SUPPORTING INFORMATION

Novel Antiviral C5-Substituted Pyrimidine Acyclic Nucleoside Phosphonates Selected as Human Thymidylate Kinase Substrates

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SI-Table 1. Structural data from X-ray of human TMP kinase

† PDB ID: 2xx3	
Data collection	
Space group	C2
Unit cell (Å)[e]	
a	102.44
b	38.32
c	73.16
Resolution (Å) [e]	35-2.2 [e]
Observed reflections	34 443
Unique reflections	15 671
Completeness (%)[1]	95.7 (94.5)**a**
I/σ(I)[1]	7.9 (1.9)
Rsym**b** (%)	11.7 (62.8)**a**
Refinement statistics	
Rcryst**c** (%)	22.9 **a**
Rfree**d** (%)	25.8e
Number of water molecules	274
R.m.s.d.	
bond lengths (Å)[e]	0.007
bond angles (°)	1.355
Ramachandran statistics**e** (%)	
most favoured regions	96.4
additional allowed regions	2.6
disallowed regions	1.0

a Numbers in parentheses represent values in the highest resolution shell (last of 10 shells).

b $R_{sym} = \Sigma_h \Sigma_I |I(h,i) - \langle I(h) \rangle| / \Sigma_h \Sigma_I I(h,i)$ where I(h,i) is the intensity value of the i-th measurement of h and $\langle I(h) \rangle$ is the corresponding mean value of I(h) for all I measurements.

c $R_{cryst} = \Sigma ||Fobs| - |Fcalc|| / \Sigma |Fobs|$, where |Fobs| and |Fcalc| are the observed and calculated structure factor amplitudes respectively

d Rfree is the same as R_{cryst} but calculated with a 5% subset of all reflections that was never used in crystallographic refinement.

e As evaluated by COOT.

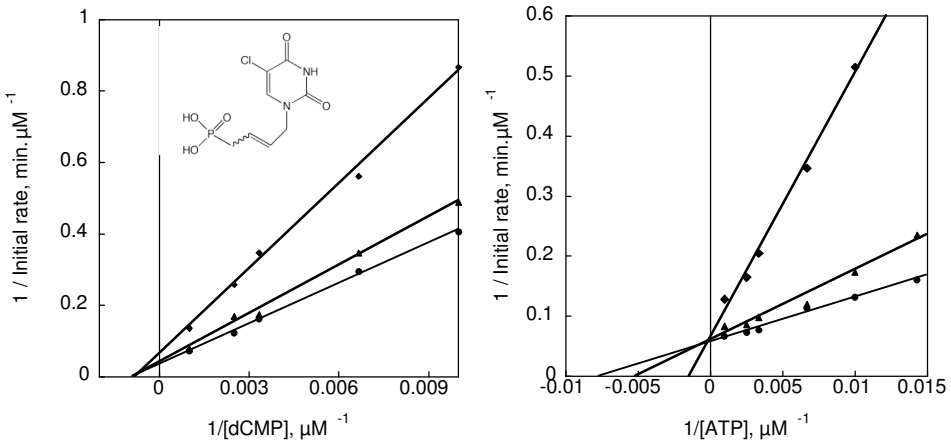
SI-Table 2: Changes in hUCK circular dichroism signal at 220 nm upon ligand binding

All circular dichroism measurements were acquired using a JOBIN-YVON CD6 spectropolarimeter. The instrument outputs were calibrated with D(+)-10-camphorsulfonic acid. The spectra were scanned using a 1-nm slit at 25 °C in a quartz optical cell with a 1 mm path length, unless specified otherwise. Spectra were recorded between 185-300 nm with 1 nm step. Typically, four scans were accumulated and averaged after buffer spectra subtraction and baseline correction. The CD spectrum of each complex hUCK-ligand was recorded in Tris-HCl buffer (50 mM KCl and 50 mM $MgCl_2$, pH 7.4). The enzyme (10 µM) was incubated with 1 mM dCMP, 2 mM cidofovir, or 2.5 mM (E)-5Cl-UbutP (**9c**) at 25°C. Spectra of the ligands were recorded and then subtracted to the corresponding enzyme-ligand spectrum. CD measurements are reported as $\Delta\varepsilon$ (M^{-1} cm^{-1}). The relative helix content was deduced according to Zhong and Johnson as the percent of helix = [$\Delta\varepsilon_{222\,nm}$ x -10], where $\Delta\varepsilon_{222\,nm}$ is the dichroic increment at 222 nm per residue.

	$\Delta\varepsilon$ ($M^{-1}cm$)
hUCK	- 0.268
hUCK + dCMP	- 0.385
hUCK + cidofovir	- 0.321
hUCK + *(E)*-5Cl-UbutP (**2d**)	- 0.206

hUCK spectrum without ligand shows the characteristic shape of a α-helix-rich protein with minima at 220 nm and 208 nm and a maximum at 192 nm. Addition of ligands (CMP and dCMP) resulted in significant changes in intensity of the double minima, most strikingly in a decrease of the CD signal around 220 nm. This variation, usually understood, as an increase in α-helix content, should be correlated here to the closure of NMP and LID domains, involving rotation of several α-helices, as proposed from crystallographic data.[21] Addition of cidofovir also induced a signal decrease, although weaker. Addition of 2 mM (*E*)-5Cl-UbutP (**9c**) increased slightly the CD spectrum in the 220 nm region. The decrease of the CD spectrum at 220 nm seems to correlate with enzyme activity as substrates decrease the signal and a non-substrate like **9c** does not.

SI-Figure 1: Inhibition of human UCK by (*E*)-5Cl-UbutP (**9c**)



(A) (B)

Double reciprocal plots at fixed concentrations of (*E*)-5Cl-UbutP (**9c**) (A, B) show the initial velocity as a function of the dCMP concentration (A) with 0.5 mM ATP. When ATP was varied (B), dCMP was 1 mM. The human UCK concentration was 8 nM. The results shown are for a typical experiment repeated twice (15%). (●), no inhibitor (all plots); (▲)50 µM **9c** in A, 100 µM **9c** in B; (◆) 200 µM **9c** in A, 1mM **9c** in B. Surprisingly, 5Cl-UbutP (**9c**) was a non-competitive dCMP inhibitor (K_I = 0.9 mM), but an ATP competitive inhibitor (K_I = 0.26 mM). The non-competitive inhibition pattern of [*E*]-5Cl-UbutP with dCMP is explained if the two ligands (dCMP and 5Cl-UbutP) indeed binds CMP site but to different conformations of the enzyme. The acceptor sites of human UMP-CMP kinase and adenylate kinase 1, which are 40% identical, adopt an open conformation in absence of substrates.[21] The acceptor and LID domains are extremely mobile in these enzymes and close when a substrate is bound.