Design of fragment-derived, covalent inhibitors of SARS-CoV-2 main protease

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ABSTRACT

This study discusses two potential inhibitors of SARS-CoV-2 main protease, in which warheads (aldehyde and nitrile) capable of reversible covalent bond formation with the catalytic cysteine, are linked to a fragment-derived inhibitor.

INTRODUCTION

Inhibitors **1** (see assay results) and **2** (see assay results) that had been designed within the Covid Moonshot initiative by linking hits from crystallographic fragment screening of the SARS-CoV-2 main protease [1] are shown in Chart 1. The crystal structure of the main protease with **1** bound is shown in Figure 1. The pyridine ring of **1** occupies the S1 subsite and mimics the pyrrolidinone P1 substituent that is common to the molecular structures of a number of peptidomimetic inhibitors [2,3,4] and it might be anticipated that the pyridine 4-methyl substituent would stabilize the bound conformation.

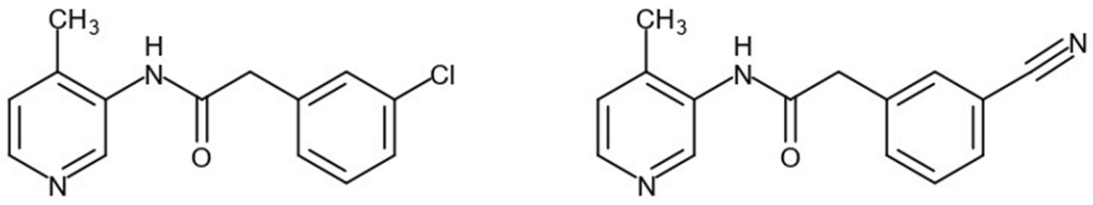


Chart 1. Fragment-derived inhibitors of SARS-CoV-2 main protease

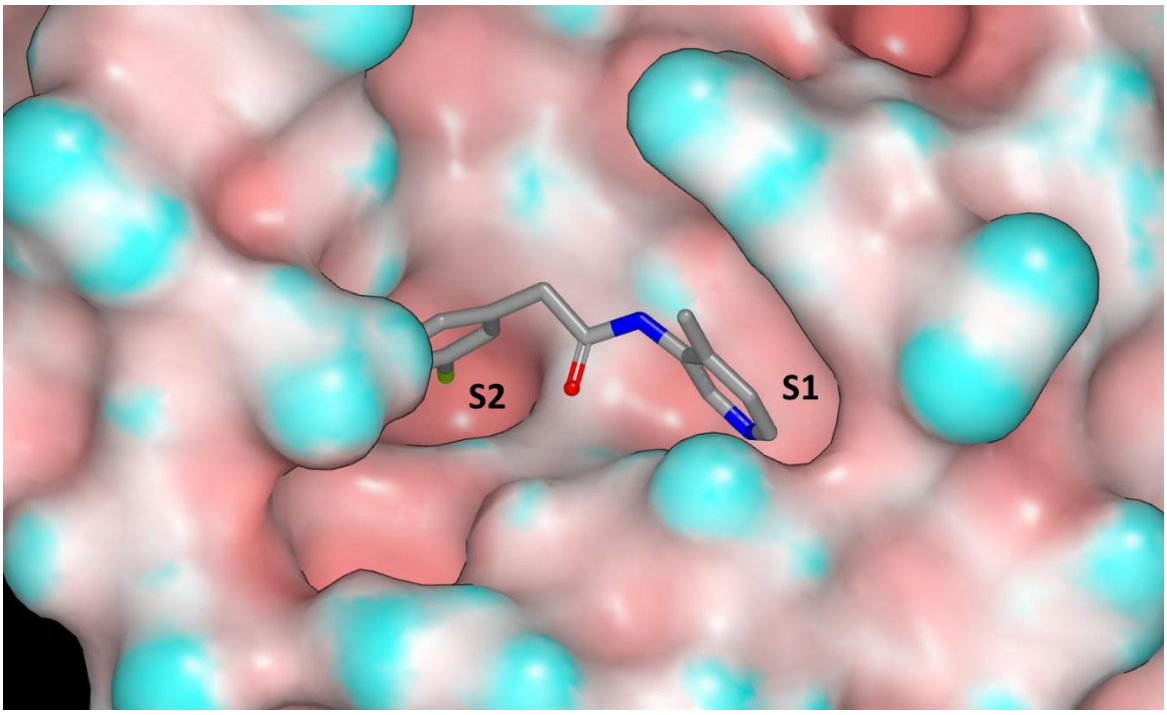


Figure 1. X-ray crystal structure of SARS-CoV-2 main protease with **1** bound. The molecular surface of protein (created using VIDA from OpenEye) is coloured by curvature.

DESIGN

As discussed previously [5], the amide nitrogen in a fragment from which **1** and **2** were derived presents opportunities for linking warheads capable of forming a covalent bond with the catalytic cysteine. Compound **1** was chosen as the starting point because it is 0.4 to 0.5 log units more potent than **2** in the enzyme inhibition assays and the nitrile substituent is likely to be associated with a greater degree of geometric constraint (a particular concern in design of covalent inhibitors). Compounds **3** (aldehyde) and **4** (nitrile) are shown in Chart 2 and potential binding modes for these compounds are illustrated in Figure 2. Aldehydes such as **3** would be expected to be more potent inhibitors of cysteine proteases than the corresponding nitriles although potency differences for inhibitors incorporating the two warheads will, in general, vary with target and substructural context [6]. It would also be anticipated that pharmacokinetic characteristics will be better for nitriles than the corresponding aldehydes which may be dosed as prodrugs [2,4,7,8]. Both **3** and **4** would be expected to be competitive, reversible inhibitors of SARS-CoV-2 main protease.

The poses for **3** and **4** were generated by editing the ligand model in the X-ray crystal structure of **1** bound to of SARS-CoV-2 main protease and have not been refined using molecular mechanics.

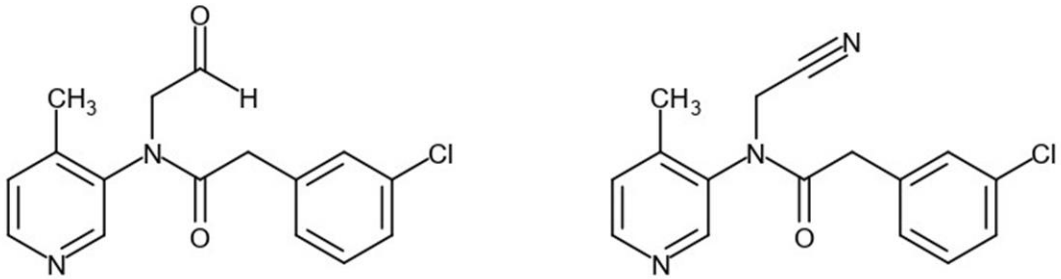


Chart 2. Potential inhibitors of SARS-CoV-2 main protease designed by linking warheads to **1**

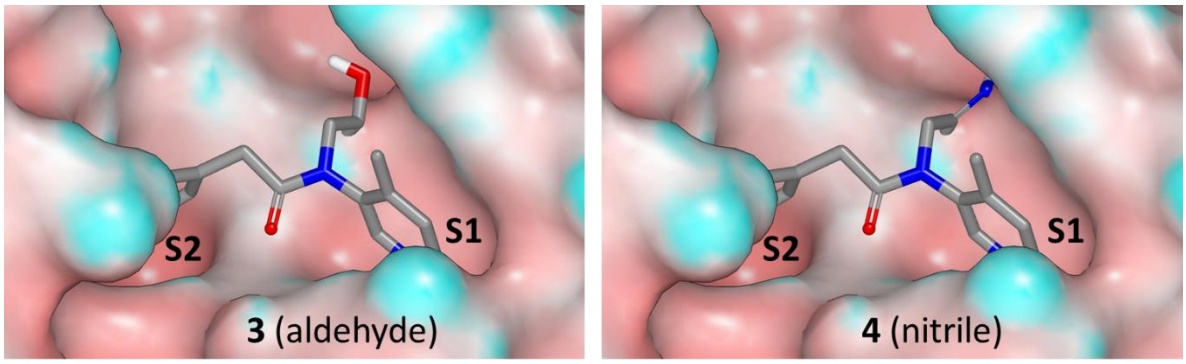
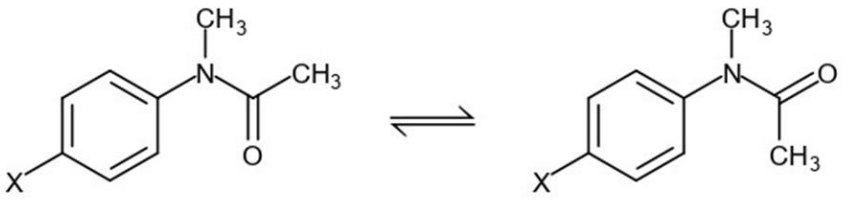


Figure 2. Potential binding modes for **3** and **4**. The molecular surface of protein (created using VIDA from OpenEye) is coloured by curvature and corresponds to the X-ray crystal structure of **1** bound to SARS-CoV-2 main protease.

One factor that needs to be considered in design is the effect on the amide geometric preference (*trans* versus *cis)* of linking a saturated carbon to the amide nitrogen. N-Methylacetanilide exists primarily in the *cis* conformation and the measured ΔG° values (in CD_2Cl_2 at 213 K) given in Table 1 illustrate how substitution on the phenyl ring can modulate the relative stability of the *cis* and *trans* forms [9]. The calculated values [10] corresponding to some of these measurements suggest that these calculations tend to over-predict energy differences.

Table 1. Relative stabilities of *cis* and *trans* forms of substituted N-methylacetanilides [9]



trans *cis*

X	ΔG°[*trans*→*cis*]
H	–1.35
NMe_2	–1.64
Br	–1.13
CN	–0.67
CF_3	–0.92
NO_2	–0.55

The ΔG° value of measured at 213 K for N-methylacetanilide corresponds to a log unit of potency at normal human body temperature (neglecting heat capacity differences between the *cis* and *trans* forms). This figure quantifies the cost in potency for converting the *cis* conformation of N-methylacetanilide (most stable in solution) to the *trans* confirmation (corresponding to the bound conformation of **1**). Aza-substitution of the benzene ring of N-methylacetanilide would be expected to result in a reduction in the difference in energy between the *cis* and *trans* conformations since it is equivalent to adding an electron-withdrawing substituent to the ring [9]. In cases where the bound conformation is relatively unstable, it may be possible to modify the molecular structure so that it is locked into the bound conformation [11]. One way in which **3** and **4** could be locked into their proposed bound conformations would be to make the amides cyclic.

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