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A comparative study of warheads for design of cysteine protease inhibitors

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ABSTRACT

The effects on potency of cruzain inhibition of replacing a nitrile group with alternative warheads were explored. The oxime was almost an order of magnitude more potent than the corresponding nitrile and has the potential to provide access to the prime side of the catalytic site. Dipeptide aldehydes and azadipeptide nitriles were found to be two orders of magnitude more potent cruzain inhibitors than the corresponding dipeptide nitriles although potency differences were modulated by substitution at P1 and P3. Replacement of the α methylene of a dipeptide aldehyde with cyclopropane led to a loss of potency of almost three orders of magnitude. The vinyl esters and amides that were characterized as reversible inhibitors were less potent than the corresponding nitrile by between one and two orders of magnitude.

Cysteine proteases have been linked to a range of human diseases.[1–4] In particular, members of this target class are seen as potential targets for therapeutic intervention in the treatment of parasitic diseases such as Chagas disease,[5] African sleeping sickness[6] and leishmaniasis.[7] A commonly employed tactic in design of cysteine protease inhibitors is to incorporate an electrophilic moiety that can form a covalent bond between the catalytic cysteine and the inhibitor.[3,4,6–13] These functional groups are commonly termed 'warheads' and the electrophilic center is typically an unsaturated carbon atom. Formation of a covalent bond between inhibitor and enzyme allows the cysteine thiol to be exploited as a molecular recognition element even when the associated part of the protein molecular surface is suboptimal for formation of non-covalent interactions.[10] For example, the molecular surface associated with the catalytic thiol of a cysteine protease is typically saddle-shaped (i.e. concave in one direction but convex in another).[11] The nitrile group[3,11–14] is considered to be a particularly useful warhead for cysteine protease inhibition on account of its metabolic stability, polarity and small contribution to molecular size.

Although covalent binding to proteins is often believed to be irreversible, this does not have to be the case. While reversibility of binding may reduce the risk of adverse effects, irreversible binding can result in prolonged duration of action.[3,9,10] Selectivity can be achieved[15] with irreversible inhibitors but irreversibility to the extent that a drug remains covalently bound to peptide fragments after protein degradation is generally undesirable because it can lead to immunogenicity.[11] The functional behavior of warheads is sometimes assumed to be determined entirely by electrophilicity although atoms bonded to the electrophilic center can form non-covalent interactions with target proteins that also modulate affinity. Examples of these non-covalent interactions are shown in Fig. 1 for inhibitors based on nitrile[16] and α-ketoamide[17] warheads. Fig. 1 also illustrates how a 1,2-dicarbonyl

Abbreviations: Boc, *tert*-butyloxycarbonyl; Cbz, carboxybenzyl; CC_{50}, half maximal cytoxicity concentration; EC_{50}, half maximal effective concentration; EDC, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; IBX, 2-iodoxybenzoic acid; K_i, inhibitory constant; pCC_{50}, $-\log_{10}(CC_{50}/M)$; pEC_{50}, $-\log_{10}(EC_{50}/M)$; pK_i, $-\log_{10}(K_i/M)$; SAR, structure activity relationship; ΔpK_i, change in pK_i resulting from a structural transformation.

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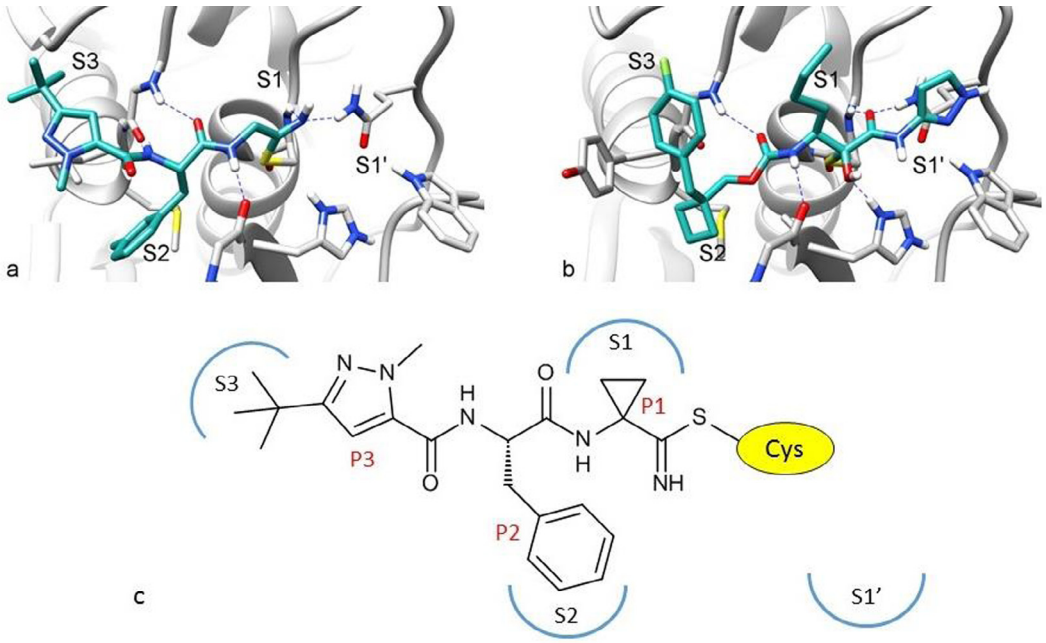


Fig. 1. (a) Binding mode of nitrile inhibitor to cathepsin L (PDB refcode: 3HHA). (b) Binding mode of a ketoamide inhibitor to cathepsin K (PDB refcode: 1TU6). (c) Schematic view of a dipeptidyl nitrile bound to a papain-like cysteine protease.

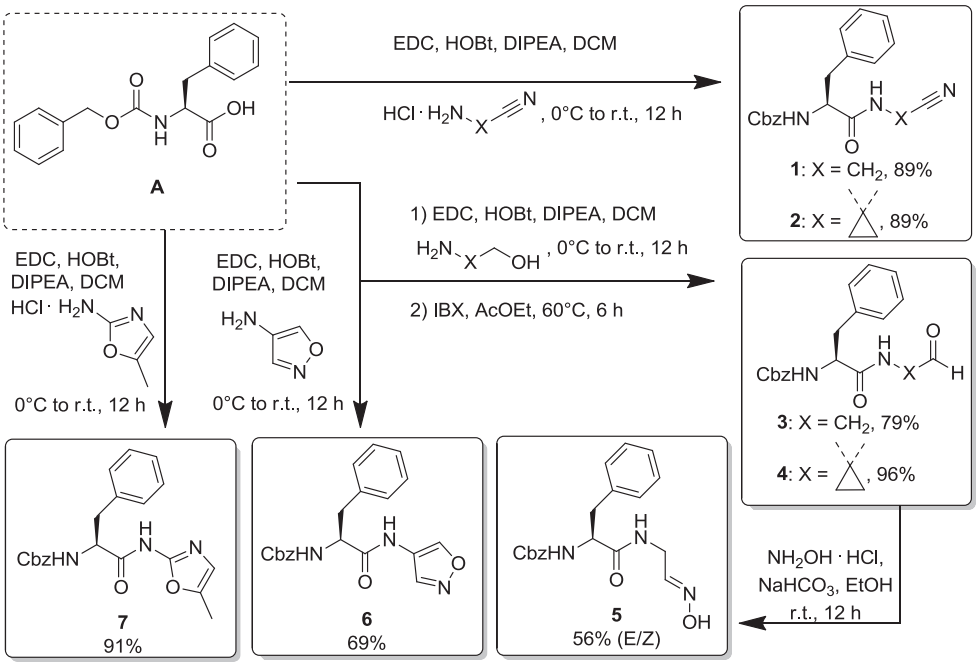
warhead provides easier access than the nitrile to the prime side of the catalytic site.

Warhead exchange can be seen as analogous to bioisosteric substitution[18] and knowledge of potency differences between warheads is potentially valuable in situations where activity has been observed for a cysteine protease inhibitor in a cell-based assay but the target is unknown.[19] The exchange of one warhead with another can be seen as a structural transformation and these can be written[20] as [X → Y] where the labels X and Y may be structure numbers, warhead names (e.g. nitrile) or substructures encoded in line notation (e.g. C#N). The potency difference, ΔpK_i, corresponding to the [X → Y] transformation can be written as:

$$\Delta pK_i[X \rightarrow Y] = pK_i[Y] - pK_i[X]$$

This study has three objectives. First, to investigate the transferability of published effects of warhead replacement, for example [nitrile → aldehyde], to cruzain which is currently of interest as an antichagasic target. Second, to evaluate warheads such as the oxime that are based on a carbon-nitrogen double bond. Third, to explore the potential of substitution for mitigating potential liabilities (e.g. instability, irreversible binding) associated with warheads.

Synthesis of compounds is summarized in Schemes 1–3. Compounds **1**–**4**, **6**, **7** were synthetized from commercially available amines and the *N*-Cbz-protected L-phenylalanine activated by



Scheme 1. Synthesis of nitrile, aldehyde, oxime and heteroaromatic warheads.