Differential Inhibition of APOBEC3 DNA-Mutator Isozymes by Fluoro- and Non-Fluoro-Substituted 2’-Deoxyzebularine Embedded in Single-Stranded DNA

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The APOBEC3 (APOBEC3A-H) enzyme family is part of the human innate immune system that restricts pathogens by scrambling pathogenic single-stranded (ss) DNA by deamination of cytosines to produce uracil residues. However, APOBEC3-mediated mutagenesis of viral and cancer DNA promotes its evolution, thus enabling disease progression and the development of drug resistance. Therefore, APOBEC3 inhibition offers a new strategy to complement existing antiviral and anticancer therapies by making such therapies effective for longer periods of time, thereby preventing the emergence of drug resistance. Here, we have synthesised 2’-deoxy nucleoside forms of several known inhibitors of cytidine deaminase (CDA), incorporated them into oligodeoxynucleotides (oligos) in place of 2’-deoxycytidine in the preferred substrates of APOBEC3A, APOBEC3B, and APOBEC3G, and evaluated their inhibitory potential against these enzymes. An oligo containing a 5-fluoro-2’-deoxyzebularine (5FdZ) motif exhibited an inhibition constant against APOBEC3B 3.5 times better than that of the comparable 2’-deoxyzebularine-containing (dZ-containing) oligo. A similar inhibition trend was observed for wild-type APOBEC3A. In contrast, use of the 5FdZ motif in an oligo designed for APOBEC3G inhibition resulted in an inhibitor that was less potent than the dZ-containing oligo both in the case of APOBEC3G and in that of full-length wild-type APOBEC3G.

Introduction

APOBEC3 (A3) enzymes are important components of the innate immune system that protect against pathogens by catalysing the deamination of cytosine residues in the single-stranded DNA (ssDNA) of the invading viral genome to form uracil residues (Scheme 1A).

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Introduce
A3 enzymes and cytidine deaminase (CDA) share a similar structural topology (despite very low sequence identity), together with, more importantly, structurally homologous zinc-containing active sites. These active sites each include a crucial Glu residue that functions as a general acid/base in the hydrolysis of cytosine. Consequently, A3s and CDA share a similar mechanism of cytosine deamination. However, CDA accepts only individual nucleosides as substrates, whereas A3 enzymes have minimum ssDNA substrates of two or three nucleotides flanking the cytosine.

To date, no selective small-molecule inhibitors of A3A or A3B have been reported. We recently developed the first rationally designed competitive inhibitor of A3 enzymes by incorporating a known inhibitor of CDA—2'-deoxyzebularine (dZ, Scheme 1B)—into ssDNA oligonucleotides. We demonstrated that dZ does not inhibit A3 enzymes when present as the free nucleoside, but becomes a low-micromolar inhibitor if, and only if, it is incorporated into ssDNA. This key observation represents support for a mechanism in which the ssDNA delivers the dZ into the active site for inhibition.

We propose that the inhibitory potential of ssDNAs can be further improved through the incorporation of potent inhibitors of CDA (also an enzyme of pharmaceutical interest) into ssDNA. Here we have considered several cytidine derivatives known to inhibit CDA and incorporated them into ssDNA as possible inhibitors of A3 enzymes (Scheme 1B). 3-Deazauridine (the ribose analogue of 3dadU) has been reported as a weak inhibitor of human liver CDA (Kᵢ = 100 μM). 5-Fluorozebularine has been shown to be a more potent inhibitor of mouse kidney CDA than zebularine (Kᵢ = 0.3 μM versus 2.3 μM, respectively). However, RNA molecules are not preferred substrates of A3 enzymes.

Herein, we report the first syntheses of the 2'-deoxy forms of 3-deazauridine and 5-fluorozebularine (3dadU and 5FdZ, respectively). We also report the incorporation of these nucleosides into ssDNA and their evaluation as A3 inhibitors with the aid of our previously described NMR-based and fluorescence-based enzymatic assays. 3-Deaza-2'-deoxyzebularine (3dadZ, Scheme 1B) has a CH motif instead of the N3 atom in comparison with dZ and so can be used to evaluate the importance of protonation of the N3 atom in dZ in its inhibitory mechanism. Our results indicate subtleties in inhibition of cytosine deamination catalysed by different A3 enzymes, and support our general strategy of using known inhibitors of CDA to guide the design of ssDNAs as inhibitors of A3 enzymes.

### Results and Discussion

**Synthesis of modified nucleosides, their DMT-protected phosphoramidites and corresponding oligos**

The synthesis of modified nucleosides started from heterocycles 1a–c and Hoffer's chlorosugar (Table 1). For the synthesis of 3dadZ (compounds 2–5a) and its incorporation into DNA, we followed previously described procedures with some modifications as described in the Supporting Information.

The pure β-anomer of 3dadU (compound 2b) was obtained by use of a silyl modification of the classical Hilbert–Johnson reaction by treating silylated 2,4-dihydroxy pyridine with Hoffer's chlorosugar in boiling CHCl₃ (Supporting Information). Double recrystallisation from EtOH provided 2b in 50% yield. Cleavage of the tolouyl protecting groups was accomplished in MeOH/NH₂OH to provide nucleoside 3b, which was then converted into 4b by selective installation of the 4,4'-dimethoxytrityl (DMT) group on the 5'-end of the nucleoside followed by benzoyl protection of the 4-hydroxy group of the nucleobase (81% yield over three steps from 2b). Phosphitylation of 4b was performed under standard conditions with N,N-diisopropylamino-2-cyanoethoxychlorophosphine and Et₃N in CH₂Cl₂ in 84% yield after silica gel column chromatography.

The synthesis of 5FdZ as a free nucleoside has been performed in the past through enzymatic conversion of dC in the presence of heterocycle 1c with trans-N-deoxyribosylase from Lactobacillus acidophilus. Later, 5FdZ was synthesised from 5-fluoro-2'-deoxyuridine in six steps. We found both protocols to be unsatisfactory in terms of potential scalability, complex procedures and overall yield. As with the syntheses of dZ₄ and 3dadU, we first used a Lewis-acid-free variant of the silyl-Hilbert–Johnson reaction for the preparation of 5FdZ from silylated heterocycle 1c and Hoffer's chlorosugar; this procedure failed.

Instead, it was necessary to use freshly distilled SnCl₄ and low temperatures (−35 °C) to obtain 3',5'-bis-O-tolouyl-protected 5FdZ (2c) in a good yield, although this product was contaminated with the α-anomer (β/α 9:1). Use of a slow step-
of a “cyclic” nucleoside. The \(^{1}H,^{13}C\) HMBC spectrum, which shows three-bond correlations, was particularly helpful during the assignment (Figure 1B). The H6–C5’ crosspeak, seen in the right-hand upper corner of Figure 1B, confirms the existence of a three-bond linkage between H6 of the nucleobase and C5’ of the sugar in the “cyclic” nucleoside. At the same time, the H6 proton cross-talks with the other carbon atoms of the nucleobase (C2, C4, C5) and with the C1’ carbon atom of the sugar moiety; this is possible only for an 5’–C6 “cyclic” nucleoside.

We observed that the ratio between “open” and “cyclic” forms changes in different solvents. In D$_{2}$O, the “open” form predominantly exists, whereas in [D$_{6}$]DMSO, CD$_{3}$CN and [D$_{6}$]THF both forms are present. This indicates that “open” and “cyclic” forms of the 5FdZ nucleoside are in dynamic equilibrium, which complicates purification but allows the transformation of an equilibrium mixture of nucleosides into the single 5’-DMT-modified product 4c. Consequently, “open” and “cyclic” forms of nucleoside 3c, without any purification after the removal of toluoyl groups from 2c, were treated with DMT-Cl in pyridine; compound 4c was obtained in 60% yield over two steps from 2c. Finally, phosphorylation of 4c gave phosphoramide 5c in 89% yield.

We incorporated the modified nucleosides at the location of dC in the preferred A3 substrate motifs. A3B and A3A prefer the TCA motif (oligo1, Table 2) whereas A3G preferentially catalyses deamination of the CCCA motif (oligo2, in which the underlined C is deaminated first). The synthesis of DNA oligos was performed with an automated DNA synthesiser and use of an increased coupling time for phosphoramidites 5a–c, from 1.5 min for standard phosphoramidites to 5 min.

In the cases of oligos containing 3dadU and 3dadZ, cleavage from the solid support and deprotection of phosphates and nucleobases was accomplished in concentrated aqueous NH$_{4}$OH. Unfortunately, the same procedure led to degradation of 5FdZ-containing oligos, as is evident from the reversed-phase HPLC profile in Figure 1C (red line). Attempted deprotection with saturated NH$_{4}$OH in MeOH was also unsuccessful (blue line, Figure 1C). We found that on-column deprotection of 5FdZ-oligo in organic solvents led to the least amounts of by-products (black profile, Figure 1C). Here, 5FdZ-oligo on the CPG support was treated with 10% Et$_{3}$NH in acetonitrile for 5 min, followed by incubation of the support in an ethylenediamine/toluene mixture for 2 h at room temperature, allowing subsequent release of the deprotected oligo in H$_{2}$O. All oligos were purified by reversed-phase HPLC. Their compositions were confirmed by ESI-MS (see the Supporting Information).

**Evaluation of oligos as inhibitors of A3 enzymes by using an NMR-based activity assay**

To assess the inhibition of A3 enzymes directly, we used a previously described NMR-based activity assay in which the DNA substrate deamination is monitored by \(^{1}H\) NMR spectroscopy in the presence of enzyme with and without inhibitors.\(^{7a,8,13}\) The NMR-based inhibition assay is a direct assay using just A3 enzymes; it does not require a secondary enzyme, such as

### Table 1. Synthesis of modified nucleosides.

| X    | Y    | Z    | R'   | R''  | Yield   |
|------|------|------|------|------|---------|
| 2a   | CH   | H    | Tol  | Tol  | 11%     |
| 2b   | CH   | OH   | Tol  | Tol  | 50%     |
| 2c   | N    | H    | F    | Tol  | 45%     |
| 3a   | CH   | H    | H    | H    | n.d.    |
| 3b   | CH   | OH   | H    | H    | quant.  |
| 3c   | N    | H    | F    | H    | n.d.    |
| 4a   | CH   | H    | DMT  | H    | 58% from 2a |
| 4b   | CH   | OBz  | H    | DMT  | 81% from 2b |
| 4c   | N    | H    | F    | DMT  | 60% from 2c |
| 5a   | CH   | H    | DMT  | P(NP)$_{2}$OCH$_{2}$CH$_{2}$CN | 93% |
| 5b   | CH   | OBz  | DMT  | P(NP)$_{2}$OCH$_{2}$CH$_{2}$CN | 84% |
| 5c   | N    | H    | DMT  | P(NP)$_{2}$OCH$_{2}$CH$_{2}$CN | 89% |

\(\text{Hoffer’s chlorosugar, KOH, CH$_{3}$CN, 15 min; ii) hexamethyldisilazane, (NH}$_{4}$\text{)$_{2}$SO$_{4}$ (cat), reflux 1 h; iii) Hoffer’s chlorosugar, CHCl$_{3}$, distill., 15 min; iv) HMDS, (NH}$_{4}$\text{)$_{2}$SO$_{4}$ (cat), reflux 1 h; v) Hoffer’s chlorosugar, SnCl$_{4}$, 1,2-dichloroethane, overnight then Bz$_{2}$O, pyridine, 0°C; vi) for 4b: DMT-Cl, pyridine, 0°C; vii) for 4c: DMT-Cl, pyridine, 0°C; viii) overnight then Bz$_{2}$O, pyridine, 0°C; ix) DNA synthesis and purification.}
By introducing different inhibitors of cytidine deamination into the A3 recognition motif preferred by the particular A3 enzyme, we expected that the trend in inhibition for all A3 enzymes would roughly parallel the trend observed for CDA inhibition, because the active site and therefore the deamination mechanism are conserved. We evaluated the inhibitory activity of our modified DNAs by using active A3 enzymes that displayed reliable expression and stability over time and had also been characterised previously in the NMR-based activity assays in our laboratory. This allows reliable determination of the inhibitory potential of modified oligos through comparison of $K_m$ values of the substrates with $K_i$ values of inhibitors determined under identical conditions (enzyme and substrate concentrations, buffer and ionic strength). The enzymes chosen—A3B CTD-AL1swap (hereafter simplified to A3B CTD-AL1) and GST-fused A3G CTD—were recombinantly expressed and purified from *Escherichia coli*. To compare the inhibitory effect of oligonucleotides between A3G CTD and full-length A3G (flA3G) we used flA3G that was purified from human cells grown planktonically [24] (see description of these enzymes and their purification in the Supporting Information).

Oligos containing 3dadZ and 3dadU in place of the target dC component in the preferred TCA-recognition motif for A3BCTD-AL1 had no effect on the initial speed of deamination catalysed by A3B CTD-AL1 (Figure 2). These oligos fail to inhibit A3 enzymes under experimental conditions. These data are in line with previous findings that 3dadU, as an individual ribose-based single nucleoside, is a very weak inhibitor of human liver CDA ($K_i = 100 \mu M$). [10] Although higher concentrations of 3dadU-oligo might result in inhibition of A3B CTD-AL1, the use of such concentrations would provide a weaker basis than our

![Figure 1](image)

**Figure 1.** A) NMR assignment of "open" (structure in black) and "cyclised" (structure in green) forms of 5FdZ. Chemical shifts (d in ppm) are shown for $^1$H in blue and for $^{13}$C in red. B) $^1$H/$^{13}$C HMBC spectrum recorded in [D$_6$]DMSO at RT, showing two- to four-bond correlations and coexistence of "open" (black labels) and "cyclised" (green labels) forms of 5FdZ. *, **, and ***: single-bond correlations of H6–C6 ("cyclised" form), H1′–C1′ ("open" form) and H1′–C1′ ("cyclised" form), respectively. C) RP-HPLC profiles of 5FdZ-oligo cleaved from the support and deprotected under different conditions. The major peak isolated after treatment with Et$_3$NH/CH$_3$CN followed by ethylenediamine/toluene gave the expected mass (ESI-MS) of 2680.6 Da (calcd. for [M]: 2680.5 Da).

| Oligonucleotides used in this study. | Name | Sequence 5′−3′ |
|------------------------------------|------|---------------|
| Oligos used in NMR-based activity assay | oligo1 | ATTT-5FdZ-ATTT |
| oligo2 | ATCC-5FdZ-ATTT |
| dZ-oligo | ATTT-dZ-ATTT |
| 3dadZ-oligo | ATTT-3dadZ-ATTT |
| 3dadU-oligo | ATTT-3dadU-ATTT |
| 5FdZ-oligo | ATTT-5FdZ-ATTT |
| C5FdZ-oligo | ATCC-5FdZ-ATTT |

| Oligos used in fluorescence-based activity assay | Name | Sequence 5′−3′ |
|-----------------------------------------------|------|---------------|
| T4-dZ-oligo | TTTT-dZ-AT |
| T4-5FdZ-oligo | TTTT-5FdZ-AT |

[a] Prepared as in ref. [8].
The action of the enzyme. Determination of $K_{\text{app}}$ inhibition constants ($K_{\text{im}}$) improved from 30-fold ($dZ$) to nearly 100-fold ($5FdZ$) if the off-targets were repeated multiple times in the same laboratory and with the same time interval. Mean values are plotted. The error bars report standard deviations.

current strategy for the development of modified 3dadU-oligos as inhibitors.

On the other hand, inhibition of A3B_{CTD}-AL1 by 5FdZ-oligo was more powerful than that by dZ-oligo under identical conditions. Previously, we had confirmed that dZ-oligo is a competitive inhibitor of this enzyme. By monitoring the reaction in the presence of inhibitor at various concentrations, we obtained the inhibition constant ($K_i$) for 5FdZ-oligo ($2.1 \pm 0.8$ $\mu$m, Supporting Information); this was 3.5 times lower than the $K_i$ of dZ-oligo ($7.5 \pm 1.7$ $\mu$m). The overall inhibition effect was improved from 30-fold (dZ) to nearly 100-fold (5FdZ) if the apparent inhibition constants ($K_i$) of dZ- and 5FdZ-containing oligos are compared with the $K_{\text{im}}$ of the ssDNA substrate 5'-ATT-C-ATTT ($K_{\text{im}} = 200$ $\mu$m). This means that 5FdZ-containing oligos can potentially be used in cells in the low-micromolar range to inhibit A3A and A3B. Thanks to the presence of the electron-withdrawing F, the heterocycle component in 5FdZ is more activated towards the nucleophilic addition of $H_2O$ than its counterpart in the case of dZ (Scheme 2), as is evident from the existence of 5FdZ in equilibrium between "open" and "closed" forms (Figure 1A). This probably explains why 5FdZ, once embedded in the ssDNA, is a better inhibitor of A3B_{CTD}-AL1 than dZ. Formation of reversible covalent adducts with the enzyme is also possible. Similar adducts between zebularine and DNA methyltransferases have been described.

Our observations parallel those reported earlier for CDA: that is, that 5-fluorzebularine is a better inhibitor than zebularine and 3-deazaauridine ($K_i = 0.3, 2.3^{[11]}$ and 100 $\mu$m, respectively). These results indicate that the structure of the nucleoside used in place of dC in the preferred ssDNA substrate determines the inhibitory potential of the oligos and that the trend of A3B_{CTD}-AL1 inhibition correlates with $K_i$ values reported earlier for individual nucleosides against CDA. This result also opens the possibility of further improvement of inhibition by introducing other inhibitors of cytidine deamination into ssDNA sequences.

The fact that 3dadZ does not inhibit A3B_{CTD}-AL1 highlights the importance of protonation of N3 in dZ by the conserved glutamic acid residue present in the active site of A3s (and CDA). This protonation makes C4 in dZ more electrophilic and more susceptible to nucleophilic attack by OH⁻/H₂O coordinated to the Zn²⁺+, which converts dZ into a tetrahedral transition-state analogue of cytidine deamination (Scheme 2). This mechanism is inoperative in the case of 3dadZ, because the C=C double bond of 3dadZ is inactive towards water addition. Moreover, the nuclease of 3dadU is planar and does not mimic the tetrahedral geometry of C4 in the transition state of cytidine deamination.

Next, having two active A3G enzymes—the C-terminal domain (A3G_{CTD}) with the wild-type sequence and full-length A3G (fA3G)—we decided to test whether inhibition of A3G_{CTD} is a good model for investigation of inhibition of two-domain enzymes such as fA3G. Our studies were performed with two oligos: an A3G-preferred CC5FdZ-oligo in which the dC residue that is first deaminated by A3G was changed to 5FdZ, and the previously reported inhibitor CCdZ-oligo. Our data show that inhibition of A3G deaminase activity by targeting only the catalytically active C-terminal domain, A3G_{CTD}, accurately translates to the overall inhibition of fA3G (Figure 3A). This is consistent with the fact that the N-terminal domain of A3G completely lacks deaminase activity. Accordingly, the specificity of ssDNA binding to the full-length A3G, and by implication A3B, lies in the C-terminal domains, and the catalytically inactive N-terminal domains enhance ssDNA deamination efficiency at the C-terminal domain and regulate processivity of enzymes. 

Interestingly, the CC5FdZ-oligo ($K_i = (71 \pm 14)$ $\mu$m, Figure 3B) did not cause greater inhibition of A3G_{CTD} in comparison with CCdZ-oligo ($K_i = (53 \pm 10)$ $\mu$m), in contrast to the trend observed above for A3B_{CTD}-AL1. Nonetheless, the fact that both CcdZ-oligo and CC5FdZ-oligo are inhibitors supports our strategy of targeting the catalytically active C-terminal domains of A3 enzymes with our DNA-based inhibitors as a means to inhibit full-length enzymes.
Plausible explanation of differences in inhibition of A3A/A3B\textsubscript{CTD} and A3G by d\textsubscript{z}- and 5FdZ-containing oligos

The fact that the trend of inhibition by d\textsubscript{z}- and 5FdZ-containing oligos is varied for A3A/A3B\textsubscript{CTD} and A3G should not be completely unexpected, because A3 family members differ strongly in their preferences for deamination of methylated cytosine residues in ssDNA.\textsuperscript{[32]} The selectivity of different A3 family members for nonmethylated versus 5-methylated 2'-deoxycytidine (5MedC) can be significantly changed by swapping loop 1 and loop 7 between the enzymes.\textsuperscript{[6b, 33]} This property suggests subtle control of the active site specificity for deamination of 5MedC, because the swapped amino acids are not in direct contact with the target cytosine moiety. Similar effects can be relevant to interaction between A3 enzymes and 5-fluoro-2'-deoxyxycytidine (5FdC) or 5FdZ. Recently, we have also observed that 5-methyl-2'-deoxycytidylate (SmedC) is a worse inhibitor than d\textsubscript{z} in the context of an oligo designed to inhibit A3G\textsubscript{CTD}-\textsuperscript{[8]}

Nevertheless, we have compared active sites of A3A, A3B and A3G to find possible differences in amino acids in proximity to the target cytosine residue. Such differences might explain the preferences of A3 enzymes towards various substrates and inhibitors.

Structural analysis of the A3B\textsubscript{CTD}-AL1 complex with ssDNA\textsuperscript{[31]} and sequential alignment of A3A, A3B\textsubscript{CTD} and A3G\textsubscript{CTD} revealed that not only the zinc-coordinating residues, but most of the residues in the active site close to the target cytosine moiety, are well conserved between these proteins. However, one residue in the substrate-binding pocket differs: an isoleucine residue, Ile\textsubscript{279}/Ile\textsubscript{96} in A3B\textsubscript{CTD}/A3A is a threonine residue (Thr\textsubscript{283}) in the corresponding position of the A3G\textsubscript{CTD} sequence. The side chain of Ile\textsubscript{279} is \(\approx 4.4\) Å distant from the NH\textsubscript{3} group of the target cytosine moiety in the inactive, substrate-binding E255A mutant of A3B\textsubscript{CTD} (PDB ID: 5TD5). On the other hand, the Thr\textsubscript{283} hydroxy group makes hydrogen bonds to a neighbouring Thr residue and to the main chain that forms part of the substrate/inhibitor binding pocket (PDB ID: 3V4J). This interaction might reduce the conformational flexibility needed to accommodate a substituent larger than hydrogen in the 5'-position of cytosine. We note that in the A3B\textsubscript{CTD}-AL1 structure
the bound cytidine residue is tightly supported by Tyr313 (Tyr315 in A3G<sub>CTD</sub>), and that this in turn is buttressed by a conserved Trp residue (Trp285 in the case of A3G<sub>CTD</sub>) on the loop that, in the case of A3G<sub>CTD</sub>, is locked in place by hydrogen bonding to Thr283. Interestingly, mouse CDA (PDB ID: 2FRS) has Ile87 in a similar position in the three-dimensional structure to that of Ile279 in A3B<sub>CTD</sub> (Figure S3 in the Supporting Information). Thus, the Ile versus Thr substitution might play a role in the differences seen between 5FdZ and dZ inhibition of A3G<sub>CTD</sub> and A3B<sub>CTD</sub>-Al1. AID, mouse APOBEC1 and mouse APOBEC3 catalyse the deamination both of 5FdC and of 5MedC less efficiently than for dC.<sup>[10]</sup> These results were explained in terms of steric effects, because F and Me are larger than H. As discussed above, the active sites around the target cytosine residue are very similar, with the exception of Ile/Thr, in the cases of A3A/A3B<sub>CTD</sub> and of A3G<sub>CTD</sub>. The dynamics of the active sites might allow better accommodation of 5FdZ in A3A/A3B<sub>CTD</sub> than in A3G<sub>CTD</sub>. In any event, the substrate and inhibitor binding and the deamination mechanism vary subtly between A3s and CDAs. Examples of highly homologous enzymes with significantly different transition states are well-established.<sup>[30]</sup>

Conclusion
The structures of modified nucleosides dZ and 5FdZ embed in the otherwise identical DNA sequence determine the inhibitory effects on human A3A, A3B<sub>CTD</sub>, and A3G<sub>CTD</sub> as well as on full-length A3G. On the other hand, the 2'-deoxyriboseyl derivative of 3-deazauridine, a previously described weak inhibitor of CDA, cannot inhibit A3 upon its incorporation into ssDNA under the conditions tested. Our results indicate that some correlation between CDA and A3 inhibition exists when CDA inhibitors replace the deamination-susceptible cytidine moiety in the ssDNA sequence. Our results also highlight the importance of protonation of the N3 atom in dZ for its inhibitory behaviour. Note-worthy differences in inhibition profiles among different A3 enzymes observed here point to possibilities of obtaining highly specific A3 inhibitors, thereby supporting our approach to development of oligonucleotide-based A3 inhibitors with the aid of chemically modified nucleosides, the structures of which can stall enzymatic cytosine deamination.<sup>[43]</sup> Future work will continue to focus on the chemical optimisation of our ssDNA-based A3 inhibitors and their evaluation in vitro and in vivo. Nucleotides flanking the target dZ and 5FdZ motifs can be further modified to improve inhibitory potential and to enhance the lifetimes of oligonucleotides in biological media.

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Conflict of Interest
D.A.H. is a co-founder, shareholder and consultant of ApoGen Biotechnologies, Inc.

Keywords: antitumor agents · APOBEC3 · enzyme catalysis · fluoro deoxyzebulurine · inhibitors

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