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Design of bivalent ligands using hydrogen bond linkers: synthesis and evaluation of inhibitors for human β-tryptase

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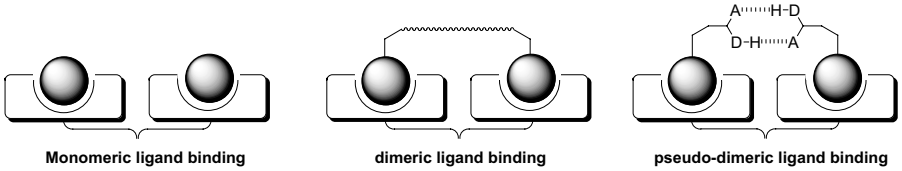
Abstract—We exploit the concept of using hydrogen bonds to link multiple ligands for maintaining simultaneous interactions with polyvalent binding sites. This approach is demonstrated by the syntheses and evaluation of pseudo-bivalent ligands as potent inhibitors of human β-tryptase.

Symmetrical molecular assembly of identical subunits is essential for many biological functions such as ion channel conductance,[1] signal transduction,[2] gene expression,[3] and enzyme catalysis.[4] There are often symmetry related multiple binding sites in these assemblies that are targeted by drug or modulator molecules. Thus, chemically symmetrical drugs involving identical functional groups separated by a spacer may be of significance in order to provide optimal activity.[5]

As an alternative to the common approach of covalently linking identical functional groups in a drug molecule,[6] van der Waals interactions such as hydrogen bonds may be invoked in constructing a molecular cluster that is optimal for the corresponding biological molecule as illustrated in Scheme 1. In comparison to a covalent linkage, a hydrogen bond linker may offer greater tolerance in terms of both distance and vector of bonding. It remains the relative size of individual monomers, which aggregates ideally under the cooperative template effect only upon binding with the biological molecule.[7] We report here an example of this approach from the synthesis and evaluation of amide derivatives as potent inhibitors of human β-tryptase.

Human β-tryptase is a trypsin-like serine protease specific to mast cells and is implicated in the pathogenesis of allergic and inflammatory diseases.[8] The X-ray analysis of its crystal structure revealed a tetrameric architecture consisting of four quasi-identical monomers.[9] The



Scheme 1.

Keywords: Hydrogen bonds; Tryptase; Enzyme inhibitors; Structure-based design.
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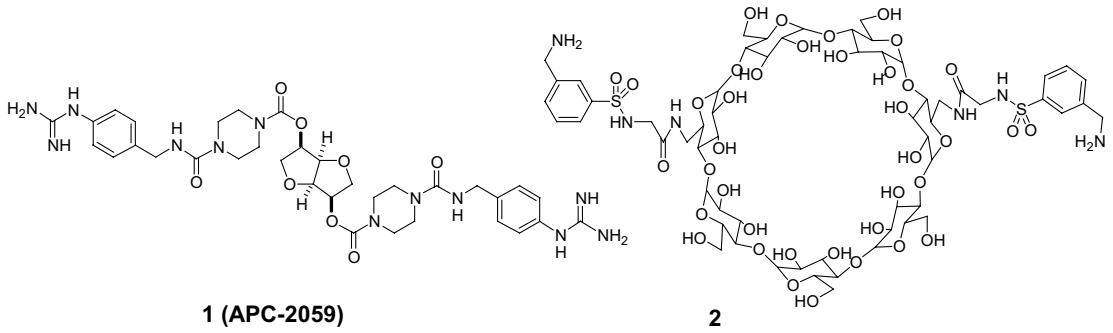


Scheme 2.

tetramer is believed to be the enzymatically active form of human β-tryptase, although its monomeric form has recently been reported to be catalytically active.[10]

Many symmetrical inhibitors with dibasic units covalently linked by various bridging skeletons have recently been reported among potent inhibitors of human mast cell tryptase.[11–13] For example, compound **1**, APC-2059, had reached the stage of clinical development,[11b] whereas compound **2** incorporated a rigid cyclodextrin template as a spacer (Scheme 2).[12a]

Our approach is based on the structural information obtained from the crystal structure of tetrameric β-tryptase (Fig. 1).[9,13h] It revealed close contact between two identical monomeric inhibitors. We envisioned that incorporation of a primary amide group at the specific position would provide an almost perfect bridge to link two inhibitors at the binding sites by forming bifurcated hydrogen bonds (Scheme 3).

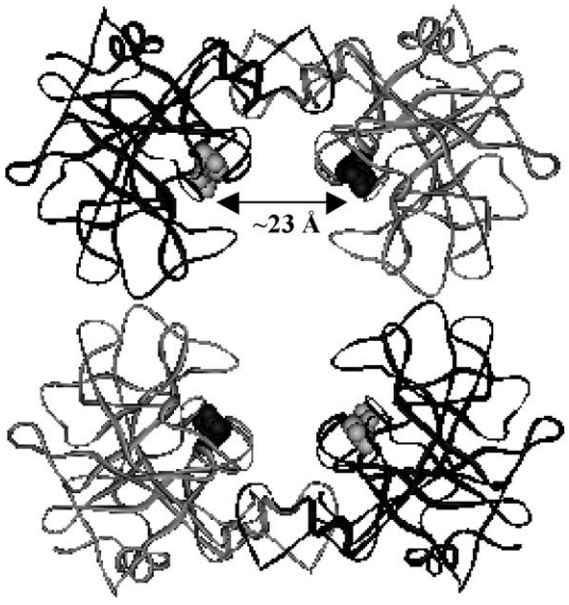
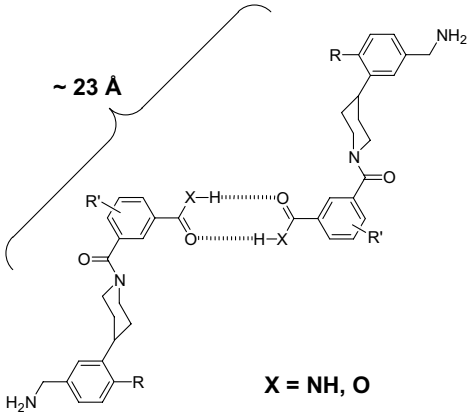


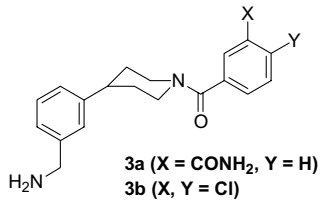
Figure 1. Ribbon rendered tetrameric structures of β-tryptase complexed with inhibitors shown in space-filled spheres (PDB entry: 1AOL).



Scheme 3. Dimeric assembly via hydrogen bond. The distance between the centroids of benzylamine aromatic rings is about 23 Å.

We first made compound **3a** (Scheme 4). To our surprise, it was a weaker inhibitor of β-tryptase than the similar compound **3b** where the bifurcating primary amide is replaced by a chloro atom.[13d] We speculate that intermolecular hydrogen bonding is insufficient to compensate for the desolvation energy that is required for compound **3a** binding to the enzyme.

Subsequently, compounds **4a**–**e** (Table 1) were synthesized according to Scheme 5. Thus, the acid **5** was condensed with thiophene *N*-hydroxy-acetamidine **6**, prepared by refluxing thiophene acetonitrile with hydroxylamine in methanol, to produce oxadiazole **7**. After hydrolysis of the ester, the acid **8** was obtained. This acid set the stage for the condensation reaction with amine template **9** to give compound **10**. Removal of the protection group yielded the desired compound **4a**–**e**.

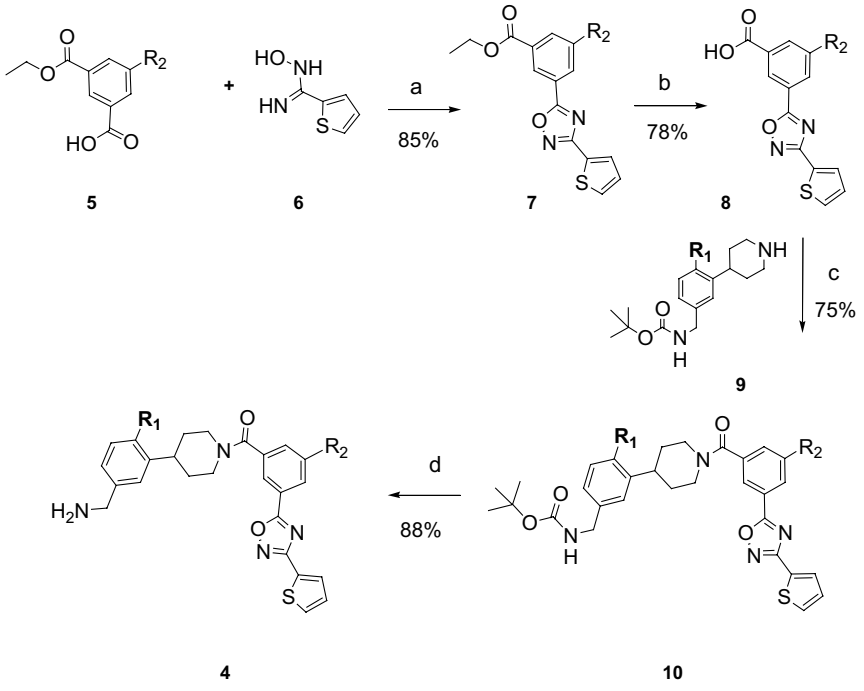


Scheme 4. Structures of β-tryptase inhibitors.

Table 1. Tryptase inhibitors binding potency

Compound	R_1	R_2	K_i (nM) Tryptase	IC_{50} (μM)		
				hERG	CYP2C9	CYP2C19
4a	F	H	4.3	0.8	0.48	2.19
4b	F	CO_2Et	59	ND[a]	ND	ND
4c	F	CO_2H	88	76.8	>50	>50
4d	H	$CONH_2$	1.5	>30	ND	ND
4e	F	$CONH_2$	1.3	17.1	5.38	9.69

[a] ND = not determined.



Scheme 5. Syntheses of the pseudo-dimer compounds. Reagents and conditions: (a) (1) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), (2) toluene, 120 °C; (b) (1) LiOH, dioxane, H_2O, rt, 2 h, (2) NH_4Cl, DMF, *O*-[(ethoxycarbonyl)cyanomethylenamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TOTU), Pr_2NEt, 0 °C to rt, overnight, (3) LiOH, dioxane, H_2O, rt, 2 h; (c) DMF, TOTU, Pr_2NEt, 0 °C to rt, overnight; (d) HCl, dioxane, rt.

Compounds **4a**–**e** were tested in a tryptase assay[14] and the results are summarized in Table 1. From Table 1, it is apparent that the compounds with the hydrogen bond formation capability showed increased potency (compare **4a**–**b** vs **4d**–**e**). Since carboxylic groups are seldom found to be in proximity in protein structures, except for those involving special functions as in the active site of an aspartyl protease, a favorable interaction between two ionized carboxylic acids is unlikely. Thus, it is not surprising that **4c** is less potent than the corresponding amide **4e**. Preliminary X-ray crystallographic study of the complex between β-tryptase enzyme and **4e** revealed indeed the assembly of the expected dimer via hydrogen bonds between the two inhibitors, which contributed to the increased potency.

To assess the potential likelihood of drug-induced QT prolongation and drug–drug interactions, compounds **4a**–**e** were profiled in hERG channel and CYP inhibition assays. According to the predictions of our QSAR CoMSiA model,[15] the amides were expected to have improved profile against the hERG channel blockage. Indeed, the experimental measurements[16] indicated these compounds, as compared to compound lacking the bifurcating amide/acid substituent, are less potent inhibitors against the hERG channel, CYP 2C9 and 2C19 isozymes.

In conclusion, we have demonstrated that hydrogen bonds, instead of a covalent bond, may be invoked in constructing a molecular cluster that is optimal for the corresponding biological molecule. As compared to dimers linked by a covalent bond, the pseudo-dimer approach via hydrogen bond may offer some advantages in terms of lower molecule weight, preferable solubility and pharmacokinetic property.

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