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Structure-based design and optimization of potent inhibitors of the adenoviral protease

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ABSTRACT

Adenoviral infections are associated with a wide range of acute diseases, among which ocular viral conjunctivitis (EKC) and disseminated disease in immunocompromised patients. To date, no approved specific anti-adenoviral drug is available, but there is a growing need for an effective treatment of such infections. The adenoviral protease, adenain, plays a crucial role for the viral lifecycle and thus represents an attractive therapeutic target. Structure-guided design with the objective to depeptidize tetrapeptide nitrile **1** led to the novel chemotype **2**. Optimization of scaffold **2** resulted in picomolar adenain inhibitors **3a** and **3b**. In addition, a complementary series of irreversible vinyl sulfone containing inhibitors were rationally designed, prepared and evaluated against adenoviral protease. High resolution X-ray co-crystal structures of representatives of each series proves the successful design of these inhibitors and provides an excellent basis for future medicinal chemistry optimization of these compounds.

Adenoviruses are non-enveloped, double-stranded DNA viruses with more than 55 serotypes identified that have been classified into seven subgroups (A–G).[1] Specific serotypes are linked to a number of acute, highly contagious infections such as gastrointestinal illness, disseminated adenovirus disease in immunocompromised patients and ocular conjunctivitis such as epidemic keratoconjunctivitis (EKC).[2] The occurrence of severe adenoviral infections is increasing due to the growing numbers of transplantations. To date, there are no approved anti-adenoviral therapeutics available, which contrasts with a growing unmet medical need for specific anti-adenoviral drugs, in particular for immunosuppressed patients or for an effective treatment of EKC.[3–5]

Adenoviruses, as all viruses, encode their own protease and depend on its activity for the development of infectivity. The protease of adenovirus, the cysteine protease adenain[6,7] plays a key role in several steps of the viral life cycle.[8] Adenain is involved in the uncoating of virus particles during viral entry,[9] it is essential for the cleavage of seven core precursor proteins that are required for the formation of mature virions[10] and it is also implicated in the cleavage of cytokeratins, which leads to host cell lysis.[11,12] In addition, adenain represents an attractive therapeutic target as it lacks human homologues. Thus, selective inhibitors of the adenoviral protease may offer efficacious treatments for adenoviral infections.

The increasing need for specific and effective anti-adenoviral therapies prompted us to engage in the development of inhibitors of adenain, in particular for the treatment of EKC caused by serotypes 8, 37, and 64. We recently disclosed our initial efforts in this area, which led to the discovery and structure-based optimization of glycine- and pyrimidine nitrile inhibitors of the adenoviral protease. Among other structures our previous hit discovery campaign identified tetrapeptide nitrile **1** as a potent inhibitor of adenain.[13] As part of our ongoing efforts to optimize this compound we aimed at further reducing the peptidic character of **1** (Fig. 1) by designing a novel replacement of the P4–P3 amide bond and by investigating irreversible inhibitors.

While tetrapeptide nitrile **1** proved to be a low nM inhibitor of adenain in vitro, it was inactive in a viral replication assay. It was thus the primary objective of our work to develop compounds with increased permeability and, hence, improved cellular potency. Guided by the X-ray co-crystal structure of adenain and bound **1** we designed a novel peptidomimetic scaffold, where the NH–CH(CH_2Ph) unit of the central Phe residue is replaced by a meta-substituted phenyl ring. Molecular modelling suggested that the original P4–P3 binding mode could be conserved by introducing an ethanone linker between this phenyl moiety and the P4 phenyl group together with an ortho-substituent on the central phenyl

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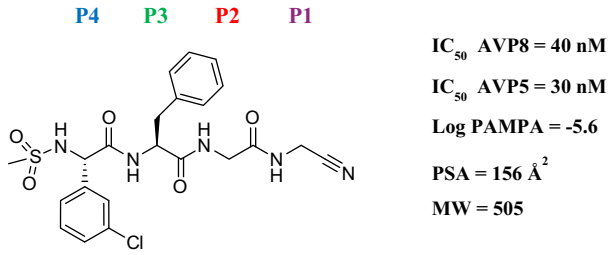


Figure 1. Structure of tetrapeptide nitrile inhibitor **1** and profiling data.

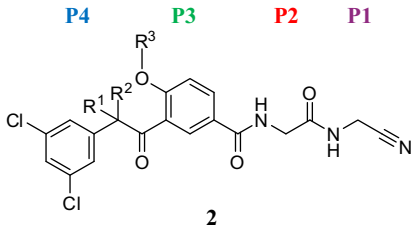


Figure 2. New designed scaffold **2**.

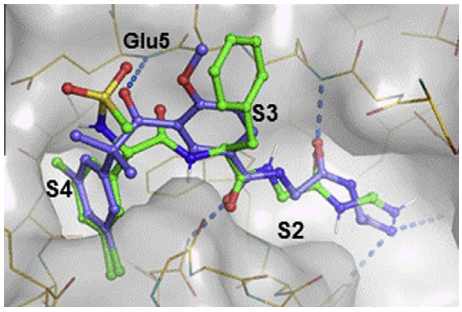


Figure 3. Superposition of the putative docking pose of **2c** (blue) with the X-ray co-crystal structure of **1** (green, PDB code 4PIE). The picture was produced using PyMol.

ring. This led to structures of type **2** (Fig. 2) as targets for synthesis and biological evaluation. The keto group of the ethanone linker was predicted to form a hydrogen bond with the amide NH of Glu5, while the ortho-substituent would force the carbonyl group out of the plane of the phenyl ring for optimal orientation. In addition, the modelling also suggested that for proper positioning of the 3,5-dichlorophenyl moiety into the S4 subsite, gem-dimethyl substitution at the benzylic position would be highly advantageous. The 3,5-dichloro substitution at the P4 phenyl ring enhances hydrophobic interactions in S4, as we have already shown in our previous work.[13]

Figure 3 illustrates the binding model of our newly designed peptide mimetic **2** to adenain and its overlay with the X-ray co-crystal structure of **1**.

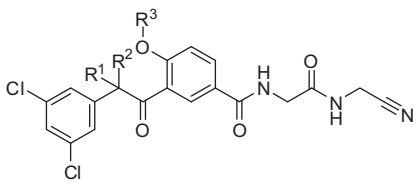
The SAR established for this new template **2** is summarized in Table 1. Compound **2a**, which does not bear a substituent at the benzylic position of the P4 group (R^1, R^2 = H), is only a 3 μM inhibitor of adenain 8 (AVP8). Attachment of one methyl group at this position gave derivative **2b**, which displayed 3-fold improved potency. Importantly, and quite intriguingly, disubstitution at the benzylic position as in compound **2c** resulted in an IC_{50} of 40 nM, which makes this analog equipotent with the initial peptide nitrile **1**. This outcome is in accordance with the original modelling predictions; most likely, the gem-dimethyl substitution locks inhibitor **2c** in the preferred conformation for binding to the enzyme. Compound **2c** combined high potency with good permeability (Table 1), but it exhibited rather low solubility. Inspection of the structure indicated that larger alkoxy groups than methoxy should be tolerated at the central phenyl ring, thus offering an option to introduce solubilizing groups. This idea resulted in the design of compound **2d**, which maintained the potency of **2c** and displayed greatly improved solubility.

Unfortunately, when tested in our anti-viral cytopathic effect (CPE) assay both compounds **2c** and **2d** exhibited only weak activity.

The X-ray co-crystal structure of inhibitor **2c** bound in the active site of adenoviral protease 8 (AVP8) is depicted in Figure 4 and provides experimental proof for the predicted binding mode. The inhibitor is covalently bound to the enzyme by thioimidate formation between the cyano group and the active site cysteine 122. The thioimidate NH forms a hydrogen bond with the carbonyl oxygen of the side chain of glutamine 115. The new scaffold links positions P2 through P4 in the non-primed part of the binding cleft and displays four hydrogen bonding interactions with the active site of adenain.

To further depeptidize scaffold **2** we replaced the P2–P1 unit by a 4-aminomethyl-2-cyano-pyrimidine, following a similar logic as for the optimization of a different lead series.[13] The resulting achiral peptidomimetics **3a** and **3b** are both exceptionally potent inhibitors of adenain (Table 2) only incorporating a single amide bond. Figure 5 captures the X-ray co-crystal structure of inhibitor

Table 1
SAR and profiling data for inhibitors **2a–d**



Compd	R^1	R^2	R^3	IC_{50} AVP8[a] [μM]	IC_{50} AVP5[a] [μM]	Log PAMPA [10^{-6} cm s^{-1}]	Sol[b] [mM]	PSA [Å^2]
2a	H	H	CH_3	2.74	1.1	nd[c]	nd[c]	108
2b	H	CH_3	CH_3	0.95	0.31	nd[c]	nd[c]	108
2c	CH_3	CH_3	CH_3	0.04	0.03	−5.0	0.05	108
2d	CH_3	CH_3	$(CH_3)_2N(CH_3)_2$	0.01	0.007	−5.5	>1	111

[a] Inhibition of AVP8 and AVP5[14] in an FLT assay using Ac-WLRGG*ARC(PT14)-NH2 as substrate. Data represent mean of at least 2 experiments performed in duplicate. Individual data points in each experiment were within 2-fold range of each other.
[b] High throughput equilibrium solubility at pH 6.8.
[c] Not determined.