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Delineation of the molecular mechanisms of nucleoside recognition by cytidine deaminase through virtual screening

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Abstract

Cytidine deaminase (3.5.4.5, CDA), an enzyme of the pyrimidine salvage pathways, is responsible for the degradation and inactivation of several cytidine-based antitumoral drugs, such as cytarabine, gemcitabine, decitabine, and azacytidine. Thus, CDA inhibitors are highly sought after as compounds to be co-administered with said drugs in order to improve their effectiveness.

Alternatively, the design of antitumoral drugs not susceptible to the action of CDA is also regarded as an attractive solution. Here, we describe a virtual screening for CDA ligands based on chemical similarity and molecular docking. The campaign led to identification of three novel inhibitors and one novel substrate, with a 19% hit rate, and allowed a significant extension of the structure-activity relationships, also in light of the compounds that resulted inactive. The most active compound identified through the screening was the inhibitor pseudisocytidine, which has the potential to serve as a lead for highly stable compounds. The study also delineated the detrimental effect of 5-aza and 6-aza substitutions, the incompatibility of the presence of an amino group at the 3'-position, as well as the presence of very strict steric requirements around the 2'-arabino position and, even more, the *N4*-position. Importantly, these features can be exploited for the design of novel antineoplastic agents resistant to the action of CDA.

Keywords

Cancer; Enzymes; Hydrolases; Inhibitors; Virtual screening

Introduction

Cytidine deaminase (3.5.4.5, CDA) is an intracellular enzyme of the pyrimidine salvage pathways that catalyzes the deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively. Structurally, human CDA is a homotetrameric enzyme with an overall *C*₂₂₂ symmetry and four independent active sites, each of which containing a Zn²⁺ ion. We dubbed the four subunits A1, A2, B1, and B2 – see Figure 4 of Costanzi et al.^[1] for further explanation. The enzyme is active only as an intact tetramer, since three different monomers concur to the formation of each of the four active sites.^[1–5] For example, the

active site enclosed within subunit A1 is also lined by residues of subunits B1 and B2 (Figure 1).

Importantly, human CDA recognizes as substrates a number of cytidine-based antitumoral drugs – such as cytarabine, gemcitabine, decitabine, and azacytidine – leading to their inactivation. For this reason, CDA inhibitors are highly sought after as compounds to be co-administered with said drugs in order to improve their effectiveness.^[6] For instance, it has been recently reported that CDA inhibition by the investigational drug zebularine enhances the antineoplastic activity of decitabine (5-aza-2'-deoxycytidine), a drug approved for the treatment of patients with myelodysplastic syndromes (MDS).^[7]

Here, we describe a virtual screening for CDA ligands based on chemical similarity and molecular docking, conducted with the primary intent of shedding further light onto the structural requirements of nucleoside recognition in order to assist the future design of novel inhibitors or antitumoral drugs not susceptible to deamination. The database of compounds subjected to screening was the “Open Chemical Repository Collection” of the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI) – henceforth NCI database. Although the human enzyme has been crystallized,^[3] we chose to work with its murine homologue because its structure was solved at a higher resolution and with lower B-factors. In particular, our docking experiments targeted the crystal structure of the murine enzyme crystallized in complex with cytidine (PDB ID: 2FR6),^[8] which, within the active site, shares 95% of sequence identity with its human homologue (Figure 1) – overall, human and murine CDA share a sequence identity of 81.5% and have a distance of 21.5 PAM (percentage of accepted mutations).^[1] The only non conserved residue within a radius of 5 Å from the bound nucleosides is residue 64, which is an isoleucine in the human and a valine in the mouse enzyme (Figure 1). However, the sidechain of Ile/Val64 points away from the active site and is not involved in interactions with the ligands. Notably, our virtual screening campaign led to the identification of three novel inhibitors and one novel substrate, and allowed a significant extension of the structure-activity relationships, also in light of the compounds that resulted inactive.

Results

Virtual screening: a) molecular fingerprint-based filtering

A collection of 260,071 molecules of the NCI database was subjected to a molecular fingerprint analysis aimed at identifying all compounds showing similarity with cytidine, uridine and tetrahydrouridine, taken as compounds representative of substrates, products and analogues of the transition state of the CDA catalyzed reaction. This analysis led to a subset of 23,741 compounds that showed a least a 50% overlap with one or more of the three above mentioned molecules, as calculated according to the Tanimoto coefficients. Further elimination of all compounds with a molecular weight higher than 600 Dalton and/or more than 5 chiral centers with unknown or unspecified chirality, led to a final subset of 20,694 compounds – henceforth “filtered NCI database”.

Virtual screening: b) molecular docking

For each molecule of the filtered NCI database, multiple alternative structures were generated to account for stereocenters with unknown or unspecified chirality. Furthermore, for each compound all the protonation and tautomeric states accessible between pH 5 and pH 9 were generated. The resulting collection of 172,605 molecular entries was then subjected to molecular docking experiments. The database was spiked with 22 known CDA ligands, divided into 8 substrates and 14 inhibitors (Table 1), in order to allow monitoring of the screening process through receiver operating characteristic (ROC) analysis. Briefly, an

ROC analysis of a docking-based virtual screening experiment can be performed sorting the screened compounds on the basis of their docking rank and subsequently plotting the fraction of retrieved active compounds (true positive fraction, TPF) versus the fraction of retrieved inactive compounds (false positive fraction, FPF); a random experiment would yield an area under the curve (AUC) of 0.5, while a perfect experiment, in which all the active compounds scored better than the inactive ones, would yield an AUC of 1.

After completion of the docking experiments, for each docked molecule only the top scoring protonation and tautomeric state was retained, leading to a ranked database composed of 42,522 unique compounds. As shown in Figure 2, our ROC analysis suggested a good ability of the screening to prioritize ligands versus inactive compounds, as indicated by an area under the curve (AUC) of 0.83. Notably, 32% and 77% of the known active compounds were retrieved within the top scoring 1,000 and 10,000 compounds, respectively, i.e. within the top 2.3% and 23% of the ranked database. Thus, the 10,000 top scoring compounds were subjected to a careful visual inspection that led to the selection of about 70 candidate molecules, 25 of which were actually available from the NCI and were acquired. Of note, of the 25 selected compounds 21 were of unknown activity. Of the remaining four compounds, two were known CDA inhibitors, namely tetrahydro-2'-deoxyuridine (**1**) and 3-deazacytidine (**2**), one was a known CDA substrate, namely 6-azacytidine (**6**), and one was a known inactive molecule, namely α -L-cytosine arabinoside (**8**). These four compounds were acquired and tested as a control.

In vitro assays

The 25 compounds were tested in vitro to determine their ability to act as substrates or inhibitors of CDA, through the spectrophotometric assay method described in the Materials and Methods. These tests led to the identification of three novel inhibitors, namely pseudoisocytidine (**3**), an imidazole-based nucleoside (**4**), and the 4-deoxy derivative of tetrahydrouridine (**5**), and a novel substrate, namely 5-aza-aracytidine (fazarabine, **7**), with a total hit rate of 19%, i.e. 3 active molecules out of 21 tested compounds with unknown activity (Table 2 and Figure 3). The assays also led to the confirmation of the activity of compounds **1**, **2**, and **6**, as well as the inactivity of compound **8**, which, as mentioned, were tested as control compounds. The structures of the remaining 17 compounds that resulted inactive are illustrated in Figure 4.

Discussion

With a K_i of 28 μ M, pseudoisocytidine was the most active compound identified through this screening. Despite its similarity to cytidine, pseudoisocytidine (**3**) turned out to be an inhibitor, and not a substrate, of the enzyme. The reason behind this behavior has to be found in the fact that, in its most probable tautomeric state, pseudoisocytidine features a protonated nitrogen at position 3 of the pyrimidine ring ($N3$). This state, which our Epik calculation indicated to have a probability of existence of 92%, is incompatible with the mechanism of the CDA catalyzed reaction, according to which the $N3$ nitrogen accepts a hydrogen bond from a protonated glutamic acid residue, namely Glu67 in the human enzyme (Figure 5). In the transition state, the hydrogen is subsequently transferred from the glutamic acid residue to the bound nucleoside. Importantly, pseudoisocytidine is a compound with anti-leukemic properties that was known not to be a substrate of CDA.^[9, 10] However, here we report for the first time that it is a CDA inhibitor. Notably, pseudoisocytidine has been shown to be stable at pH 7.4 for 6 days at 22° and for at least 3 days at 37°.^[9] This exceptional stability is probably due to the substitution of the canonical glycosidic bond with a carbon-carbon bond. Furthermore, pseudoisocytidine has been reported to have a low cytotoxicity.^[10] In fact, hematological or intestine toxicity and depression of white blood cell count were not observed in leukemic patients treated with

pseudoisocytidine. Hepatic toxicity proved to be dose limiting.^[10] In light of its great chemical stability and its low cytotoxicity pseudoisocytidine may be more suitable for in vitro and in vivo applications than other CDA inhibitors, despite its somewhat lower potency. Pseudoisocytidine may be also a good lead for the design of more potent inhibitors. In particular, pseudoisocytidine bears a nitrogen atom at position 5 of the aromatic ring (N5). Notably, 5-aza substitutions, as well as 6-aza substitutions, while somewhat tolerated by CDA are detrimental for the affinity of the compounds – see for instance cytidine and 5-azacytidine (Table 1) and 6-azacytidine (compound **6**, Table 2). The lower activity of the azanucleosides with respect to the analogs that bear carbon atoms at positions 5 and 6 (C5 and C6) is likely to be caused by a significant weakening of the T-stacking between the pyrimidine nucleobase and Phe137. As revealed by the crystal structure (PDB ID: 2FR6), cytosine-based nucleosides favorably interact with the electron cloud of Phe137 through aromatic hydrogen atoms bearing partial positive charges at position 5 and 6 of the nucleobase (Figure 6, panels A and B). However, in 5-azacytosine and, to an even higher extent, 6-azacytosine derivatives, this interaction is disrupted by the presence of aromatic nitrogen atoms bearing partial negative charges (Figure 6, panels C and D). In light of these considerations, the affinity of pseudoisocytidine for CDA can probably be enhanced by substituting the nitrogen at position 5 of the nucleobase with an aromatic carbon.

The inhibitory activity shown by compound **4** indicates that CDA is rather tolerant to the replacement of the pyrimidine ring with appropriately decorated imidazoles. However, the inactivity of compounds **12** (showdomycin), **14**, and **24**, all based on non-imidazole five-membered rings, suggests that the enzyme might be selective for specific heterocycles only. Interestingly, among the tested five-membered ring compounds, **4** is the only one that, according to our Epik calculations, does not feature a protonated nitrogen within a physiologically relevant pH range. This observation suggests that compound **4** binds to the enzyme as a substrate analogue.

Compound **5** is the 4-deoxy derivative of tetrahydrouridine, a potent CDA inhibitor that acts as an analogue of the transition state. Compound **5** is about 3000 fold less potent than its parent compound, which is not surprising in light of the loss of the hydrogen bond between the absent 4-OH group and Glu67. However, Glu67 maintains its hydrogen bond with the protonated N3 of compound **4**. This feature seems an essential element for the recognition of transition state and product analogs (Figure 5), as also indicated by the inactivity of uricytin (**15**), the N3 of which is not protonated. The importance of the interaction between N3 and Glu67 is also corroborated by the lack of activity of compounds **9**, **10**, **17**, and **21**, all of which are unable of establishing such an interaction.

Another essential determinant of ligand recognition seems to be the presence of a 3'-OH in the β -configuration, which, as revealed by the crystal structures, interacts simultaneously with glutamate and an asparagine residue, namely Glu56 and Asn54 (Figure 5). This is testified by the inactivity of the tested glucofuranosyl derivatives **13**, **20**, and **22**, which, unlike their ribose-based analogs, feature a 3'-OH in the α -configuration. The absence of this key interaction is likely also the cause of the inactivity of compounds **11**, **23**, and **25**, in which the ribose is substituted by acyclic chains. Of note, the inactivity of 3'-amino-2',3'-dideoxycytidine (**19**) suggests the importance of the simultaneous interaction of the 2'-group with both Glu56 and Asn54. In fact, the 2'-amino group of **19** could interact favorably with the glutamate but not with the asparagine residue.

Importantly, our study revealed that the combination of the 2'-arabino and 5-aza substitutions shields pyrimidine nucleosides from the enzymatic activity of CDA more effectively than the respective single substitutions. Accordingly, compound **7** – 5-azacytosine arabinoside, also known as fazarabine is only a weak substrate of human CDA,

with significantly high K_m and V_{max} values. Conversely, cytarabine (cytosine arabinoside AraC) and 5-azacytidine, which are approved antineoplastic drugs, have K_m values only subtly higher than the natural substrate cytidine and, thus, are significantly subjected to deamination (see Table 1). Thus, fazarabine could potentially offer a better lead for the design of antineoplastic compounds insensitive to the action of CDA. As mentioned, the significant resistance of fazarabine to deamination is due to the substitutions that the compound bears at position 5 of the nucleobase (*N*5 rather than *C*5) and at position 2' of the sugar moiety (2'-OH in the arabinose rather than in the ribose configuration). We have already discussed the reasons behind the effect of 5-aza substitutions. Concerning the effect of substitutions at the 2'-position, the solved crystal structures indicate the presence of two cysteine residues, namely Cys59 and Cys65, in close proximity to the *C*2' (Figure 7). This observation hints particularly strict steric requirements around the 2'-arabino position and suggests that the introduction of relatively bulky 2'-arabino substituents could be used as a means to confer CDA resistance to cytidine-based antineoplastic compounds. In this context, it is worth noting the complete resistance of compound **16** – 2'-bromo-2'-deoxycytosinearabinoside – to the action of cytidine deaminase.

Lastly, it is worth noting that the complete inactivity of compound **18** is likely due not only to the 6-aza substitution, but also to the presence of a hydroxyl substituent on the exocyclic amino group (*N*4). In fact, the solved crystal structures indicate a very limited steric tolerance at this position (Figure 8), which could also be exploited to design CDA-resistant antineoplastic drugs.

Conclusions

Through a virtual screening campaign based on chemical similarity and molecular docking, we identified three novel inhibitors and one novel substrate of human CDA, with a 19% hit rate. Notably, this study allowed a significant extension of the understanding of the requirements for recognition by CDA of substrates and inhibitors, also in light of the compounds that resulted inactive. The most active compound resulting from the screening was the inhibitor pseudoisocytidine (**3**) that, in light of its carbon-carbon nucleosidic bond, is significantly more stable than canonical nucleosides^[9] and offers a new lead for the design of potent stable inhibitors to be co-administered with cytidine-based antineoplastic drugs. In particular, pseudoisocytidine features a nitrogen atom at position 5 of the nucleobase and our structure-activity relationships suggest that its affinity might be substantially enhanced by the substitution of this atom with an aromatic carbon. This study also demonstrated: (a) the detrimental effect on ligand recognition of 5-aza and 6-aza substitutions; (b) the incompatibility of the presence of an amino group at the 3'-position, where a group capable of simultaneously acting as a hydrogen-bond donor and acceptor is required; (c) the presence of very strict steric requirements around the 2'-arabino position, where bulky substituents are not tolerated; and (d) an even stricter tolerance around the *N*4-position, where even small substituents cause complete inactivity. Importantly, these features can be exploited for the design of novel antineoplastic agents resistant to the enzymatic degradation caused by CDA.

Experimental Section

The NCI database

A database of 260071 compounds belonging to the “NCI/DTP Open Chemical Repository Collection” was downloaded from the web site of the NCI/CADD group (<http://cactus.nci.nih.gov/download/nci/>), in SDF format.

Fingerprint-based similarity searches

Structural fingerprints were calculated for all the compounds in the NCI database on the basis of MDL MACCS keys (public subset of 166 bit keys),^[11] through the Molecular Operating Environment (MOE).^[12] Subsequently, the fingerprints were used to calculate the overlap of each NCI compound with cytidine, tetrahydrouridine, and uridine, on the basis of the Tanimoto coefficient.

Molecular descriptors-based filtering

Molecular weights and number of atoms with undefined chirality were calculated in MOE.^[12]

Generation of stereoisomers, protonation states and tautomeric states

The filtered NCI-database was subjected to two rounds of preparation with the LigPrep module of the Schrodinger package,^[13] in order to produce low-energy three-dimensional structures minimized with the OPLS 2005 forcefield. In the first round, all possible stereoisomers of each compounds were generated, varying in a combinatorial manner the configuration of all the chiral centers with unspecified chirality. In the second round, for each of the stereoisomers all possible tautomeric and protonation states accessible between pH 5 and pH 9 were generated with the Epik engine.^[14, 15] To monitor the molecular docking process, the NCI database was spiked with 22 known ligands (Table 1), which were also prepared according to the same procedure.

Preparation of the CDA structure for the molecular docking experiments

The coordinates of the X-ray structures of the mouse CDA in complex with cytidine (PDB ID: 2FR6) were retrieved from the protein data bank (www.rcsb.org). The structure was then subjected to the Protein Preparation Wizard workflow implemented in the Schrodinger package. This added hydrogen atoms, which were subsequently minimized using the OPLS 2005 force field and the Impact molecular mechanics engine. Furthermore, it optimized the protonation state of His residues and the orientation of hydroxyl groups, Asn residues, and Gln residues. A cubic docking grid, with a size of 21 Å, was then generated through the Glide module of the Schrodinger package.^[16] Specifically, the grid was centered on the cytidine molecule crystallized within the active site formed principally by residues of chain A. No scaling factors were applied to the receptor atoms.

Molecular docking

The filtered NCI-database, prepared as indicated above, was then subjected to molecular docking through the Glide module of the Schrodinger package with the OPLS 2005 forcefield.^[16] Specifically: the molecules were docked in Standard Precision (SP) mode; partial charges were calculated according to the OPLS 2005 forcefield; a scaling factor of 0.8 was applied to the van der Waals radius of all the ligand atoms with partial charge absolute value lower than 0.15; five poses per ligand were subjected to post-docking minimization and rescored in Extra Precision (XP) mode; Epik state penalties and a strain correction term were applied to the final scoring. After the scoring, for each stereoisomer the highest scoring instance was retained, while the remaining tautomeric and protonation states and the lower scoring poses were discarded.

Visual inspection and selection of the compounds

When visually inspecting the docked compounds, for each entry we analyzed the correspondence of the stereochemistry with the molecular names registered in the NCI database, whenever these were present, and discarded all those in disagreement. Furthermore, for all nucleotides for which stereochemistry was unspecified in the NCI

database, we assumed a β -D configuration, and discarded the entries with different stereochemistry.

Calculation of electrostatic potentials

The compounds 1-methyl-cytosine, 1-methyl-5-azacytosine and 1-methyl-6-azacytosine were sketched in MOE.^[12] They were then subjected to energy minimization at the semiempirical level with the MOPAC 7 engine, as implemented in MOE,^[12] until reaching a potential energy gradient of 1 kcal mol⁻¹ Å⁻¹). The PM3 hamiltonian was used, with an SCF convergence limit of 0.00001 kcal mol⁻¹. Molecular surfaces were then generated in MOE^[12] and colored according to the molecular electrostatic potential calculated on the basis of the PM3 charges.

Figures

Figures 1 and 5 were produced with the Schrodinger package. Figures 2, 6, 7 and 8 were produced with MOE.

In vitro assays

CDA activity was assayed spectrophotometrically at 37°C by following the decrease in absorbance as described previously.^[17] The standard reaction mixture contained the substrate, 100 mM Tris-HCl, pH 7.5, in a total volume of 1 ml and the reaction was initiated by addition of 0.02–0.04 U of enzyme. One enzyme unit is defined as the amount of enzyme which catalyzes the deamination of 1 μ mol of cytidine per minute at 37°C. The compounds (**6**), 6-azacytidine and (**7**), fazarabine, were assayed at 305 nm ($\Delta\epsilon_{305} = 0.8$) and 260 nm ($\Delta\epsilon_{260} = 0.744$) respectively. In kinetic studies an appropriate amount of 0.1 M KCl was added to the buffer to quench the changes in ionic strength due to the different amounts of substrate and compound used. Deoxycytidine ($\Delta\epsilon_{282} = 3.6$) was used as substrate at final concentrations of 0.3; 0.5; 0.7; 1.0; 2.0 mM.

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Figure 1.

A structural superimposition of the human (1MQ0) and murine (2FR6) cytidine deaminase reveals that 20 out of the 21 residues located within 5 Å from the ligands are conserved in the two enzymes, resulting in 95% sequence identity. All the residues shown in the figure belong to subunit A1, unless differently specified in parentheses.

Figure 2.

Receiver operating characteristic (ROC) curve relative to our docking-based virtual screening, with the true and false positive fractions (TPF and FPF) in the ordinate and abscissa, respectively. Known substrates and inhibitors employed as spike compounds to monitor the screening are indicated by green and red dots, respectively. The five inhibitors retrieved through this screening, two of which known and three of which new, are indicated by brown dots. The two substrates retrieved through this screening, one of which known and one of which new, are indicated by blue dots.

(1), Tetrahydro-2'-deoxyuridine

3-Deazacytidine (2)

(3), Pseudoisocytidine

(4)

(5)

(6), 6-Azacytidine

(7), Fazarabine

(8) α -L-Cytosine arabinoside

Figure 3.
Molecular structures of the compounds listed in Table 2.

Figure 4.
List of the compounds tested in this study and found inactive.

Figure 5.

View of the catalytic site of mouse cytidine deaminase in complex with the substrate cytidine (panel A, PDB ID: 2FR6), the transition state analogue tetrahydrouridine (panel B, PDB ID: 2FR5), and the product uridine (panel C, PDB ID: 2FR6).^[8] For most residues only the side chains are shown. However, for those that are hydrogen-bonded to the bound nucleosides through their backbone (Ser66 and Ser97 of subunit A1 and Ala58 of subunit B2), only the backbone is shown. All the represented residues are conserved in the human homologue. In panel A, the atom numbering typical of pyrimidine nucleosides is also indicated. All the residues shown belong to monomer A1, unless differently specified in parentheses.^[1]

Figure 6.

Panel A: As revealed by the crystal structure (PDB ID: 2FR6), cytidine interacts with F137 through an aromatic T-stacking. Panel B, C, and D: Electrostatic potentials of 1-methylcytosine, 1-methyl-5-azacytosine, and 1-methyl-6-azacytosine, plotted on the molecular surfaces of the compounds – negatively charged areas colored in red and positively charged areas colored in blue.

Figure 7.

View of the complex between CDA (PDB ID: 2FR6) and fazarabine (**7**), as resulting from molecular docking: the proximity of two cysteine residues causes very limited steric tolerance around the 2'-arabino position. The molecular surfaces of the two cysteine residues are colored according to atom type. For clarity, only the side chains of the residues are shown. All the residues shown belong to monomer A1, unless differently specified in parentheses.^[1]

Figure 8.

View of the complex between CDA and cytidine (PDB ID: 2FR6): a number of residues closely surround the *N4* position, precluding the binding of *N4*-substituted compounds. The molecular surfaces of the residues are colored according to atom type. For clarity, only the side chains are shown, with the exception of Ser97, for which the backbone carbonyl is also shown. All the residues shown belong to monomer A1, unless differently specified in parentheses.^[1]

Table 1

Docking scores and experimentally determined kinetic parameters for the set of know CDA substrates and inhibitors used to monitor the virtual screening.^[a]

Substrates	Docking Rank ^[b]	K_m (μM)	V_{max} (U/mg) ^[c]
Deoxycytidine	621	3.9	45.54
Cytidine	126	3.9	68.30
5-Methylcytidine	307	4.0	35.00
5-Methyldeoxycytidine	7676	8.0	40.8
5-I-deoxycytidine	18388	10.0	91.07
Cytosine arabinoside	217	11.0	42.0
2'-F-deoxycytidine	483	16.0	146.2
5-azacytidine	512	28.0	38.0

Inhibitors ^[c]	Docking Rank ^[b]	K_i (μM)
3,4,5,6-Tetrahydrouridine (THU)	3067	0.15
5-Fluorozebularine	1476	0.3
Zebularine	933	1
5,6-Dihydrouridine	6226	40
3-Deazauridine	3073	110
5-Fluoro-2'-deoxyuridine	21267	180
5,6-Dihydrothymidine	41744	280
2-Thio-6-azauridine	6919	290
5-Bromouridine	8935	380
Uridine	3437	400
5-Fluorouridine	3720	430
5-Methyluridine	5566	440
2'-Deoxyuridine	18345	440
CMP	40724	500

^[a] Experimental data obtained from recombinant human CDA^[17] and human CDA purified from placenta.^[18]

^[b] Glide XP

^[c] Units are defined as micromoles of substrate deaminated per minute at 37°C.

^[d] All inhibitors were competitive.

Table 2

Experimentally measured kinetic parameters and docking scores of the three novel inhibitors (**3–5**) and one novel substrate (**7**) identified in this study.^[a] The structure of the compounds are given in Figure X.

Compound	NSC number	Docking Rank ^[b]	K _i (μM)	K _m (μM)	V _{max} (U/mg) ^[c]
(1), Tetrahydro-2'-deoxyuridine	613315	2979	0.037	==	==
(2), 3-Deazacytidine	133115	788	0.5	==	==
(3), Pseudoisocytidine	279852	21	28	==	==
(4)	124167	3545	256	==	==
(5)	153788	6740	440	==	==
(6), 6-Azacytidine	524767	53	==	1014	43.82
(7), Fazarabine	281272	693	==	2300	148
(8), α-L-Cytosine arabinoside	143922	1027	==	==	==

^[a]The table lists also the two known inhibitors tetrahydro-2'-deoxyuridine (**1**) and 3-deazacytidine (**2**), the known substrate (**6**) and the known inactive compound α-L-cytosine arabinoside (**8**), which were tested in this study as control compounds.

^[b]Glide XP

^[c]Units are defined as micromoles of substrate deaminated per minute at 37°C.