Synthesis and Characterization of the Heterocyclic Inhibitors and Control.

Method A

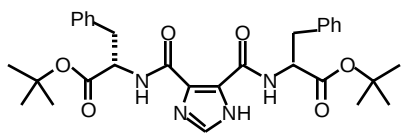
4,5-Bis{[(1,1-dimethylethoxy)-(*S*)-phenylalanyl]carbonyl}-1*H*-imidazole, **1**. To a dry roundbottom flask was added 10 mL of anhydrous CH_2Cl_2 followed by the addition of imidazole-4,5-dicarboxylic acid (0.5 g, 3.20 mmol), 1-hydroxybenzotriazole monohydrate (0.87 g, 6.41 mmol), and L-phenylalanine *t*-butyl ester hydrochloride (2.06 g, 8.01 mmol) under a blanket of inert gas. This stirred suspension was cooled to 0 °C and triethylamine (1.12 mL, 8.01 mmol) was added dropwise. This helped solubilize some of the remaining solids, but not all of them. Finally, dicyclohexylcarbodiimide (1.39 g, 6.72 mmol) was added all at once. The suspended imidazole-4,5-dicarboxylic acid slowly dissolved and gave way to precipitated dicyclohexylurea. The reaction was stirred for 24 h at which time the precipitated solids were removed by filtration. The dichloromethane was diluted with 80 mL of ethyl acetate before washing the solution with 20 mL each of 5% citric acid, 1 M $NaHCO_3$, H_2O, and a saturated NaCl solution. The organic fraction was dried over anhydrous $MgSO_4$, filtered, and concentrated to a white foam. Final purification was done on a silica gel column by gravity chromatography with ethyl acetate/hexanes (50/50) as the eluant. The fractions containing the desired product were combined and concentrated to yield 455 mg of pure material for a 25% yield.

Method B

4,5-Bis{{[(1,1-dimethylethoxy)-(*S*)-valyl]-(*S*)-phenylalanyl}carbonyl}-1*H*-imidazole, **2**. To a dry roundbottom flask containing **1** (157 mg, 0.278 mmol) was added 2 mL each of anhydrous dichloromethane and trifluoroacetic acid. This solution was stirred for 4 h after which the starting material was entirely consumed as determined by TLC analysis. The dichloromethane and trifluoroacetic acid were removed under vacuum. The resulting solid was again dissolved in dichloromethane, and the solvent removed under vacuum, to help remove any remaining traces of trifluoroacetic acid. This later process was repeated 3 more times before coupling the material to L-valine *t*-butyl ester hydrochloride by method A outlined above to provide the desired compound following workup and purification.

The remaining compounds, **3**-**6**, were prepared by using the appropriate starting material and the methods outlined above. Yields obtained following purification were as follows: **1**, 25%; **2**, 14%; **3**, 8%; **4**, 16%; **5**, 2%; and **6**, 11%. The yields are lower than expected based on the simplicity of the reaction. This may involve several factors including the insolubility of the starting materials or products. Additional details regarding the synthesis and characterization of this class of compounds will be published separately. Mass spectrometry confirmed the presence of the parent ions. The ^{1}H and ^{13}C NMR spectra for compounds **1**-**6** follow.

1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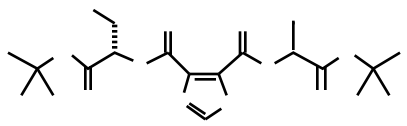

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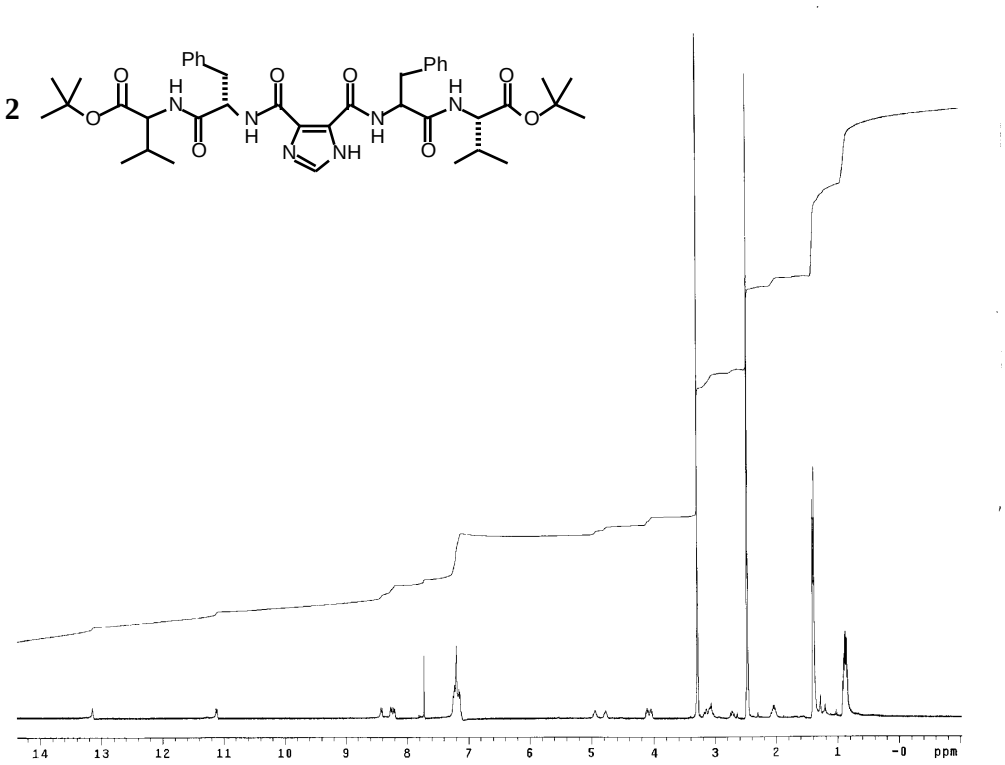
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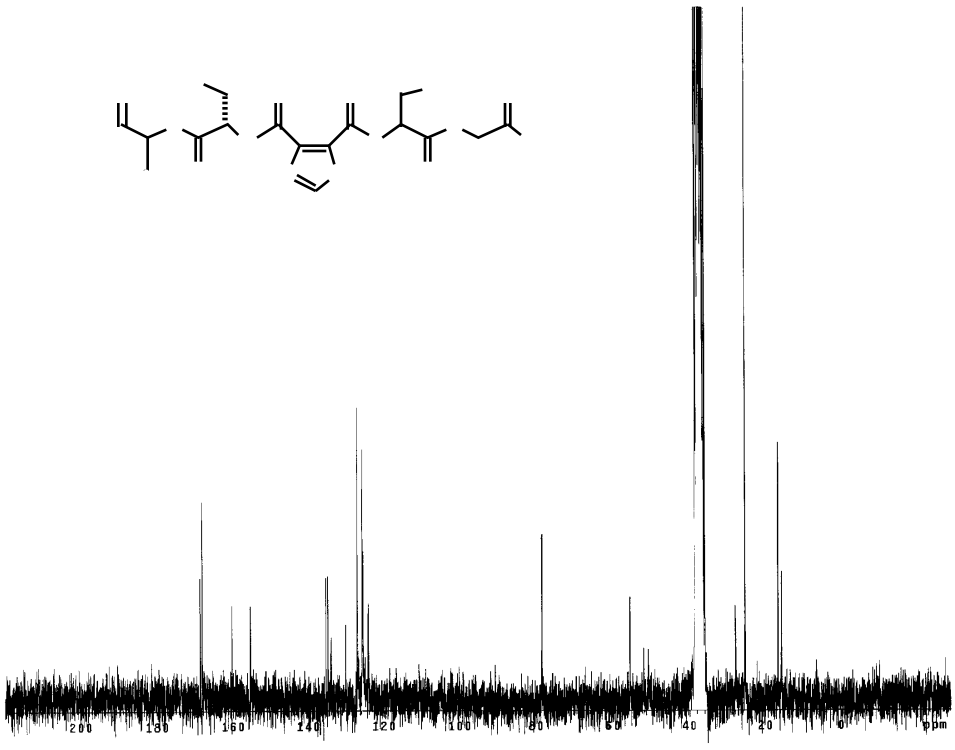
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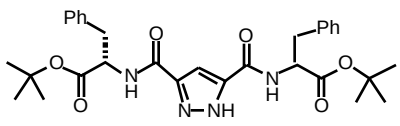
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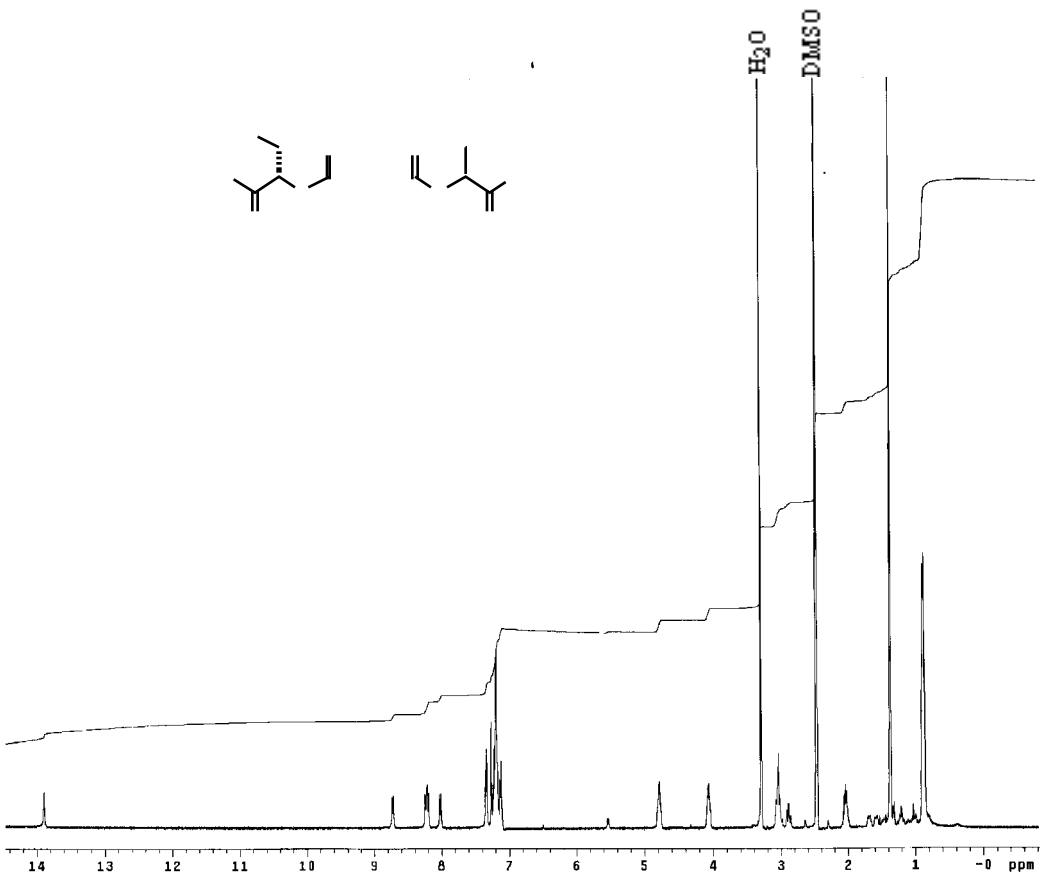
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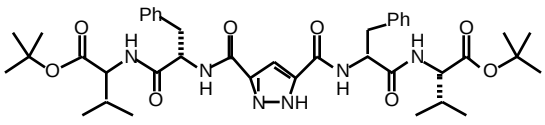
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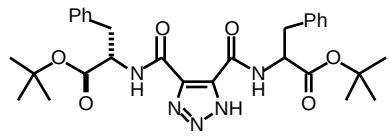
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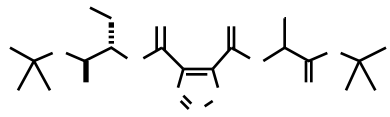

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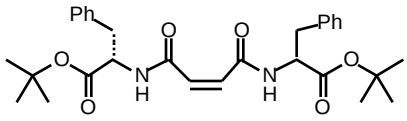
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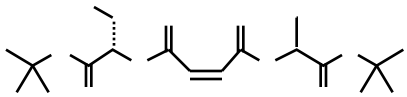

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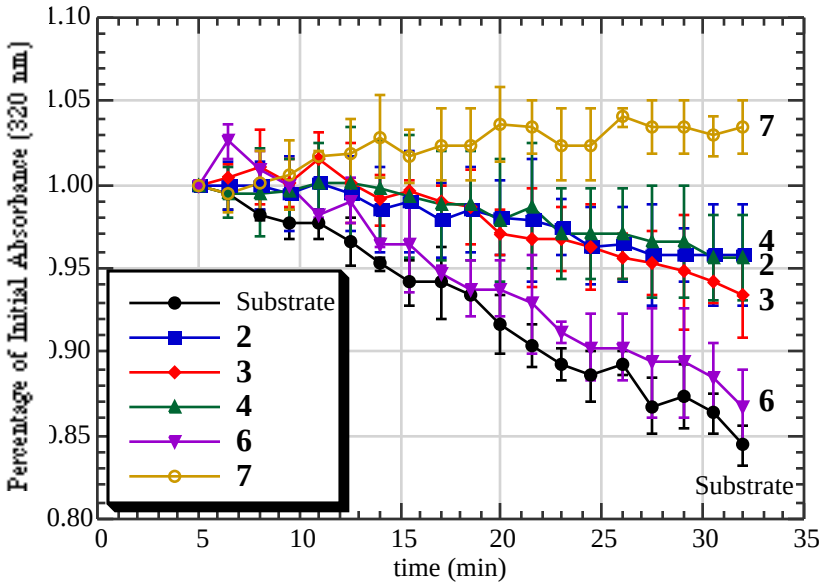
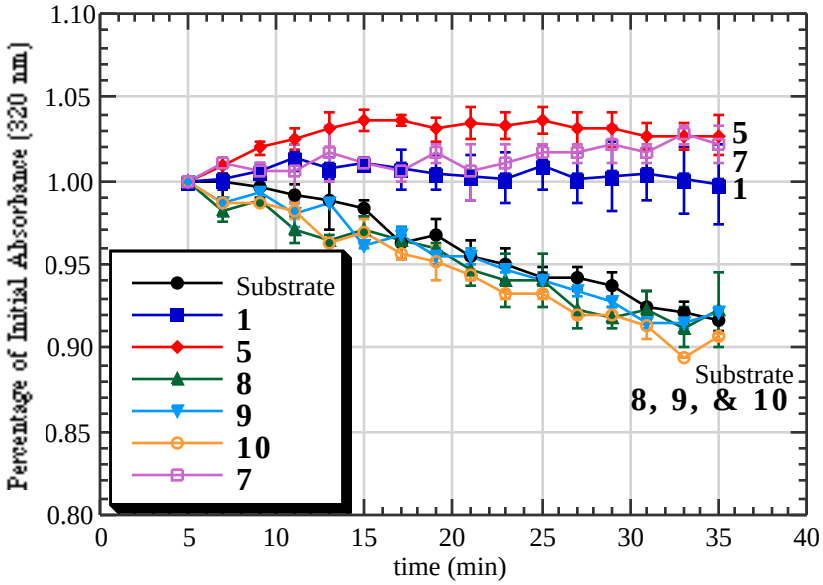
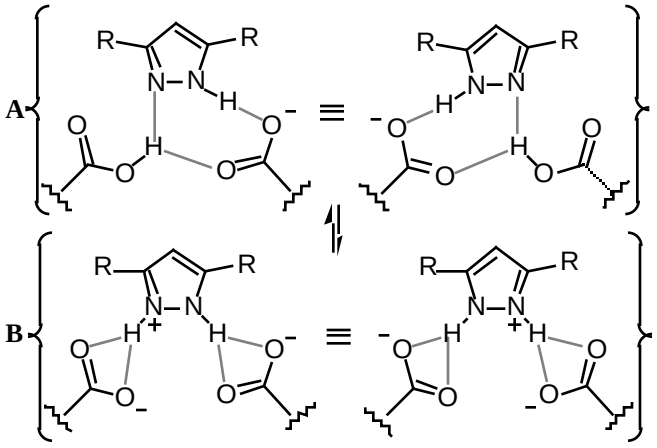


Supplementary Figure 1. Time course assay of inhibitors at 1 μM .



Supplementary Figure 2. Time course assay of inhibitors at 1 μM .



Supplementary Figure 3. Proposed interactions between the carboxylic acid residues in the active site and a pyrazole core structure. Similar to the imidazole-based inhibitors in the text, these structures are all interchangeable by hydrogen transfer. The tautomers in the pyrazole inhibitors are also equivalent by symmetry. The most significant differences are the pK_a's of the heterocycles, the absence of the acidic C2 hydrogen in the pyrazole ring as compared to the imidazole ring, and the different locations of the hydrogen bond donors and acceptors in the two heterocycles.