Cyclohexyl Ketone Inhibitors of Pin1 Dock in a Trans-Diaxial Cyclohexane Conformation

Guoyan G. Xu, Carla Slebodnick, Felicia A. Etzkorn*

Department of Chemistry, Virginia Tech, Blacksburg, Virginia, United States of America

Abstract

Cyclohexyl ketone substrate analogue inhibitors (Ac–pSer–Ψ–[C = OCH]–Pip–tryptamine) of Pin1, the cell cycle regulatory peptidyl-prolyl isomerase (PPIase), were designed and synthesized as potential electrophilic acceptors for the Pin1 active site Cys113 nucleophile to test a proposed nucleophilic addition-isomerization mechanism. Because they were weak inhibitors, models of all three stereoisomers were docked into the active site of Pin1. Each isomer consistently minimized to a trans-diaxial cyclohexane conformation. From this, we hypothesize that Pin1 stretches substrates into a trans-tryrrolidone conformation to lower the barrier to isomerization. Our reduced amide inhibitor of Pin1 adopted a similar trans-tryrrolidone conformation in the crystal structure. The molecular model of 1, which mimics the trans-π-Ser-π-Pro stereochemistry, in the Pin1 active site showed a distance of 4.4 Å, and an angle of 31° between Cys113-S and the ketone carbon. The computational models suggest that the mechanism of Pin1 PPIase is not likely to proceed through nucleophilic addition.

Introduction

Pin1 (peptidyl-prolyl isomerase (PPIase)) interacting with never-in-mitosis A kinase-1 was discovered in 1996 as a PPIase enzyme that regulates mitosis [1]. The two domains of Pin1, a WW and a PPIase domain, are connected by a flexible linker that serves as a communication conduit between the domains [2]. Both of these domains recognize the phospho-Ser/Thr-Pro bonds present in mitotic phosphoproteins [3]. Pin1 is distinct from two other PPIase families, cyclophilin and FK506 binding protein (FKBP) [4], since Pin1 only has PPIase activity for phosphorylated substrates [3].

Pin1 catalyzes prolyl cis-trans isomerization to function as a molecular timer regulating the cell cycle, cell signaling, gene expression, immune response, and neuronal function [5]. Pin1 is overexpressed in many cancer lines, and plays an important role in oncogenesis [6]. Because of its significant role in cell cycle regulation by a unique mechanism, Pin1 represents an intriguing diagnostic and therapeutic target for cancer [7,8]. Several promising classes of Pin1 inhibitors have been synthesized as potential lead compounds [7], including designed inhibitors [9,10,11,12,13,14], and natural products [15,16].

The mechanisms of the PPIases, cyclophilins and FKBP are shown to go through a twisted amide transition state. Evidence included secondary deuterium isotope effects, molecular modeling, mutagenesis, and bound inhibitor structure [17,18,19,20,21,22,23,24]. There are two proposed mechanisms for Pin1 catalysis: (1) the twisted-amide mechanism [25], and (2) the nucleophilic-addition mechanism (Figure 1) [26].

In this work, we describe the synthesis, bioassay, and docking of ketones 1, Ac–π-pSer–Ψ–[C = OCH]–α-pipecolyl (Pip)–tryptamine, and rac-2, enantiomeric Ac–π-pSer–Ψ–[C = OCH]–α-Pip–tryptamine and Ac–π-pSer–Ψ–[C = OCH]–π-Pip–tryptamine. These inhibitors were designed as electrophilic acceptors of the Pin1 active site Cys113 thiol nucleophile to mimic the enzyme-bound tetrahedral intermediate (Figure 1C).

On the other side of the coin, we have described reduced amides designed as twisted-amide transition-state analogues 3 and 4 (Figure 2) [27]. The evidence for a nucleophilic addition mechanism included the proximity of Cys113 to the substrate in the X-ray crystal structure, and the attenuation of activity for Pin1 mutants: 20-fold for C113S and 120-fold for C113A [26]. We anticipated that the ketones would be poor inhibitors, while the reduced amides, as twisted-amide analogues, would fare better. Indeed, the reduced amide 3 is a better Pin1 inhibitor than a similarly substituted substrate analogue (Z-alkene isostere 5 (Figure 2) [13,27]. Our crystal structure of reduced amide 4 bound to the Pin1 catalytic site adopted a trans-tryrrolidone conformation, supporting the twisted-amide mechanism [27].

Ketones have been widely used as analogues of aldehydes or carboxylic acids to inhibit serine, cysteine [28,29], and asparagyl proteases [30,31]. Substrate-analogue ketones have not yet been developed as inhibitors of Pin1. Juglone is a ketone natural product that was shown to be a non-specific inhibitor of Pin1 through Michael addition to a surface Cys thiol of Pin1, resulting in unfolding [15]. Daum et al developed a series of aryl indanyl ketone inhibitors of Pin1; the best inhibitor had an IC30 value of 0.2 µM [11]. These inhibitors were reversible and cell penetrating, and they showed biological activities against p53 and β-catenin [11]. Daum et al proposed that the aryl indanyl ketones mimic the transition state of the twisted amide, based on the conformation in a crystal structure [11]. ψ-Ketoamides 6a and 6b were designed as...
potential transition state analogue inhibitors of Pin1, but their weak inhibition could not be used either the twisted-amide or the nucleophilic-addition mechanism (Figure 2) [14].

Results

Design of Inhibitors

Ketone 1 was designed as a tetrahedral intermediate analogue, incorporating an electrophilic ketone to act as an acceptor for the Pin1 active site Cys113 thiol (Figure 1). Ketone 1 was designed based on substrate and peptide inhibitor specificities [12,32]. The stereoisomer obtained as a side product during synthesis, rac-2, was also tested for Pin1 inhibition because Wildeman et al. found that α-Thr containing peptide inhibitors were more potent than 1-Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12]. The carbocyclic analogue of Pip, a cyclohexyl ring, was chosen based on the 100-fold improved inhibition of peptides with Thr [12].

Synthesis

In the synthesis of ketones 1 and rac-2, addition of cyclohexenyl lithium to a Weinreb amide was used to form the ketone functionality (Figure 3). α,β-Unsaturated ketone 7 was obtained by deprotonation of Boc-Ser(Bn)-N(OMe)Me Weinreb amide with i-PrMgCl, followed by addition of cyclohexenyl lithium [34]. The lithium reagent was prepared in situ by treating amide with obtained by deprotonation of Boc-Ser(Bn)-N(OMe)Me Weinreb methylenolactocin [36]. We first attempted the Michael addition compared with Fmoc analogues for enzyme assays [13].

Figure 1. Ketone inhibitors were designed to mimic the tetrahedral intermediate of proposed mechanism B. (A) Proposed Pin1 hydrogen-bond assisted twisted amide mechanism [25]. (B) Pin1 Cys113 nucleophilic-addition mechanism tetrahedral intermediate proposed by Ranganathan et al [26]. (C) Electrophilic ketone inhibitor designed to mimic the proposed tetrahedral intermediate upon Cys113-S nucleophilic addition. doi:10.1371/journal.pone.0044226.g001

with BF$_2$Et$_2$O and H$_2$O gave a mixture of diastereomeric carboxylic acids 10 [36]. Without further purification, acids 10 were coupled to tryptamine with EDC to generate the ketone diastereomeric mixture of (1S,3R,4R)-11 and rac-11, which were separated by silica flash chromatography (Figure 3).

The two diastereomers were carried on separately to the final compounds 1 (Figure 3), and rac-2. The major diastereomer (1S,3R,4R)-11 was treated with BCl$_3$ to remove the benzyl group and form alcohol (1S,3R,4R)-12 [37,38]. Phosphorylation with dibenzylphosphoramide gave dibenzyl phosphate (1S,3R,4R)-13 [10,39]. Phosphorylation were also attempted with di-tert-butyl or dicyanoethyl phosphoramidites to produce di-tert-butyl or dicyanoethyl instead of dibenzyl phosphate. Neither of these phosphates was stable on silica gel, and β-elimination products were obtained after chromatography. TFA deprotection of crude di-tert-butyl phosphate, and NH$_4$OH deprotection of crude dicyanoethyl phosphate both gave β-elimination products as well. Thus, the dibenzyl phosphate was chosen to carry through to the final products 1 and rac-2.

Hydrogenation of the crude dibenzyl phosphate (1S,3R,4R)-13 went very slowly, giving a complex crude mixture. Thus, (1S,3R,4R)-13 was purified by reverse-phase semi-preparative high performance liquid chromatography (HPLC). With pure dibenzyl phosphate, hydrogenation at atmospheric pressure worked very well, and gave a very clean final product 1, similar to our experience with α-ketoamides [14].

X-ray crystallography

During the synthesis of the inhibitors, Michael addition of trithiomethyl methide to an α,β-unsaturated ketone 8 produced three stereoisomers of 9, which could not be readily separated (Figure 3). Two diastereomers of a subsequent synthetic intermediate, (1S,3R,4R)-11 and rac-11, were separated by chromatography. Each diastereomer was crystallized, and the relative stereochemistry was determined. The absolute configuration of the major diastereomer was assigned to be (1S,3R,4R)-11, with
the original Ser configuration intact (Figure 4). The minor isomer, rac-11, proved to be a racemic mixture. The absolute configurations were assigned as (1R,3R,4R)-11 and (1S,3S,4S)-rac-11, in which the stereocenter of the Ser analogue was partially epimerized to the syn-Ser-trans-cyclohexyl configuration (Figure 4).

Pin1 PPIase Enzyme Assays

The α-chymotrypsin protease-coupled assay was used to evaluate inhibition of Pin1 by compounds 1 and rac-2 with the same substrate concentration as described previously [10,14]. The IC50 values of the two diastereomers were determined to be 260 ± 30 μM for 1, and 61 ± 8 μM for rac-2. Preincubation with Pin1 for 15 minutes did not result in improved inhibition.

Molecular modeling

Each of the three cyclohexyl ketone inhibitors was docked flexibly, with geometry minimization, into the Pin1 active site. The resulting docked stereoisomers, (1S,3R,4R)-1, (1R,3R,4R)-2, and (1S,3S,4S)-2, are shown in Figure 5. The total energies, Cys113–S=C=O ketone distances, and angles are reported in Table 1. The distance between 1,2-diequatorial carbonyl groups was 2.93 Å, while the distance between 1,2-diaxial carbonyl groups in 1,2-cyclohexanecarboxylic acid was 3.79 Å after geometry optimization. The distance between the carbonyl carbons of Ac-cis-Pro–OH

Figure 3. Cyclohexyl ketone inhibitor 1 was synthesized by the method shown.
doi:10.1371/journal.pone.0044226.g003

Figure 4. X-ray crystal structures of intermediates (1S,3R,4R)-11 and rac-11 are shown above as displacement ellipsoid drawings (50%). The positional disorder of the benzyl group in rac-11 is shown as lighter lines. Hydrogen atoms are omitted for clarity. Structural depiction of the stereochemistries of (1S,3R,4R)-11 and rac-11 are shown below each crystal structure. doi:10.1371/journal.pone.0044226.g004

after geometry optimization was 3.16 Å; with the trans-pyrrolidine torsion angle fixed during geometry optimization, the distance was 3.67 Å (Figure 6).

Discussion

Stereochemical results of inhibitor synthesis

Thermodynamic control in the Michael addition resulted in the anti-Ser-trans-cyclohexyl stereoisomer of 9 as the major product (Figure 4). The chiral center adjacent to the Ser carbonyl was easily epimerized due to the electron-withdrawing effects of both the α-amide and α-ketone, resulting in an enantiomeric mixture of a second diastereomer, rac-9. Because the unnatural d-Thr-
containing inhibitors were more potent than the L-Thr in work by Zhang et al [32], both diastereomers 1 and rac-2 were tested for Pin1 inhibition. Inhibitor 1, corresponding to the native L-Ser-L-Pro stereochemistry of Pin1 substrates, had an IC$_{50}$ value of 260 µM, while rac-2, an enantiomeric mixture of d-Ser-L-Pro and t-Ser-d-Pro analogues, had an IC$_{50}$ value of 61 µM. Preincubation did not result in improved inhibition, suggesting that they are not slow-binding inhibitors. We obtained a crystal structure of the similarly substituted, reduced amide inhibitor 4 bound in the Pin1 active site, suggesting that the ketones also bind in the active site [27].

Insights into the Pin1 enzymatic mechanism

To better understand the mechanism of Pin1 PPIase activity, each of the three stereoisomers was docked into the Pin1 active site (Figure 5). Curiously, in each case the inhibitor minimized to a conformation with a trans diaxially substituted cyclohexyl ring. Attempts to force a trans diequatorial conformation on the starting structure resulted in conversion to either a twist boat or a diaxial conformation again. Clearly, the preferred conformation of these cyclohexyl substrate analogues in the Pin1 active site is diaxial. In the crystal structures of intermediates (1S,3R,4R)-11 and rac-11, the cyclohexyl rings were in the diequatorial chair conformation (Figure 4), which are likely to be the low-energy, solution-phase conformations as well. These inhibitors would thus undergo an unfavorable diequatorial to diaxial conformational change in order to bind to the Pin1 active site.

We hypothesize that the binding interactions of the enzyme with the phosphate and the aromatic group are strong enough to stretch the cyclohexyl rings into the less stable diaxial conformation upon binding (Figure 6). The difference in the distances between diequatorial and diaxial carbonyl groups on a cyclohexane ring was 0.86 Å, an elongation of the structure. The corresponding difference between the planar Ac–Pro–OH conformation, and the trans-pyrrolidine Ac–Pro−OH conformation was 0.51 Å (Figure 6). This effect of stretching the ring conformation may provide insight into the mechanism of Pin1. In either of the proposed mechanisms: (1) nucleophilic-addition [26], or (2) twisted-amide [25], the nitrogen of the prolyl ring must become pyramidalized and deconjugated from the carbonyl in the transition state [22, 24, 25]. If binding of substrate to the catalytic site forces the Pro ring into a trans-pyrrolidine conformation, the nitrogen lone pair and the carbonyl π-bond would no longer be conjugated (Figure 6). The substrate would be destabilized, lowering the barrier to rotation around the amide bond. This proposed stretching action is consistent with the twisted-amide mechanism, providing a more detailed description of how the isomerization might proceed.

Stereoisomer (R,R,R)-2, with the ketone carbonyl carbon 4.4 Å from the proposed Cys113-S nucleophile, and the S—C = O angle of 102°, had the lowest energy of the three stereoisomers (Table 1). The angle of 102° is close to the optimum angle for nucleophilic addition, i.e. close to the Burgi-Dunitz angle of 107° [27]. Despite this, the inhibition results suggest that covalent modification, i.e. suicide inhibition, of Pin1 does not occur. Ketones 1 and rac-2 were designed as tetrahedral-intermediate analogues based on the nucleophilic-addition mechanism; they do not appear to behave as such. The IC$_{50}$ values are in the range of substrate analogue inhibitors. These results argue against the proposed nucleophilic-addition mechanism for Pin1 [14].

### Stereochemical effects on inhibition

The stereochemistry affected the inhibition, since the racemate rac-2 was about 4-fold more potent than diastereomer 1. Molecular modeling provides insight into the stereochemical preferences of the Pin1 active site. The relative (not absolute) energies of the three models can be compared because they are all stereoisomers bound into the same Pin1 active site (Table 1). These inhibitors are substituted with tryptamine, comparable to our ground-state alkene isosterie inhibitor 5 with an IC$_{50}$ value of 25 µM (Figure 2) [13], and with Ac and naphthylethylamine comparable to our α-ketoamide inhibitors 6, with IC$_{50}$ values of 100 and 200 µM [14]. The Pin1-(S,S,S)-1 complex, with an intermediate energy, corresponds to the native L-Ser-L-Pro configuration, yet it had very poor inhibition (260 µM), comparable to the similarly substituted α-ketoamides 6 [14]. The Pin1-(S,S,S)-2 complex, which corresponds to the t-Ser-d-Pro configuration, had the highest energy of the three, while Pin1-(R,R,R)-2, corresponding to a t-Ser-d-Pro configuration had the lowest energy. This is consistent with the t-Thr-L-Pip in the most potent peptide inhibitors of Pin1 [12,32]. We expect that (R,R,R)-2 isomer would be more potent than the IC$_{50}$ value of 61 µM for rac-2 indicates, and (S,S,S)-2 is likely to be less potent than 61 µM, because the IC$_{50}$ value represents a weighted average of the two. The most potent that either enantiomer could possibly be

Table 1. Comparison of cyclohexyl ketone inhibitor-Pin1 complex molecular models.

<table>
<thead>
<tr>
<th>Stereoisomer:</th>
<th>(1S,3R,4R)-1</th>
<th>(1R,3R,4R)-2</th>
<th>(1S,5R,4S)-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mimics:</td>
<td>L-Ser-L-Pro</td>
<td>D-Ser-L-Pro</td>
<td>L-Ser-D-Pro</td>
</tr>
<tr>
<td>Color in Figure 5</td>
<td>orange</td>
<td>blue</td>
<td>green</td>
</tr>
<tr>
<td>Total E (kcal/mol)</td>
<td>−477</td>
<td>−518</td>
<td>−494</td>
</tr>
<tr>
<td>Cys113−S−C = O (Å)</td>
<td>4.4</td>
<td>4.4</td>
<td>5.0</td>
</tr>
<tr>
<td>Cys113−S−C = O /</td>
<td>31°</td>
<td>102°</td>
<td>59°</td>
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doi:10.1371/journal.pone.0044226.t001
is 30 μM if the other was not an inhibitor at all. This is highly unlikely, but it serves to show that these ketone inhibitors behave as substrate analogues.

Conclusions

Three stereoisomeric ketone analogues of Pin1 substrates were synthesized, modeled, and assayed as Pin1 inhibitors. Molecular modeling shows that the inhibitors have a preference for trans-diaxial-cyclohexane conformations upon binding to Pin1. This led us to propose a stretching mechanism to attain pyramidalization of the prolyl nitrogen, consistent with the preferred twisted-amide mechanism [25]. The molecular models of the three stereoisomers in the active site of Pin1 confirmed the stereochemical preferences of Pin1 for inhibitors seen in other inhibitors [12,14,27,32]. We attribute the weaker binding of these inhibitors to a combination of: (1) the conformational change required for binding, and (2) the inability of these ketones to act as electrophilic acceptors for the Pin1 Cys113 thiol. The weak inhibition of the ketones, and the correspondingly stronger inhibition by similarly substituted reduced amide inhibitors [27], provides evidence against the nucleophilic addition mechanism for Pin1.

Materials and Methods

Synthesis

Unless otherwise indicated, all reactions were carried out under dry N2 in flame-dried glassware. THF was distilled from Na-benzophenone, and CH2Cl2 was dried by passage through dry alumina. Anhydrous DMF (99.8%), MeOH, and DIEA were used directly from sealed bottles. Brine (NaCl), Na2S2O3, NaHCO3, and NH4Cl refer to saturated aqueous solutions, and HCl refers to a 1 N aqueous solution, unless otherwise noted. Flash chromatography was performed on 230–400 mesh silica gel with reagent grade solvents. Analytical HPLC were obtained on a 4.6 mm x 25 cm column, in vacuo. The crude product was purified by chromatography on silica (eluant: 8% EtOAc/hexanes) to yield ketone 7 (4.3 g, 68%) as a colorless oil. Anal. HPLC, 254 nm, 7.3 min, 98.2%; 1H NMR δ 7.29 (m, 5H), 6.91 (m, 1H), 5.59 (d, J = 0.3, 1H), 5.13 (dt, J = 4.4, 8.3, 1H), 4.54 (d, J = 12.4, 1H), 4.42 (d, J = 12.4, 1H), 3.66 (dd, J = 4.4, 9.3, 1H), 3.66 (dd, J = 4.4, 9.6, 1H), 2.36 (m, 1H), 2.21 (m, 2H), 2.12 (m, 1H), 1.62 (m, 4H), 1.44 (s, 9H); 13C NMR δ 197.8, 155.5, 141.9, 137.8, 137.4, 128.0, 127.7, 127.6, 79.0, 73.1, 71.3, 54.3, 28.4, 26.2, 23.4, 21.8, 21.5; ES+ HRMS m/z 382.1998 [M+Na]+. Calculated for C21H24N2O3Na: 382.1994.

Acetyl-ketone 8. Boc-ketone 7 (1.1 g, 4.2 mmol) was dissolved in CH2Cl2 (20 mL), and Pr3SiH (0.2 mL) and TFA (20 mL) were added. The mixture was stirred at rt for 0.5 h. The reaction mixture was then concentrated under reduced pressure. The residue was triturated with hexanes (3×25 mL). After evaporation in vacuo for 2 h, the ammonium salt obtained was dissolved in CH2Cl2 (20 mL), and Ac2O (2 mL) and DIEA (2 mL) were added. The reaction mixture was stirred at rt for 1 h. After dilution with CH2Cl2 (30 mL), the mixture was washed with HCl (2×25 mL), 1 N NaOH (2×25 mL), and brine (25 mL). The organic layer was dried over Na2SO4, filtered and evaporated. The residue was purified by flash chromatography on silica (step gradient: 25% then 50% EtOAc/hexanes) to yield 8 (1.1 g, 90%) as a pale, yellow oil. Anal. HPLC, 254 nm, 5.1 min, 100%; 1H NMR δ 7.35-7.20 (m, 5H), 6.83 (m, 1H), 6.62 (br, 1H), 5.42 (m, 1H), 4.52 (d, J = 12.3, 1H), 4.40 (d, J = 12.3, 1H), 3.70 (m, 2H), 2.39-2.04 (m, 4H), 2.02 (s, 3H), 1.62 (m, 4H); 13C NMR δ 197.3, 169.8, 142.3, 137.3, 132.7, 128.5, 127.9, 127.6, 73.2, 71.1, 53.3, 26.2, 23.44, 23.38, 21.8, 21.5; ES+ HRMS m/z 302.1760 [M+H]+. Calculated for C19H21NO3S: 302.1756.

Orthothroioformate 9. n-Butyl lithium (2.5 M in hexane, 6.81 mL, 17.0 mmol) was added dropwise to a solution of CH(SMe)2 (2.68 g, 17.0 mmol) dried over 4 Å molecular sieves in THF (65 mL) at −78 °C. The solution was stirred at −78 °C for 2 h. A solution of the acetyl ketone 8 (0.790 g, 2.62 mmol) dissolved over 4 Å molecular sieves in THF (50 mL) was added dropwise via cannula. The reaction mixture was stirred at −78 °C for 2 h, and quenched with NH4Cl (80 mL). The resulting mixture was extracted with EtOAc (3×150 mL). The organic layer was dried over Na2SO4, filtered and evaporated. The crude product was purified by chromatography on silica (step gradient: 0% then 20% EtOAc/hexanes) to yield the orthothioformate 9, a mixture of two diastereomers, (0.60 g, 50%) as a colorless oil. The mixture was used in the next reaction without separation. The major diastereomer was partially separated for characterization. Major diastereomer: 1H NMR δ 6.30 (m, 5H), 6.60 (d, J = 7.5, 1H), 5.26 (dd, J = 3.4, 4.2, 7.6, 1H), 4.52 (d, J = 12.1, 1H), 4.49 (d, J = 11.8, 1H), 3.89 (dd, J = 3.3, 10.2, 1H), 3.79 (dd, J = 4.4, 9.9, 1H), 3.11 (dd, J = 3.8, 10.5, 11.5, 1H), 2.39 (dd, J = 5.6, 10.4, 11.8), 2.09 (m, 1H), 2.07 (s, 9H), 2.02 (s, 3H), 1.82 (m, 1H), 1.73 (m, 1H), 1.63 (m, 2H), 1.38 (m, 1H), 1.31 (m, 1H), 1.22 (m, 1H), 1.06 (m, 1H); 13C NMR δ 207.0, 169.4, 137.9, 128.5, 127.9, 127.8, 75.3, 73.4, 68.9, 59.5, 51.5, 48.0, 31.6, 28.6, 25.6, 25.1, 23.6, 15.2; ES+ HRMS m/z 478.1530 [M+Na]+. Calculated for C22H23NO5S2Na: 478.1520.

Ac-Ser(OBn)–[C = OCH]–2-(indol-3-yl)-ethylamine (13,3R,4R)-11 and rac-11. A mixture of compound 9 (0.30 g, 0.66 mmol) and HgO (0.70 g, 3.2 mmol) was suspended in 4:1 THF:H2O (45 mL), and BF3·Et2O (1.2 mL, 9.6 mmol) was added. The mixture was stirred at rt for 3 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3×50 mL). The organic layer was dried over Na2SO4, filtered and evaporated. The residue was filtered through silica to remove HgO, and the solvent was evaporated in vacuo. The crude carboxylic acid 10 was dissolved in a mixture of CH2Cl2 (100 mL) and DMF (15 mL), and tritylmamine (0.27 g,
CH2Cl2 (8 mL). The solution was cooled to 512.2530 [M+Na]−; 7.35-7.25 (m, 5H), 7.19 (d, J = 7.2, 1H), 6.91 (d, J = 5.8, 1H), 6.32 (d, J = 3.3, 9.9, 1H), 3.76 (dd, J = 4.4, 9.9, 1H), 3.44 (m, 2H), 3.05 (ddd, J = 3.3, 10.7, 12.4, 1H), 2.84 (m, 2H), 2.37 (m, 1H), 2.13 (m, 1H), 1.99 (s, 3H), 1.80 (m, 3H), 1.49 (m, 1H), 1.26 (m, 2H), 1.08 (m, 1H); 13C NMR δ 209.5, 174.7, 170.0, 137.9, 136.5, 128.3, 127.9, 127.8, 127.4, 122.4, 122.2, 119.5, 118.9, 112.9, 111.4, 77.4, 73.4, 68.3, 58.4, 47.8, 47.7, 39.6, 30.0, 28.6, 25.3, 23.4; ESI+ HRMS m/z 512.2590 [M+Na]+. Calculated for C28H33NO4Na; 512.2525.

Minor isomer rac-11: HPLC, 254 nm, 5 min, 96.3%; 1H NMR δ 8.25 (br, 1H), 7.56 (d, J = 7.0, 1.1), 7.32 (m, 1H), 7.06 (d, J = 7.1, 8.0, 1.1), 7.01 (s, 1H), 6.97 (d, J = 7.9, 1.0, 8.0, 1.1), 6.93 (d, J = 8.2, 1.1, 7.0), 5.18-5.04 (m, 1H), 5.04 (d, J = 6.6, 2.0, 5.20 (d, J = 6.1, 2.0), 4.93 (dd, J = 3.8, 6.8, 1.8), 4.53 (ddd, J = 5.9, 6.9, 10.8, 1.8), 4.16 (d, J = 7.1, 8.2, 1.1, 11.1), 3.37 (m, 2H), 2.95 (ddd, J = 3.2, 10.8, 12.2, 1.1), 2.85 (m, 2H), 2.51 (m, 1H), 1.94 (s, 3H), 1.85 (m, 1H), 1.78 (m, 2H), 1.32 (m, 3H), 1.10 (m, 1H); 13C NMR δ 209.5, 177.2, 173.2, 138.1, 137.1, 130.7, 129.73, 129.68, 129.23, 129.19, 128.8, 123.5, 122.3, 119.6, 113.9, 113.2, 112.2, 71.0, 67.0 (d, 3JPC = 3.5), 50.91 (d, 3JPC = 7.5), 50.6, 47.3, 41.3, 31.1, 30.2, 26.6, 26.5, 26.3, 22.4; 31P NMR (202 MHz): δ = 0.40; ESI+ HRMS m/z 660.2846 [M+H]+. Calculated for C43H37O11P 660.2839.

Ac-Ser–Ψ[C = OCH]–Pip–2–(indol-3-yl)-ethylamine (1S,3R,4R)-11 and rac-12. Ac-Ser(OBn)–Ψ[C = OCH]–2–(indol-3-yl)-ethylamine (1S,3R,4R)-11 (48 mg, 0.098 mmol) was dissolved in CH2Cl2 (8 mL). The solution was cooled to −78°C, and BCl3 (1 М in CH2Cl2, 1.2 mL) was added dropwise. The reaction mixture was stirred at −78°C and warmed to 0°C over 1.5 h. The reaction mixture was cooled to −78°C, and MeOH (0.5 mL) and acq. HCl (2 N, 5 mL) were added. The solution was diluted with EtOAc (150 mL), and washed with HCl (50 mL), 5% NaHCO3 (aq), and quenched with Na2S2O3. The mixture was filtered through Celite, and washed with 13C NMR δ 210.9, 174.5, 170.1, 137.8, 136.5, 128.5, 127, 127.8, 127.4, 122.4, 122.2, 119.5, 118.9, 112.9, 111.4, 77.4, 73.4, 68.3, 58.4, 47.8, 47.7, 39.6, 30.0, 28.6, 25.3, 23.4; ESI+ HRMS m/z 490.2701 [M+H]+. Calculated for C30H33NO5P 490.2706.
$J = 7.7, 1H$, 7.32 (d, $J = 8.2, 1H$), 7.07 (m, 2H), 6.99 (dd, $J = 0.9, 7.6, 8.0, 1H$), 4.92 (m, 1H), 4.35 (m, 1H), 4.18 (m, 1H), 3.40 (m, 2H), 3.04 (dt, $J = 3.0, 11.4, 1H$), 2.89 (m, 2H), 2.52 (dd, $J = 3.4, 10.5, 12.2, 1H$), 2.22 (dd, $J = 2.2, 13.2, 1H$), 2.00 (s, 3H), 1.93 (m, 3H), 1.35 (m, 3H), 1.11 (m, 1H); $^{13}$C NMR (CD$_3$OD) δ 209.6, 177.3, 175.2, 132.6, 129.8, 123.9, 122.1, 119.5, 119.3, 113.2, 112.2, 65.5, 59.2 (d, $J_{C,P} = -125, 50.4, 47.1, 41.2, 31.2, 30.3, 26.8, 26.6, 26.2, 22.3$; $^{31}$P NMR (CD$_3$OD) δ 1.44; ESI$^+$ HRMS m/z 480.1906 [M+H]$^+$. Calculated for C$_{22}$H$_{31}$N$_3$O$_7$P 480.19.

By the same procedure, the minor isomer rac-2, ret. time 6.8 min, was obtained as a white powder (5.5 mg, 70%). Anal. HPLC, 254 nm, (gradient: 5% B for 3 min, then 5–90% B over 6 min), 6.8 min, 89%; 1HN M R (5.5 mg, 70%). Anal. HPLC, 254 nm, (gradient: 5% B for 3 min, then 5–90% B over 6 min), 6.8 min, 99.1%; $^1$H NMR (dimethylsulfoxide (DMSO)-d$_6$): δ 10.82 (br s, 1H), 8.25 (br s, 1H), 7.89 (t, $J = 5.6, 1H$), 7.50 (d, $J = 7.8, 1H$), 7.31 (d, $J = 7.7, 1H$), 7.10 (d, $J = 1.5, 1H$), 7.04 (t, $J = 7.4, 1H$), 6.96 (t, $J = 7.2, 1H$), 4.75 (m, 1H), 4.14 (m, 1H), 3.96 (m, 1H), 3.24 (m, 2H), 2.99 (t, $J = 10.4, 1H$), 2.73 (m, 2H), 2.56 (dt, $J = 3.6, 11.3, 1H$), 1.94 (m, 1H), 1.87 (s, 3H), 1.82 (m, 1H), 1.68 (m, 2H), 1.24 (m, 3H), 1.02 (m, 1H); $^{13}$C NMR (CD$_3$OD): δ 210.5, 177.2, 173.1, 138.1, 128.8, 123.6, 122.2, 119.5, 119.3, 113.3, 112.2, 65.4, 60.0, 49.4, 49.3, 41.2, 31.3, 29.3, 26.5, 26.4, 26.2, 22.6; $^{31}$P NMR (DMSO-d$_6$): δ = 2.93; ESI$^+$ MS m/z 480.18 [M+H]$^+$. Calculated for C$_{22}$H$_{31}$N$_3$O$_7$P 480.19.

**X-ray structures**

Crystal structure (1S,3R,4R)-11: Colorless needles (0.31 × 0.02 × 0.004 mm$^3$) were recrystallized from EtOAc:hexanes (1:2) at rt. The chosen crystal was centered on the goniometer of an Oxford Diffraction Nova diffractometer operating with CuK$_\alpha$ radiation. The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlis [40]. The Laue symmetry and systematic absences were consistent with the monoclinic space groups P2$_1$ or P2$_2$. Since the molecule was known to be enantiomerically pure, the chiral space group, P2$_1$, was chosen. The structure was solved by direct methods and refined using SHELXTL NT [41]. The asymmetric unit of the structure comprises one crystallographically independent molecule. The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. Since there were no heavy atoms, the absolute configuration could not be determined from the Friedel pairs; the Friedel pairs were therefore merged for the final refinement. The absolute configuration was assigned by reference to C(19) of known S-configuration. Relative to C(19), C(17) and C(12) are absolute configuration was assigned by reference to C(19) of known S-configuration. Relative to C(19), C(17) and C(12) are consistent with the monoclinic space group P2$_2$. The molecule was known to be enantiomerically pure, the chiral space group, P2$_1$, was chosen. The structure was solved by direct methods and refined using SHELXTL NT [41]. The asymmetric unit of the structure comprises one crystallographically independent molecule. The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms, and a riding model for all hydrogen atoms. The benzyl group was modeled with positional disorder, with the two positions refining to relative occupancies of 52.9(3)% and 47.1(3)% (Figure 4). Deposited Cambridge Crystallographic Data Centre (CCDC) 782064.

**Pin1 Enzyme Assays**

The Pin1 inhibition assay was performed at 4°C in 35 mM 4-[2-hydroxyethyl]piperazin-1-ethanesulfonic acid (HEPES) pH 7.8 in a total assay volume of 1.2 mL as published [10]. Inhibitors were dissolved in DM SO:H$_2$O (2:1) and 20 µL of stock was added to give final concentrations of 1: 12, 50, 100, 200, 400, 810 µM, and rac-2: 10, 20, 40, 60, 120, 240, 400 µM, pre-equilibrated with Pin1 in HEPES at 4°C for 15 min. The Pin1 final concentration in the assay was 67 nM. The final concentration of succinyl-Ala–Glu–(S)-Pro–OH, and Ac–Pro–O H with fixed rac-11/mixture was 34 µM. For each concentration, the assay was performed in duplicate. The plot of % Inhibition vs. log [I] [µM] produced sigmoidal curves by fitting all of the experimental data to Eq. 1 using TableCurve v3 for windows (Dataset S2). The IC$_{50}$ values were derived from the fitted equation at 50% inhibition of enzyme activity (Eq. 1), where a, b, c, and d are fitted constants given on the plots for compounds 1 and rac-2 (Dataset S2).

\[
\% \text{Inhibition} = a + \frac{b}{1 + (|I|/c)^d}
\]

**Computational Methods**

Models of three stereoisomeric ketones were based on the X-ray structure of peptide inhibitor, Ac–Phe–pThr–Pip–Nal–Gln–NH$_2$, bound to Pin1, protein data bank (PDB) 2Q5A, using Sybyl 8.1.1 (Figure 5) [32]. In each case, the Pip nitrogen was changed to a CH group with the appropriate stereochirality. The naphthyl (Nal) side chains were modified to indoles, and the Nal carbonyls were deleted. The Thr methyl groups, the Gln, and all except the alpha-carbon and carbonyl of the Phe residues (which became acetyl groups) were deleted. Further modification of the starting structures included drafting a diequatorial chair conformation for the cyclohexyl rings, inversion of the Ser stereocenter for (1R,3R,4R)-2, and manual rotation of torsions of (1R,3R,4S)-2 to bring the phosphate and indole groups close to these groups in the original crystal structure. Explicit waters from the crystal structure were retained. Protein termini charges, all hydrogens, and Amber FF02 atom types were added manually to the inhibitor atoms, phosphate groups, and Arg guanidines. The 3 oxygens of the phosphate groups were given formal charges of −0.67 prior to computation of Gasteiger-Marsili charges. Energy minimization, with geometry optimization of the inhibitors and all Pin1 residues within 8 Å of the inhibitors, was performed using Sybyl 8.1.1 with Gasteiger-Marsili charges, Amber FF02 force field, Powell conjugate gradient, gradient termination at 0.1 kcal/mol-A, 8 Å non-bonded cut-off, and a dielectric constant of 1.0. Typically, gradient convergence was reached within 3000 iterations. Distances and angles were measured using Sybyl 8.1.1 [42].

Cyclohexane-1,2-cis-dial, cyclohexane-1,2-trans-dial, Ac–cis-Pro–OH, and Ac–Pro–OH with fixed trans-pyrrolidine (α = −60°) conformation were geometry optimized using WebMO with Moller-Plesset 2, 6-31G(d), polarizable continuum model, and water as solvent [43]. For the twisted-amide conformation, the trans-pyrrolidine torsion angle was fixed to −155.6°, the angle found at the B3LYP STO-3G level of theory.

**Dataset Information**

**Dataset S1** HPLC chromatograms for 1 and rac-2. $^1$H, $^{13}$C, and $^{31}$P NMR spectra for compounds 1, rac-2, and 7-13 (PDF)

**Dataset S2** Pin1 inhibition plots for 1 and rac-2. Crystallographic data, CCDC 782064 and 782063 for (1S,3R,4R)-11 and rac-2 respectively, can be obtained free of charge from The
References


Author Contributions

Conceived and designed the experiments: GGX CS FAE. Performed the experiments: GGX CS FAE. Analyzed the data: GGX CS FAE. Wrote the paper: GGX CS FAE.