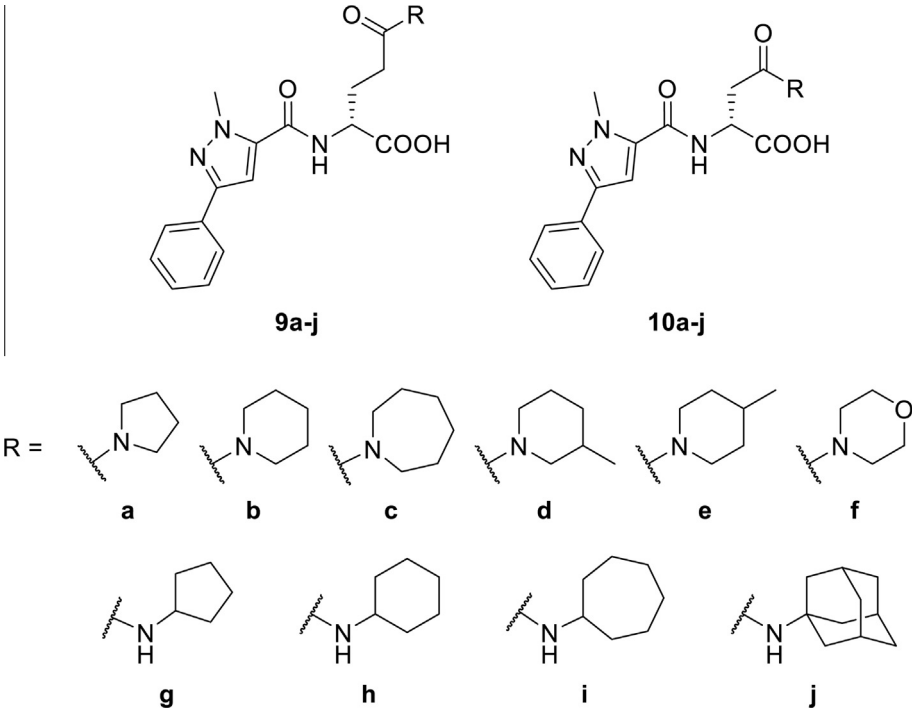
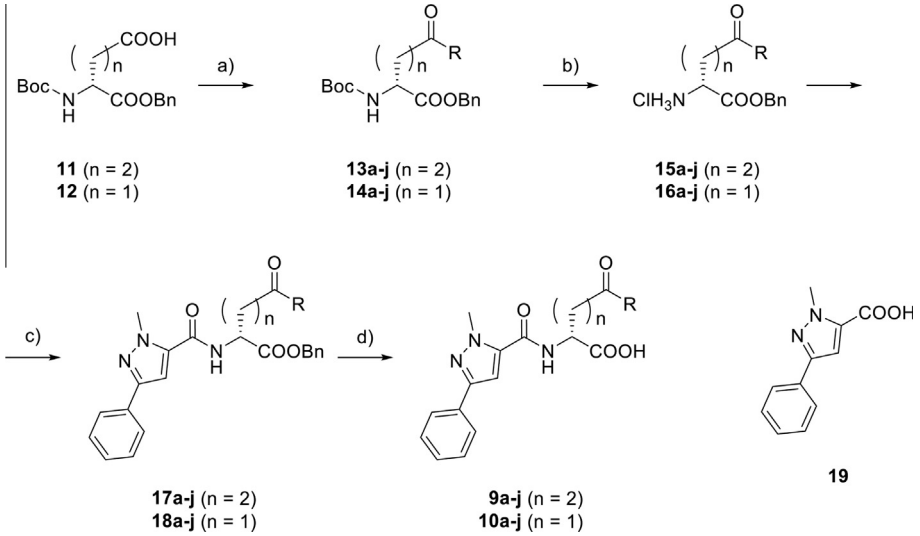


Figure 4. Molecular design for Glu/Asp-scaffold Pin1 inhibitor candidates.



Scheme 1. Synthesis of compounds **9** and **10**. Reagents and conditions: (a) amine **a**–**j**, EDCI. HOBt, DMF; (b) HCl, dioxane; (c) **19**, EDCI, HOBt, NEt_3, DMF; (d) LiOH, H_2O, MeOH, THF.

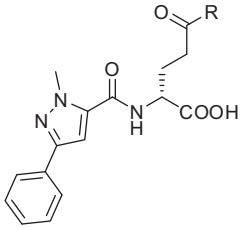
To develop new Pin1 inhibitors, we focused on interactions within the proline pocket of Pin1. In the complex of VER1 and Pin1, the pocket interacts with the naphthyl group of VER1, but it is not completely filled by the planar aromatic group. Therefore, we explored sterically bulkier aliphatic groups, in place of the naphthyl group, and designed potential inhibitors, **9a**–**j** and **10a**–**j**, bearing various cyclic or cycloalkylamine moieties (Fig. 4).

The synthetic procedures are summarized in Scheme 1. Compounds **9a**–**j** were derived from D-glutamic acid, and **10a**–**j** were derived from D-aspartic acid. Boc-D-Glu-OBzl (**11**) or Boc-D-Asp-OBzl (**12**) was subjected to condensation reaction with various amines in DMF, using EDCI and HOBt. The Boc moiety of the resulting amide (**13a**–**j**, **14a**–**j**) was deprotected with HCl-containing dioxane. The obtained amine (**15a**–**j**, **16a**–**j**) was condensed with 1-methyl-3-phenyl-1*H*-pyrazole-5-carboxylic acid (**19**), and then hydrolyzed with LiOH to afford compounds **9a**–**j** and **10a**–**j** (Table S1).

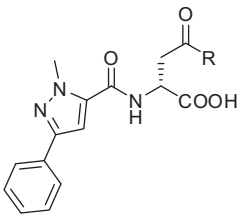
The Pin1-inhibitory activity of the synthesized compounds was evaluated by means of the proteinase-coupled assay method.[20] Briefly, 250 μM synthetic substrate peptide (suc-Ala-Glu-Pro-Phe-pNA) was preincubated with 0.2 mM DTT, 100 μg/mL BSA, and 22 nM Pin1 in 150 μL of 35 mM HEPES-KOH (pH 7.8) for 10 min at 10 °C after adding the indicated concentration of test compound (as a DMSO solution; the final DMSO concentration was 5% v/v). C-terminal hydrolysis of the substrate peptide was initiated by addition of an excess amount of α-chymotrypsin (150 μL of 0.8 mg/mL protease in 35 mM HEPES-KOH, pH 7.8). The absorbance of the released p-nitroaniline (pNA) at 390 nm was recorded for 10 min with a spectrophotometer. Initially, α-chymotrypsin rapidly digested the substrate peptides present in the *trans* form (rapid phase), and then subsequently hydrolyzed the peptides as they were slowly isomerized from *cis*- to *trans*-form by Pin1 (isomerization phase). The observed reaction rate of the isomerization phase was thus taken as the Pin1 activity. The inhibitory activity was then expressed as $((k_{(inh)} - k_0)/(k_{(noinh)} - k_0)) \times 100$ (%), where $k_{(inh)}$ is the observed pseudo-first order rate in the presence of an inhibitor, $k_{(noinh)}$ is that without inhibitor, and k_0 is that in the absence of Pin1.

The synthesized compounds were tested for Pin1-inhibitory activity in the range of 1 to 200 μM, and IC_{50} values were calculated (Table 1). As the positive control, VER1 was also synthesized as reported[16] and evaluated. Among the compounds with various amine moieties, **9f** and **10i** showed moderate to good inhibitory activity. Among the Glu-derived compounds, the compounds bearing small cyclic amines or large cycloalkylamines were ineffective (**9a**–**c**, **e**, **g**, **i**, **j**). As for Asp-derived compounds, those bearing smaller amines (**10a**–**d**, **g**) showed poor activity, and the morpholino derivative (**10f**) was also ineffective.

Table 1
Pin1-inhibitory effect of the synthesized compounds **9** and **10**



9a-j



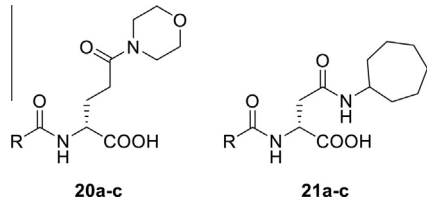
10a-j

Compd	R	IC_{50} (μM)
9a	Pyrrolidin-1-yl	>100
9b	Piperidin-1-yl	>100
9c	Homopiperidin-1-yl	>100
9d	3-Methylpiperidin-1-yl	39
9e	4-Methylpiperidin-1-yl	>100
9f	Morpholin-1-yl	10
9g	Cyclopentylamino	>100
9h	Cyclohexylamino	77
9i	Cycloheptylamino	>100
9j	Adamant-1-ylamino	>100
10a	Pyrrolidin-1-yl	>100
10b	Piperidin-1-yl	>100
10c	Homopiperidin-1-yl	>100
10d	3-Methylpiperidin-1-yl	>100
10e	4-Methylpiperidin-1-yl	81
10f	Morpholin-1-yl	>100
10g	Cyclopentylamino	>100
10h	Cyclohexylamino	67
10i	Cycloheptylamino	18
10j	Adamant-1-ylamino	91
VER1 (**6**)		4.2 (3.9[a])

[a] Ref. 16.

Structural analysis of the VER1 and Pin1 complex indicates that the distance between the proline pocket and the cation site is important, suggesting that the cyclic amine or cycloalkylamine moiety on the Glu scaffold would be too long to interact with the

Table 2
Pin1-inhibitory effect of derivatives of **9f** and **10j**



Compd	R	IC_{50} (μM)
20a	Naphth-2-yl	83
20b	Benzofuran-2-yl	91
20c	2-Phenylthiazol-4-yl	5.4
21a	Naphth-2-yl	>100
21b	Benzofuran-2-yl	>100
21c	2-Phenylthiazol-4-yl	4.3

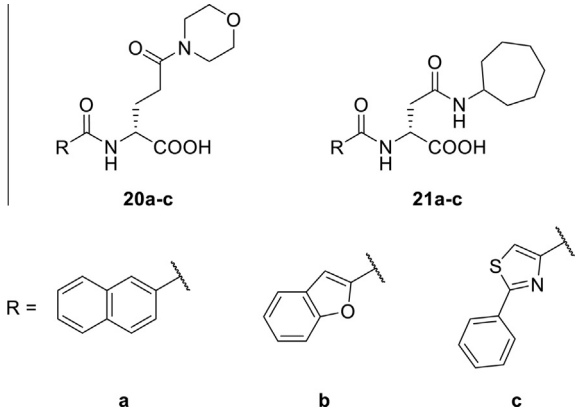
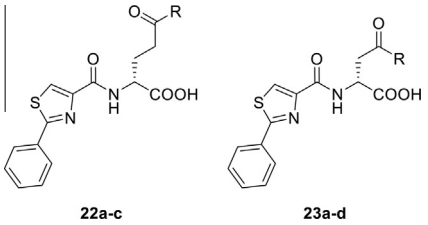


Figure 5. Synthesized derivatives of **9f** and **10i**.

two important binding sites, and those with the Asp scaffold would be too short. As for the ring size, a 7-membered cyclic amine or cycloalkylamine moiety was more effective than a 6- or 5-membered one, suggesting that it may match well the steric size of the proline pocket. This is consistent with a recent report that

Table 3
Inhibitory effect of the derivatives of **20c** and **21c**



Compd	R	IC_{50} (μM)
22a	3-Methylpiperidin-1-yl	19
22b	Azocan-1-yl	>50
22c	Cyclooctylamino	>50
23a	Cyclohexylamino	>100
23b	4-Methylpiperidin-1-yl	14
23c	Azocan-1-yl	3.0
23d	Cyclooctylamino	>100

ATRA showed good inhibitory activity toward Pin1, considering the distance between its carboxylic acid and aliphatic ring structures.[19]

The proteinase-coupled assay revealed that the compounds bearing a phenylthiazole moiety (**20a–c** and **21a–c**) showed inhibitory activity comparable to that of VER1 (Table 2). The phenylthiazole moiety was assumed to interact favorably with the hydrophobic surface of the enzyme in the case of compounds bearing an alicyclic moiety. This aryl moiety has not previously been used as a hydrophobic interacting group in small-molecular Pin1 inhibitors, so this result means that phenylthiazole compounds may be useful tools to explore the motif interacting with the hydrophobic surface of the enzyme pocket, in combination with appropriate proline-pocket-binding groups. Although the benzofuran moiety was expected to interact with residues such as Cys113 or Ser114, it did not appear to be effective. The naphthyl group has been used for hydrophobic interaction in compound **5**, another effective Pin1 inhibitor, but seems ineffective in our inhibitor scaffold (Fig. 5).

Next we explored combinations of proline pocket-binding aliphatic groups with the phenylthiazole moiety to examine the structure–activity relationship. We synthesized derivatives of **20c**

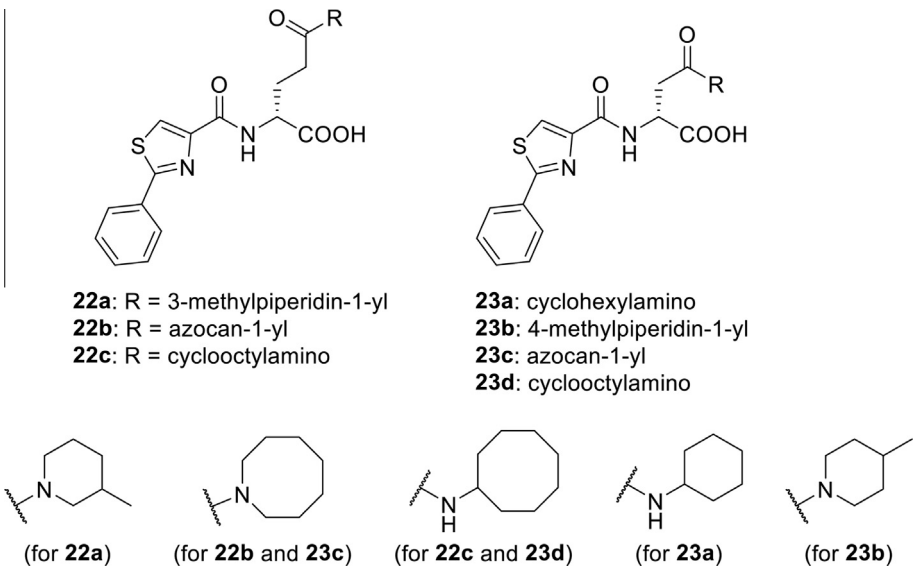


Figure 6. Synthesized derivatives of **20c** and **21c**.

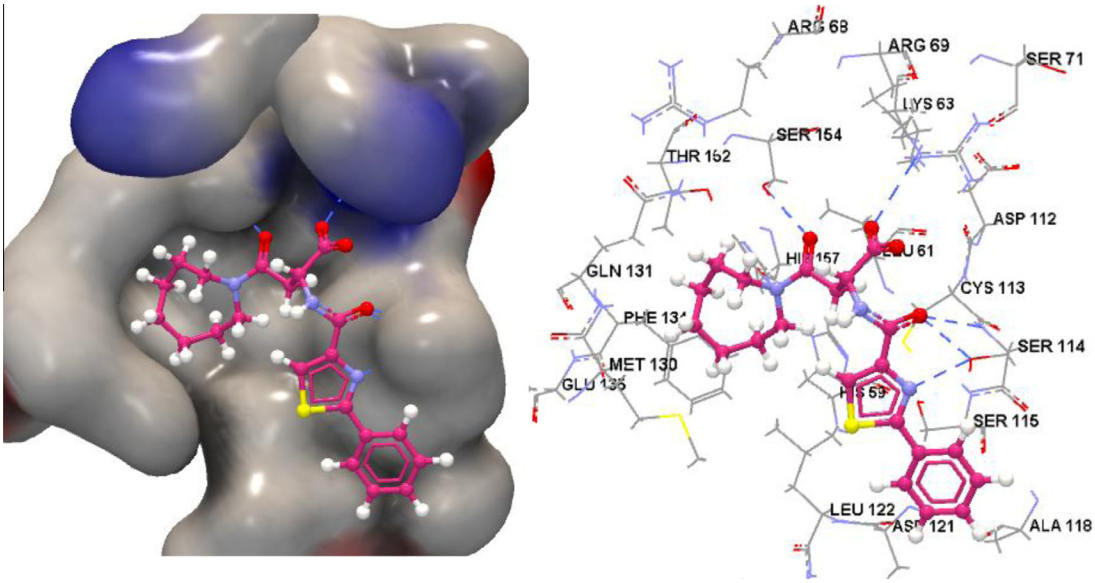


Figure 7. Docking model of compound **23c** with Pin1 catalytic site (Docking stimulation was conducted with CLC Drug Discovery Workbench (Filgen)) based on PDB 3KAI.[16]

and **21c** with different ring size and scaffold length (**22a–c** and **23a–d**, Fig. 6). These compounds were obtained by the same method shown in Scheme 1, using corresponding cyclic amines or cycloalkylamines. The proteinase-coupled assay revealed that the combination of a 1-azocanyl group and the Asp scaffold (**23c**) was as potent as VER1 (Table 3). It seems likely that appropriate folding of the azocane moiety enabled it to fill the bulky proline pocket (Fig. 7). In the Glu scaffold series, cycloalkylamino and cycloalkylimino moiety (**22a–c**) resulted in less active than morpholino moiety (**20c**) unlike the case of Asp scaffold. The morpholino moiety may have additional interaction(s) with polar residues in the relatively hydrophobic pocket.

In conclusion, we explored aliphatic cyclic amines and cycloalkylamines as proline-pocket binding motifs on a glutamic acid or aspartic acid scaffold, and discovered unique Pin1 inhibitors bearing morpholino, cycloheptylamino, and azocanyl moieties instead of the simple aromatic groups present in previously reported small-molecular inhibitors. Inhibitors containing these new moieties are expected to have improved log*P* values and decreased molecular planarity, which might be favorable for practical application. Among the various drug properties, the log*P* value is one of the importance parameters because the appropriate log*P* value is considered to elucidate the good efficiency and also provide good clearance from the body. The log*P* value of a drug is better to be less than 5 in accordance with Lipinsky's rule of five[21], and in general the value of 1 to 3 is more suitable.[22] Considering these guidance, compound **23c** showed better log*P* value (1.59) than VER1 (3.02). Furthermore, compound **23c** is bearing balkier functional group of aliphatic imine which is expected to provide a positive effect on the log*P* value through inhibiting the molecular self-stacking via planer molecular structures.[23,24] The introduction of steric groups is also expected to interact with larger surface of the proteins. The aspartic acid scaffold with a steric imide group might have good balkiness for Pin1 binding pocket.

Further work is needed to evaluate the cellular effects of these compounds, and to extend our knowledge of the structure–activity relationships.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.033.

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