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Evaluation of Substituted 1,2,3-Dithiazoles as Inhibitors of the Feline Immunodeficiency Virus (FIV) Nucleocapsid Protein via a Proposed Zinc Ejection Mechanism

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A diverse library of 5-thieno-, 5-oxo-, and 5-imino-1,2,3-dithiazole derivatives was synthesized and evaluated for efficacy against the feline immunodeficiency virus (FIV) as a model for HIV in cells. Several diverse compounds from this series displayed nanomolar activity and low toxicity, representing a potential new class of compounds for the treatment of FIV and HIV.

The continual discovery of novel therapeutics against various viral and bacterial strains is essential due to their ability to adapt and become resistant to current treatments. The human immunodeficiency virus (HIV) is a case in point, with an array of drugs used to target multiple points in the viral life cycle, but the continual development of resistance by the virus has continued to erode their efficacy.[1] HIV, which results in the development of acquired immunodeficiency syndrome (AIDS), has caused over 25 million deaths worldwide, with over 34 million people currently infected with HIV.[2] Amongst non-primate lentiviruses such as HIV-1 and HIV-2, only the feline immunodeficiency virus (FIV) causes a similar compromised immune system as that observed in humans,[3] with several FIV strains also displaying central nervous system involvement and analogous AIDS-type disease progression.[4,5]

The short basic nucleic acid binding nucleocapsid protein (NCp) of HIV and FIV is an underexplored antiviral target with no clinically used drugs, despite its involvement at multiple points of the viral replication cycle. These include annealing of the cellular primer $tRNA_3^{lys}$ to the primer binding site in reverse transcription,[6,7] promotion of dimerization, packing and organization of protein–RNA complexes within freshly created virions.[8–10] The NCp is an attractive protein target, as it has been shown to be mutation resistant with inhibition yielding noninfectious virions.[6,11] It contains a conserved double zinc finger peptide unit C-X_2-C-X_4-H-X_4-C (CCHC) that is found in nearly all retroviruses with the exception of spumaviruses,[7] including HIV-1/2,[12,13] FIV,[14] simian immunodeficiency virus (SIV),[15] equine infectious anemia virus (EIAV),[16] amongst others.[17] The development of an effective agent that could target one or both of these zinc fingers of the nucleocapsid protein would render the virus inert, as deletion or modification of either zinc finger leads to virus inactivation.[18,19]

Two different approaches have been used toward the development of nucleocapsid inhibitors: the first, based on small molecules that compete with binding of the substrate RNA nucleic acid chain has met with some success.[20,21] The second approach is based on the irreversible ejection of the structural Zn^{2+} ion, and has been more productive. Key compounds with this mechanism of action are exemplified by compounds **1**–**8**.[22–29] Common functionalities include a disulfide bridge and more recently a diselenide bridge[30] and/or a stable electronically deficient core/functional group(s) (Figure 1). As part of a longer-term program focusing on the development of novel anti-FIV/HIV agents that target the NCp via zinc abstraction, we explored the development of novel compound classes by using FIV as a surrogate model for HIV; the results of our investigations are reported herein.

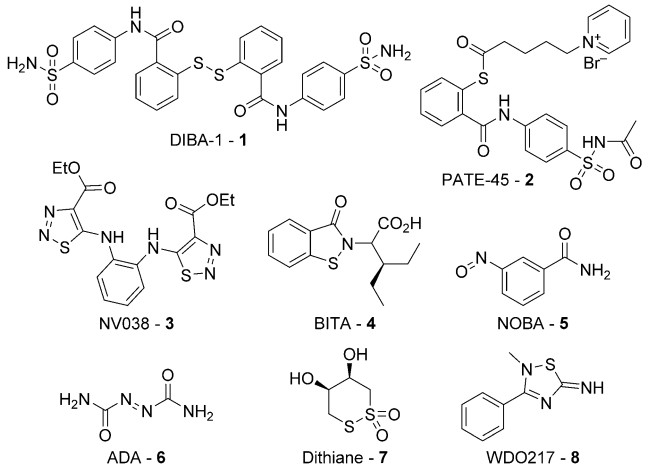

Figure 1. Previously reported zinc abstractors **1**–**8**.

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To develop novel therapeutic agents against the NCp of FIV, we used an in silico homology model derived from HIV-1 and EIAV nucleocapsid proteins to explore heteroatom-rich disulfide-containing compounds, by building on our previous knowledge relating to bis[1,2]dithiolo[1,4]thiazines and bis[1,2]-dithiolopyrrole derivatives[31] and tetrathiocine derivatives.[32] The 1,2,3-diathiazole core caught our interest, as it has been shown to have a broad biological activity profile, including antibacterial,[33–35] anticancer,[37–38] antifungal/herbicidal,[39–43] and anti-melanin activities.[44]

The 1,2,3-dithiazole scaffold is stable and has the potential to be substituted at the C4 and C5 positions, while containing an electrophilic disulfide bond. Substitutions were made to explore the propensity of the disulfide to react with the cysteine thiolates of the NCp model. Investigation of the alignment and electronic distribution of the 1,2,3-dithiazole core led to a small selection of structurally diverse compounds, which were computationally modeled using density functional theory (DFT), and a small selection of compounds were then synthesized and tested (Figure 2).

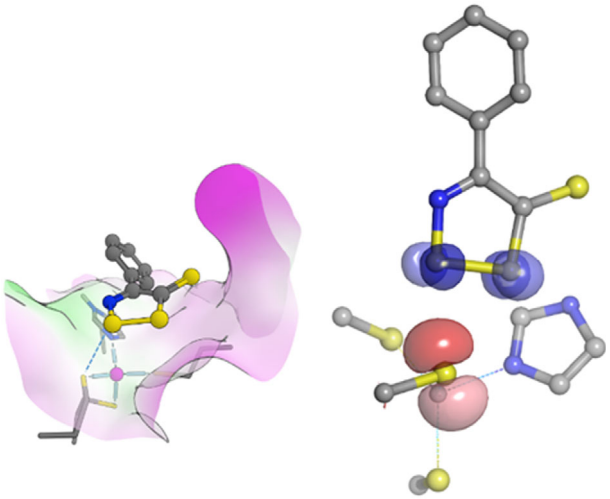
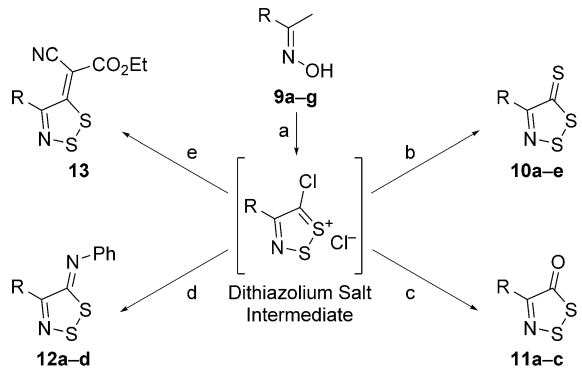


Figure 2. Virtual screening was based on both molecular docking (left) and DFT molecular orbital calculations (right). Molecular docking was used to determine if the screened compound is able to achieve a suitable docking pose. The DFT calculations were based on the docking poses and thus validated whether the frontier orbitals of the reactants are geometrically optimally located (HOMO orbitals are marked with a red surface, and the LUMO orbitals are of the blue-colored 1,2,3-dithiazole scaffold), (Schrödinger Maestro).

A series of selected 1,2,3-dithiazoles containing a disulfide bridge and our supporting mechanistic rationale demonstrate the potential of this heterocyclic system. These heterocycles are underreported in the literature due to the fact that C5-substituted derivatives are a relative synthetic challenge.[45] To date, 1,2,3-dithiazole chemistry has centered around the 4,5-dichloro-1,2,3-dithiazolium chloride salt (Appel salt) and the susceptibility of attack by nucleophiles at the S1, S2, C4, and C5 atoms.[46] We anticipated that these synthetic mechanisms to access the core could also play a role in the zinc abstraction mechanism.[47] Although chloride substitution at C5 is more commonly reported with the Appel salt chemistry, this could present an issue relating to the ability of the chloride to act as a potential leaving group in the final compound.

The syntheses of novel 4- and 5-substituted-1,2,3-dithiazoles bearing a thioketone, oxo, imino or ethyl 2-cyano-2-acetate ylidene functionality at C5 were performed using the reaction between substituted ethanone oximes and sulfur monochloride according to a one-pot protocol, generating an in situ prefunctionalized dithiazolium salt intermediate before treatment with a selected nucleophile (Scheme 1).[48,37]



Scheme 1. Synthesis of dithiazole derivatives. *Reagents and conditions*: a) S_2Cl_2/pyridine, CH_3CN, Ar, −5 to 0 °C, 15 min; b) thioacetamide, 0 to 25 °C, CH_3CN, Ar, 2 h; c) formic acid, CH_3CN, Ar, 30 min at 0 °C, 1 h at reflux; d) ethyl 2-cyanoacetate, pyridine, 25 °C, CH_2Cl_2, 5 h; e) aniline/benzyl amine, CH_3CN, Ar, 30 min at 0 °C, followed by pyridine.

The ethanone oximes **9a–g** were prepared according to published procedures in quantitative yields by heating a mixture of the corresponding ketones or aldehydes with excess hydroxylamine hydrochloride with sodium acetate in methanol at reflux.[49] The oximes were subsequently suspended in acetonitrile and reacted with disulfur dichloride, with pyridine added dropwise to form the related dithiazolium salt intermediate, which was then treated with the appropriate nucleophilic source.

There are a number of methods for the formation of 4-chloro-1,2,3-dithiazolo-5-thione, including the use of hydrogen sulfide in acetonitrile[46] or 2-cyanothioacetamide,[50] but we chose to use thioacetamide for its ease of use and low cost. Thioacetamide was added after preparation of the dithiazole salt derivative, and this selectively gave compounds **10a–e** in good yields. 5-Oxodithiazoles were synthesized by replacing thioacetamide with formic acid to produce **11a–c**. 5-Iminodithiazoles were prepared in a similar fashion by substituting thioacetamide with aniline to give **12a–d**. Compound **13** was produced by treatment of the **9a** dithiazole salt derivative with ethyl cyanoacetate; although modeling indicated that this compound was not optimal, we wanted to investigate if having a polar head group such as this cyano ester derivative would be tolerated.

Compounds **10–13** were then screened for toxicity in vitro against feline kidney cells and tested for antiviral efficacy in vitro against a chronically infected feline lymphoid cell line. Bio-

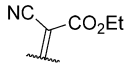
logical testing of the compounds was based on a dual approach, the first aimed at identifying any compounds with nonspecific toxicity at three higher concentrations (1–100 μM) in a short MMT cell viability assay[51] over 24 h with Crandell Rees feline kidney cells (CrFK).[52] The second provided an enhanced longer-term cytotoxicity screen and an anti-FIV profile at six concentrations (1 nM to 100 μM) over seven days, using an IL-2-independent feline lymphoblastoid cell line (FL-4).[53] FL-4 cells infected with FIV were exposed to the compounds over a period of seven days and sampled every day and at each of six concentrations to determine the extent of viral replication/suppression. Viral RNA was isolated from cell culture supernatants using the MagNA Pure LC System by the Total Nucleic Acid Isolation Kit (Roche Applied Science). Viral loads were determined by quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR).[54] The remaining cells were subjected to an MTT cell viability assay to rule out any toxicity effects, to validate that the RT-qPCR result is not caused by nonspecific toxicity, and to provide a therapeutic index for each compound.

The results obtained demonstrate that activity was observed at varying potencies across each series with nanomolar activities as observed with **10a** (Table 1). Toxicity was generally light when observed in the initial screening with CrFK cells with 24 hours exposure. This is generally a good indication of the level of toxicity that would be observed if there was a clearance mechanism in the FL-4 assay. There appears to be a roughly tenfold increase in toxicity when looking at the results for the FL-4 assay relative to the CrFK data, but this has to be taken in the context of chronic exposure in an in vitro assay and would not necessarily be truly representative of an in vivo system. Importantly, the overall results show that even with prolonged exposure to these potentially zinc abstracting agents, there is a good therapeutic index, which suggests that zinc abstraction is not intrinsically tied to cytotoxicity. The toxicity does not show any correlation with the activity displayed by the most active compounds such as **10a** and **11b**. While **12a** also displays good activity and represents an improvement on previous work within our group, the therapeutic index (CC_{50}/EC_{50}) still requires further improvement.

After the initial promising result with **10a** we looked to expand a small cluster of compounds around the main scaffold and alter structural features of the core compound to increase the therapeutic index. However, with **10b–e**, we found there to be a decrease in activity relative to **10a**, with **10d** and **10e** showing unfavorable therapeutic indexes in comparison with **10a**, combined with lower overall activity. The slightly increased EC_{50} value of **10c** could be due to limited cell penetration relative to the other counterparts, or the fact that sulfur is less electronegative than oxygen.[55] This drop in activity was not observed with the switch to the oxygen analogue **11b** which was almost as potent as **10a**, but in displaying increased toxicity, the corresponding therapeutic index was lower. We expected a boost from a nitro group substitution (**11c** and **12c**) but this modification led to an increase in toxicity with no material potency gain. The consistent substituent across each series was the *para*-methoxyphenyl group, which was used to screen the 'head group'. Whilst the results of these compounds were quite consistent, they did not demonstrate improved potency, but displayed increased toxicity over

Table 1. Results from the 1,2,3-dithiazole scaffold FIV screening.

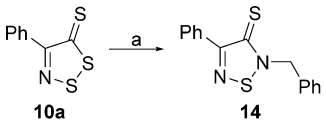
General Structure

Compd	X	R	CrFK [%][a]	CC_{50} [μM][b]	EC_{50} [μM][b]	TI[c]	cLog*P*[d]
10a	S	phenyl	>100	>100	0.023	>4000	3.53
10b	S	4-methoxyphenyl	96.43	39.24	0.456	86.1	3.45
10c	S	4-fluorophenyl	>100	42.17	2.66	15.8	3.67
10d	S	2-benzofuranyl	74.1	3.04	0.9044	6.7	4.3
10e	S	2-thiophenyl	57.2	6.02	0.1773	17.1	3.38
11a	O	4-methoxyphenyl	>100	40.84	0.193	211.6	2.16
11b	O	4-fluorophenyl	>100	63.52	0.025	>2500	2.38
11c	O	4-nitrophenyl	87.45	6.26	0.4325	14.5	1.98
12a	N-Ph	phenyl	75.66	7.49	0.5888	12.7	5.84
12b	N-Ph	4-methoxyphenyl	66.78	4.53	0.1398	32.4	5.76
12c	N-Ph	4-nitrophenyl	92.51	9.74	0.5001	19.5	5.58
12d	N-Ph	ethyl ester	76.64	28.21	0.0665	424.2	4.24
13		4-fluorophenyl	90.33	3.24	0.2397	13.5	3.36
14	S	phenyl	71.11	0.681	0.392	1.7	4.79
15	–	AZT	>100	>100	5.31	18.8	−0.16
16	–	raltegravir	>100	>100	0.01	>10000	1.16

[a] Percent viability; compound concentration of 10 μM on CrFK cells for 24 h. [b] Geometric mean, each concentration tested in triplicate after 7 days as a difference of an untreated DMSO control FL-4 cells with <10% error. [c] Therapeutic index: CC_{50}/EC_{50}, which is the ratio of toxicity to activity. [d] Calculated using ChemBioDraw Ultra 14.

10a in this in vitro system. Interestingly, an increase in cLog*P* beyond 5 did not significantly inhibit the activity or affect the solubility of compounds **12a–c**. Compounds **12d** and **13** were investigated to understand if disruption to the core structure by removal of sulfur or oxygen would be tolerated; although activity was observed, there was not a significant improvement over **10a**, due to an increased metabolic liability from the ethyl ester. The activity of azidothymidine (AZT, **15**) and raltegravir (**16**) are consistent with previous reports in FIV/HIV.[56,57]

The focus of this work builds on the idea that the disulfide bridge is pivotal to the activity of the compounds against the NCp target. We also aimed to demonstrate that the disulfide is essential for activity by synthesizing a disrupted 1,2,3-dithiazole system with a disulfide bond by treatment of **10a** with benzylamine (Scheme 2),[58,59] to give 1,2,5-thiadiazolothione



10a **14**

Scheme 2. Reaction of 5*H*-1,2,3-dithiazole-5-thiones **10a** to form **14**. *Reagents and conditions*: a) benzyl amine, THF, Ar, 3 h, RT.

14. However, we found the utility of this compound to be limited by toxicity, after prolonged cellular exposure. The EC_{50} closely mapped to the CC_{50} with a resulting therapeutic index of less than 2; this meant that this was not a definitive way to demonstrate if the disulfide bridge is essential or not. This observation may or may not support our hypothesis that the disulfide bridge is essential for the activity observed, but was not conclusive.

With the activities observed, our proposed mechanism of action of this compound class involves modification of the zinc-binding sites on the NCp of FIV which we determined by using DFT calculations. Support for this idea comes from previous NMR and MS studies on HIV NCp7 that have shown observable formation of protein–zinc thiol(ate) complexes and covalent modifications.[25] We reasoned that 1,2,3-dithiazole-mediated zinc ion abstraction ejection also occurs via an analogous mechanism to known zinc binding/disrupting compounds in which a zinc-binding cysteinyl thiol(ate) reacts with the disulfide of the core to generate a transient protein–DTA disulfide (Figure 3).[25,36,60] This can then rearrange to form an intramolecular protein disulfide with a consequent decrease in zinc ion affinity. The ejected zinc ion (or zinc 1,2,3-dithiazole complex) can then potentially complex with a second (reduced) 1,2,3-dithiazole core to form a stable complex which is analogous to work previously reported on the epidithiodiketopiperazine class of natural products.[60–62]

Observations from our calculations show that it is unlikely that thermodynamic parameters drive the observed kinetics. Once disulfide exchange occurs between the zinc finger and the inhibitor, the zinc finger in the protein is no longer able to re-form, and equilibrium to regenerate the structure is unable to be established. We therefore propose this mechanism using

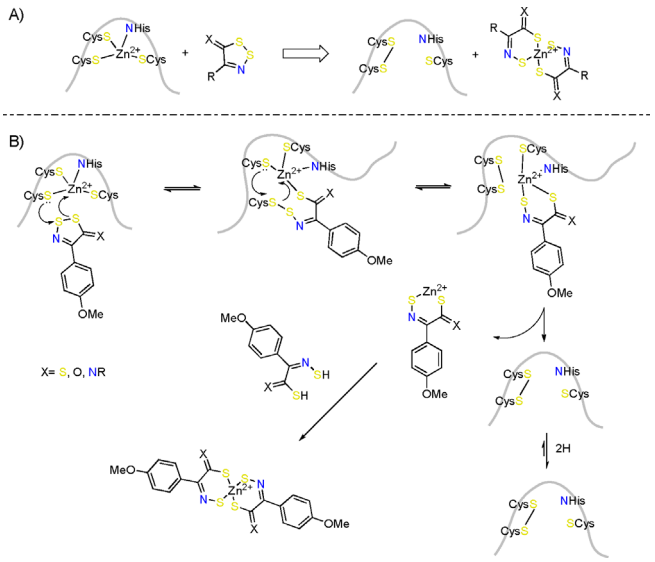


Figure 3. Proposed mechanism of action of 1,2,3-dithiazole for modification of the NCp homology model. A) Summary of the mechanism of zinc ejection from NCp/NCp7. B) A Zn^{2+}-coordinating cysteine thiol(ate) reacts with the disulfide of the 1,2,3-dithiazole core to generate a transient intermediate disulfide. The disulfide then rearranges to form an intramolecular protein disulfide with consequent decrease in zinc ion affinity. The ejected zinc ion (or part Zn^{2+} complex) can then complex with a second 1,2,3-dithiazole core (reduced) to form a stable complex.

a kinetic argument whereby the energy barrier for zinc ejection is favored and, in addition, that the formation of a stable zinc complex is potentially thermodynamically favored. When the requisite disulfide bridge in the inhibitor is interrupted in compound **14**, this disruption to the zinc finger can no longer occur, and abstraction of zinc by our proposed mechanism is not available to this compound, leading to the observed limited activity (Figure 4).

The sub-micromolar potencies of the compounds described in this study combined with the lower toxicities show a step change in progression toward the development of useful candidate compounds for targeting the FIV/HIV nucleocapsid protein. The low toxicity and high activity of the initial hit **10a** led

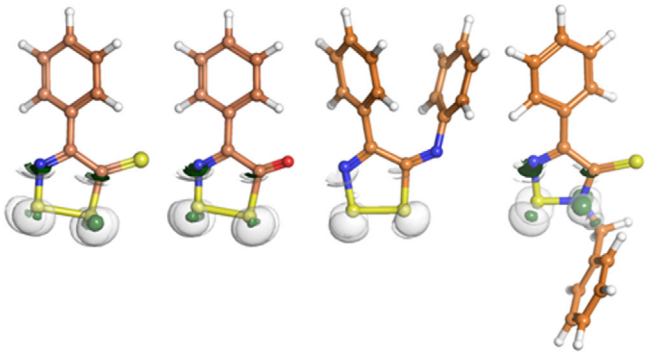


Figure 4. Global reactivity was estimated by calculation of the Fukui frontier molecular orbitals of **10**–**14** and the propensity of the protein to undergo zinc abstraction. Fukui f+ negative visualized using iso-values 0.0015 (white) and 0.005 (green), (Schrödinger Maestro).

us to believe that improved compounds with enhanced characteristics were possible. Many avenues of development exist, such as substitution at the *para* position of the pendant phenyl ring to prevent P450 metabolism while still maintaining activity. We demonstrated that **10a** strikes a good overall balance between activity and toxicity. With the quantum calculations demonstrating the viability of zinc ejection, this opens the possibility of our investigations with the aim of generating lead structures with more favorable profiles. While we have enhanced the repertoire of the 5-thieno-, 5-oxo-, 5-imino-1,2,3-dithiazole class of compounds, the hope is that this work will allow us to generate a pre-clinical candidate that can treat both FIV and HIV in an in vivo setting, and this will be reported in due course.

Experimental Section

Modeling

Molecular docking: A quantum polarized molecular docking study for a number of compounds (Figure 2) was conducted, which was similar to that previously generated using a homology model,[31] with the version used in this study based on HIV-1 PDB ID: 2JZW (NCp7)[63] and EIAV PDB ID: 2BL6 (NCp11) as a model for the FIV NCp.[16] The inhibitory mechanism of compounds is assumed to begin with coordination of compounds against more the accessible zinc finger motif of the model (Figure 3). Our previous homology modeling and docking studies suggest that inhibitory activity can be partly explained by means of facile coordination of compounds close to the more accessible zinc finger structure.

DFT molecular orbital calculations: The zinc finger structure is not well described by means of molecular-mechanics-based molecular docking; hence we used Jaguar DFT calculations for the docking-pose-derived model. The geometry of zinc finger cysteine and histidine residues were constrained to their initial geometry using Cartesian constraints to connector carbon atoms. B3LYP of theory and MSV basis set were used for geometry optimizations in the gas phase. Reactivities of selected compounds were separately estimated by unconstrained gas-phase geometry optimizations (B3LYP/6-31g**) followed by single-point Fukui frontier molecular orbital calculations (visualized in Figure 4; Schrödinger Inc., New York, NY, USA).

Biology

Initial short cytotoxicity assay: Crandell Reese feline kidney (CrFK) cells[52] were cultured in 96-well plates (TPP, Trasadingen, Switzerland) at a density of 10000 cells per well, in complete medium [RPMI 1640 medium (Sigma–Aldrich, Buchs, Switzerland) containing 10% fetal calf serum (FCS), 1% *v/v* (200 mM) glutamine, and 1% *v/v* antibiotic/antimycotic (Ab/Am) (all three from Gibco Life Technologies, Zug, Switzerland)]. The antiviral compounds were dissolved in 2% DMSO (Sigma–Aldrich, Buchs, Switzerland) to make a 1 M stock solution. The compounds were 10-fold serially diluted with complete medium. Six dilutions of the compounds (100 μM to 1 nM) were added in triplicate to the CrFK cells (200 μL) and incubated for 24 h (37 °C, 5% CO_2). The medium was removed and replaced with phenol-red-free medium (180 μL) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 20 μL, 4 mg mL^{-1}; Sigma–Aldrich, Buchs, Switzerland), and cells were incubated at 37 °C for 4 h. The medium was removed by vacuum and the cells were lysed with methanol (200 μL) to reveal a bright-purple formazan product.[51] The methanol–formazan absorbance was determined at 570 nm (BioTek Synergy HT plate reader with KC4 software, Cambridge systems), and values expressed as 50% cytotoxic concentration (CC_{50}).

FIV viral loading studies: The potential antiviral effect against FIV was tested in a cell culture assay using an IL-2-independent feline FL-4 lymphoblastoid cell line.[53] The compounds were added at concentrations ranges in triplicate as described above. The cell supernatant (100 μL) was removed, frozen at −20 °C and replaced daily for 7 days. To determine the "long-term" cytotoxicity, on day 7 the cell culture supernatant was replaced with phenol-red-free medium (80 μL), and the cells were subjected to MTT assay as before. The day 7 cell culture supernatants were centrifuged in a table-top centrifuge (10000 *g*) at maximal speed for 2 min to pellet cells and debris. The cell culture supernatants of each triplicate were pooled and frozen immediately at −20 °C until analysis. Total nucleic acids (TNA) were extracted using the MagnaPure LC TNA extraction kit (Roche, Basel, Switzerland) from 100 μL pooled solution according to the manufacturer's instructions. FIV RNA was quantitated by RT-qPCR as described for FIV, using DSMO and untreated cells as controls.[54] Results were expressed as 50% effective concentrations (EC_{50}) determined by a sigmoid dose–response curve at 50% inhibition, with sigmoid dose–response curve fitting using GraphPad Prism version 6.0 (GraphPad Inc., La Jolla, CA, USA) and an RNA standard.

Chemistry

General: Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Specord M-80 instrument in KBr pellets. ^{1}H NMR were recorded on a Bruker WM 300 spectrometer (300 MHz), and ^{13}C NMR spectra were recorded on a Bruker AM300 (75.5 MHz) in $CDCl_3$. *J* values are given in hertz. Mass spectra were recorded on a Finnigan MAT INCOS 50 instrument using electron impact ionization. Elemental analyses were performed on PerkinElmer 2400 Elemental Analyser.

General procedure A: 4-Phenyl-5*H*-1,2,3-dithiazole-5-thione (10a): Pyridine (0.243 mL, 3.0 mmol) was added dropwise at −5 to 0 °C to a stirred solution of (*E*)-1-phenylethan-1-one oxime **9a**[47] (135 mg, 1.0 mmol) and sulfur monochloride (0.160 mL, 2.0 mmol) in acetonitrile (10 mL) under an inert atmosphere of argon. The mixture was stirred at 0 °C for 15 min, and thioacetamide (83 mg, 1.1 mmol) was added in one portion. The resulting mixture was stirred at RT for 2 h, filtered, and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum/CH_2Cl_2 mixtures) to afford compound **10a** as a brown solid (155 mg, 73%): mp: 95–100 °C. Anal. calcd for $C_8H_5NS_3$ (%): C 45.47, H 2.39, N 6.63, found (%): C 45.55, H 2.43, N 6.70; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36$ (3H, m, Ph), 7.47 ppm (2H, m, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 128.0$, (2CH, Ph), 129.4 (2CH, Ph), 130.5 (CH, Ph), 131.3, 179.3 (2 sp^2 tertiary C), 208.0 ppm (C=S); MS (EI, 70 eV), *m/z* (%): 211 (100) $[M]^+$, 135 (95), 103 (30); IR (KBr): $\tilde{\nu} =$ 3056, 2920 (C–H), 2852, 2356, 2336, 1436, 1132 (C=S), 604, 499 cm^{-1} (S–S).[37]

4-(4-Methoxyphenyl)-5*H*-1,2,3-dithiazole-5-thione (10b): (*E*)-1-(4-Methoxyphenyl)ethan-1-one oxime **9b**[47] (165 mg, 1 mmol) was treated according to general procedure A to afford compound **10b** as pale-brown crystals (73 mg, 30%): mp: 68–71 °C. Anal. calcd for $C_9H_7NOS_3$ (%): C 44.79, H 2.92, N 5.80, O 6.63, found (%): C 44.95, H 3.08, N 6.04; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.87$ (3H, s,

CH_3); 6.98 (2H, d, 2CH, J=8.8 Hz), 8.98 ppm (2H, d, 2CH, J=8.8 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=55.4 (CH_3), 113.5 (2CH, Ar), 123.9 (2CH, Ar), 131.1, 161.4, 167.3 (3C_{quat}), 208.4 ppm (C=S); MS (EI, 70 eV), m/z (%): 241 (28) $[M]^+$, 210 (15), 197 (4), 133 (100); IR (KBr): $\tilde{\nu}$=2924 (C–H), 2852, 1600, 1516, 1280, 1144, 1036, 828, 752, 700 cm^{-1}.[48]

4-(4-Fluorophenyl)-5*H*-1,2,3-dithiazole-5-thione (10c): (*E*)-1-(4-Fluorophenyl)ethan-1-one oxime **9c**[47] (153 mg, 1 mmol) was treated according to general procedure A to afford compound **10c** as brown crystals (92 mg, 40%): mp: 148–152 °C. Anal. calcd for $C_8H_4FNS_3$ (%): C 41.90, H 1.76, N 6.11, found (%): C 41.83, H 1.89, F 8.29, N 6.11; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.15 (2H, t, J=8.80, Ar), 7.98 ppm (2H, m, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=115.4, 130.2 (sp^2 tertiary C), 131.7 (4CH, Ar), 150.9, 140.6, 165.7 (3sp^2 tertiary C), 208.0 ppm (C=S); MS (EI, 70 eV), m/z (%): 229 (78) $[M]^+$, 209 (10), 185(12), 153 (89); IR (KBr): $\tilde{\nu}$=2924 (C–H), 2856, 1888, 1600, 1508, 1408, 1272, 1136, 852, 792, 680 cm^{-1}.[37]

4-(1-Benzofuran-2-yl)-5*H*-1,2,3-dithiazole-5-thione (10d): (*E*)-1-(Benzofuran-2-yl)ethan-1-one oxime **9d**[47] (175 mg, 1 mmol) was treated according to general procedure A to afford compound **10d** as red crystals (138 mg, 55%): mp: 132–134 °C. Anal. calcd for $C_{10}H_5NOS_3$ (%): C 47.79, H 2.01, N 5.57, O 6.37, found (%): C 47.92, H 2.23, N 6.76; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.32 (1H, m, Bzf), 7.44 (1H, m, Bzf), 7.62 (1H, d, J=8.1, Bzf), 7.73 (1H, d, J=7.3, Bzf), 8.50 ppm (1H, s, Bzf); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=110.7, 111.4, 122.9, 123.6, 127.1 (5CH, Bzf), 132.9, 140.8, 147.6, 168.7 (4sp^2 tertiary C), 205.2 ppm (C=S); MS (EI, 70 eV), m/z (%): 251 (23) $[M]^+$, 175 (59), 143 (58); IR (KBr): $\tilde{\nu}$=1648, 1612, 1560, 1348, 1260, 1164, 1036, 828, 748, 700 cm^{-1}.[37]

4-Thien-2-yl-5*H*-1,2,3-dithiazole-5-thione (10e): (*E*)-1-(Thiophen-2-yl)ethan-1-one oxime **9e**[63] (141 mg, 1 mmol) was treated according to general procedure A to afford compound **10e** as red crystals (54 mg, 25%): mp: 89–92 °C. Anal. calcd for $C_6H_3NS_4$ (%): C 33.16, H 1.39, N 6.44, found (%): C 33.28, H 1.54, N 6.14; HRMS Anal. calcd for $C_6H_3NS_4$: 216.9148, found: 216.9151; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.14 (1H, m, Th), 7.53 (1H, d, J=5.1 Hz, Th), 8.36 ppm (1H, d, J=4.4 Hz, Th); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=127.2, 130.7, 131.7 (3CH, Th), 133.9, 164.0 (2sp^2 tertiary C), 205.6 ppm (C=S); MS (EI, 70 eV), m/z (%): 217 (73) $[M]^+$, 141 (100), 109 (53); IR (KBr): $\tilde{\nu}$=3100, 2920 (C–H), 2852, 1520, 1412, 1364, 1276, 1140, 984, 824, 784 cm^{-1}.[37]

General procedure B: 4-(4-Methoxyphenyl)-5*H*-1,2,3-dithiazole-5-one (11a): Pyridine (0.243 mL, 3 mmol) was added dropwise at −5 to 0 °C to a stirred solution of (*E*)-1-(4-methoxyphenyl)ethan-1-one oxime **9f**[47] (165 mg, 1 mmol) and sulfur monochloride (0.160 mL, 2 mmol) in acetonitrile (10 mL) under argon. The mixture was stirred at 0 °C for 15 min followed by addition of formic acid (0.189 mL, 5 mmol), and the resulting mixture was stirred at 0 °C for 30 min and heated at reflux for 1 h, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum/CH_2Cl_2 mixtures) to afford compound **11a** as yellow crystals (146 mg, 65%): mp: 59–62 °C. Anal. calcd for $C_9H_7NO_2S_2$ (%): C 47.98, H 3.13, N 6.22, O 14.20, found (%): C 48.12, H 3.31, N 6.48; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.87 (3H, s, CH_3) 6.97 (2H, d, J=8.8 Hz, Ar), 8.15 ppm (2H, d, J=9.5 Hz, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=55.5 (CH_3), 114.1 (2CH, Ar), 129.6 (2CH, Ar), 123.8, 154.4, 161.8 (3sp^2 C), 190.5 ppm (C=O); MS (EI, 70 eV), m/z (%): 225 (27) $[M]^+$, 197 (7), 165 (5), 133 (100); IR (KBr): $\tilde{\nu}$=3064 (C–H), 3008, 2920, 2840, 1656 (C=O), 1576, 1508, 1248, 1180, 832, 684 cm^{-1}.[59]

4-(4-Fluorophenyl)-5*H*-1,2,3-dithiazole-5-one (11b): (*E*)-1-(4-Fluorophenyl)ethan-1-one oxime **9c**[47] (153 mg, 1 mmol) was treated according to general procedure B to afford compound **11b** as yellow crystals (107 mg, 50%): mp: 70–72 °C. Anal. calcd for $C_8H_4FNOS_2$ (%): C 45.06, H 1.89, F 8.91, N 6.57, O 7.50, found (%): C 44.89, H 1.98, N 6.37; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.15 (2H, t, J=8.5, Ar), 8.19 ppm (2H, t, J=8.5, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=115.8 (2CH, d, J=23.0 Hz, Ar), 130.1 (2CH, d, J=7.0 Hz, Ar), 127.1, 153.8, 164.3 (C–F, s, J=251.0 Hz), (3sp^2 C), 190.1 ppm (C=O); MS (EI, 70 eV), m/z (%): 213 (14) $[M]^+$, 185 (14), 121 (40); IR (KBr): $\tilde{\nu}$=3072 (C–H), 1920, 1676 (C=O), 1596, 1512, 1408, 1272, 1164, 804, 696 cm^{-1}.[59]

4-(4-Nitrophenyl)-5*H*-1,2,3-dithiazole-5-one (11c): (*E*)-1-(4-Nitrophenyl)ethan-1-one oxime **9f**[47] (153 mg, 1 mmol) was treated according to general procedure B to afford compound **10c** as yellow crystals (84 mg, 35%): mp: 155–156 °C. Anal. calcd for $C_8H_4N_2O_3S_2$ (%): C 39.99, H 1.68, N 11.66, found (%): C 40.31, H 1.81, N 11.62, O 19.98, S 26.69; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.33 (2H, d, J=8.8 Hz, Ar), 8.40 ppm (2H, d, J=8.8 Hz, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=129.0 (2CH, Ar), 133.9 (2CH, Ar), 141.7, 153.4, 158.7 (3sp^2 C), 195.5 ppm (C=O); MS (EI, 70 eV), m/z (%): 240 (5) $[M]^+$; IR (KBr): $\tilde{\nu}$=3100 (C–H), 1667 (C=O), 1652, 1600, 1512, 1408, 1348, 1300, 1272, 860, 796, 672 cm^{-1}.[59]

General procedure C: *N*-[(5*Z*)-4-Phenyl-5*H*-1,2,3-dithiazol-5-ylidene]aniline (12a): Pyridine (0.243 mL, 3 mmol) was added dropwise at −5 to 0 °C to a stirred solution of (*E*)-1-phenylethan-1-one oxime **9a**[47] (135 mg, 1 mmol) and sulfur monochloride (0.160 mL, 2 mmol) in acetonitrile (10 mL) under an inert atmosphere of argon. The mixture was stirred at 0 °C for 15 min, whereupon aniline (1 mmol) was added, and the mixture stirred at 0 °C for 30 min, followed by the addition of pyridine (0.162 mL, 2 mmol). The reaction mixture was filtered, and solvents were evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum/CH_2Cl_2 mixtures) to afford compound **12a** as bright-yellow crystals (149 mg, 55%): mp: 73–76 °C. Anal. calcd for $C_{14}H_{10}N_2S_2$ (%): C 62.19, H 3.73, N 10.36, found (%): C 62.25, H 3.68, N 10.62; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.19 (3H, m, Ph), 7.48 (5H, m, Ph), 8.22 ppm (2H, m, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=119.0, 125.6, 128.1, 129.0, 129.8, 130.3 (10CH, Ar), 132.6, 153.1, 159.2, 165.4 ppm (4sp^2 tertiary C); MS (EI, 70 eV), m/z (%): 270 (96) $[M]^+$; IR (KBr): $\tilde{\nu}$=3048 (C–H), 1604, 1598, 1500 (C=N), 1280, 633, 488 cm^{-1} (S–S).[37]

***N*-[(5*Z*)-4-(4-Methoxyphenyl)-5*H*-1,2,3-dithiazol-5-ylidene]-*N*-phenylamine (12b)**: (*E*)-1-(4-Methoxyphenyl)ethan-1-one oxime **9b**[64] (135 mg, 1 mmol) was treated according to general procedure C to afford compound **12b** as yellow crystals (81 mg, 27%): mp: 84–85 °C. Anal. calcd for $C_{15}H_{12}N_2OS_2$ (%): C 59.98, H 4.03, N 9.33, O 5.33, found (%): C 60.21, H 4.22, N 9.32; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.88 (3H, s, CH_3), 6.98 (2H, d, J=9.5 Hz, Ar), 7.20 (3H, m, Ar), 7.46 (2H, d, J=8.8 Hz, Ar), 8.25 ppm (2H, d, J=8.8 Hz, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ=55.3 (CH_3), 113.5 (2CH, Ar), 119.0 (2CH, Ar), 129.8 (2CH, Ar), 130.6 (2CH, Ar), 125.3 (CH, Ar); 125.6, 153.1, 158.4, 161.2, 165.8 ppm (5 sp^2 C); MS (EI, 70 eV), m/z (%): 300 (25) $[M]^+$, 167 (71), 133 (100); IR (KBr): $\tilde{\nu}$=3088, 2956 (C–H), 2836, 1600, 1508, 1484, 1284, 1172, 752, 692 cm^{-1}.

***N*-[(5*Z*)-4-(4-Nitrophenyl)-5*H*-1,2,3-dithiazol-5-ylidene]-*N*-phenylamine (12c)**: (*E*)-1-(4-Nitrophenyl)ethan-1-one oxime **9f**[47] (180 mg, 1 mmol) was treated according to general procedure C to afford compound **12c** as orange crystals (85 mg, 27%): mp: 171–172 °C. Anal. calcd for $C_8H_4N_2O_2S_3$ (%): C 53.32, H 2.88, N 13.32, O 10.15, found (%): C 53.48, H 2.88, N 13.32; ^{1}H NMR (300 MHz,

$CDCl_3$): $\delta = 7.23$ (3H, m, Ph), 7.48 (2H, m, Ph), 8.31 (2H, d, $J =$ 8.3 Hz, Ar), 8.49 ppm (2H, d, $J = 8.3$ Hz, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 124.2$, 128.6, 135.3, 135.4, 131.0 (9CH, Ar), 143.5, 153.4, 157.9, 162.5, 164.0 ppm (5 sp^2 tertiary C); MS (EI, 70 eV), *m/z* (%): 315 (13) $[M]^+$, 167 (54); IR (KBr): $\tilde{\nu} = 1612$, 1600, 1580, 1576, 1492, 1276, 1076, 832, 756, 696 cm^{-1}.[37]

Ethyl (5*Z*)-5-(phenylimino)-5*H*-1,2,3-dithiazole-4-carboxylate (12 d): Ethyl (*E*)-2-(hydroxyimino)propanoate **9 g**[65] (131 mg, 1 mmol) was treated according to general procedure C to afford compound **12 d** as a red oil (154 mg, 58%): Anal. calcd for $C_{11}H_{10}N_2O_2S_2$ (%): C 49.61, H 3.78, N 10.52, found (%): C 49.88, H 4.02, N 10.42; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.43$ (3H, t, $J = 7.3$ Hz, CH_3), 4.38 (2H, q, $J = 7.3$ Hz, CH_2), 7.16 (3H, m, Ph), 7,46 ppm (2H, m, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.2$ (CH_3), 63.0 (CH_2), 119.3 (2CH, Ph), 126.4 (CH), 129.8 (2CH, Ph), 139.4, 152.1, 153.3, 160.4 ppm (4 sp^2 tertiary C); MS (EI, 70 eV), *m/z* (%): 266 (40) $[M]^+$, 167 (80); IR (KBr): $\tilde{\nu} = 3076$, 2960 (C–H), 2924, 2852, 1744 (C=O), 1700 (C=O), 1584, 1540, 1484, 1368, 1216, 1144, 740, 676 cm^{-1}.[37]

Ethyl (*Z*)-2-cyano-2-(4-(4-fluorophenyl)-5*H*-1,2,3-dithiazol-5-ylidene)acetate (13): Sulfur monochloride (0.32 mL, 4 mmol) and pyridine (0.48 mL, 6 mmol) were successively added dropwise to a solution of (*E*)-1-(4-fluorophenyl)ethan-1-one oxime (**9 c**; 153 mg, 1 mmol) in CH_2Cl_2 (15 mL) at $-7\,^\circ C$ under argon. The reaction mixture was kept at $-2\,^\circ C$ for 20 min and a solution of ethyl 2-cyanoacetate (1.13 g, 10 mmol) in acetonitrile (10 mL) was added dropwise at $-15\,^\circ C$, and the resulting mixture was stirred for 1 h. Pyridine (0.32 mL, 4 mmol) was added dropwise at $-15\,^\circ C$, the temperature steadily increased to room temperature, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel Merck 60, light petroleum and then light petroleum/CH_2Cl_2 mixtures) to afford compound **13** as orange crystals (49 mg, 16%): mp: 188–193 °C. Anal. calcd for $C_{13}H_9FN_2O_2S_2$ (%): C 50.64, H 2.94, F 6.16, N 9.09, found (%): C 50.49; H 3.10; N 9.12; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.38$ (3H, t, $J =$ 7.3 Hz, CH_3), 4.37 (2H, q, $J = 7.3$ Hz, CH_2), 7.21 (2H, t, *J* 8.5, Ar), 7.46 ppm (2H, m, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.3$ (CH_3), 63.2 (CH_2), 116.1 (2CH), 131.5 (2CH), 90.3, 113.1, 128.6, 162.3, 163.1, 166.3, 167.5 ppm (7 sp^2 C); MS (EI, 70 eV), *m/z* (%): 308 (62) $[M]^+$, 235 (100), 153 (22); IR (KBr): $\tilde{\nu} = 2928$ (C–H), 2204 (CN), 1664, 1520, 1444, 1280, 1104, 824, 792, 700 cm^{-1}.

2-Benzyl-4-phenyl-1,2,5-thiadiazole-3(2*H*)-thione (14): Benzyl amine (0.109 mL, 1 mmol) was added to a solution of 4-phenyl-5*H*-1,2,3-dithiazole-5-thione (**10 a**; 0.106 mg, 0.5 mmol) in THF (4 mL) at room temperature. The reaction mixture was stirred for 3 h at room temperature, the alkylammonium hydrogen sulfide was filtered off, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (Silica gel Merck 60, light petroleum, and then light petroleum/CH_2Cl_2 mixtures) afford compound **14** as a yellow crystalline solid (80 mg, 56%): mp: 95–97 °C; Anal. calcd for $C_{15}H_{12}N_2S_2$: C 63.35, H 4.25, N 9.85, S 22.55, found (%): C 63.45, H 4.39, N 10.05, S 22.68; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.31$ (2H, s, CH_2), 7.49 (8H, m, Ar), 8.42 (2H, m, Ar); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 53.8$ (CH_2), 128.1 (2CH, Ar), 129.1 (2CH, Ar), 129.5 (2CH, Ar), 129.9 (2CH, Ar), 129.9 (CH, Ar), 130.5 (CH, Ar), 133.0 (sp^2 C), 133.2 (sp^2 C), 160.5 (sp^2 C), 177.4 ppm (C=S); MS (EI, 70 eV), *m/z* (%): 284 (25) $[M]^+$, 251 (13); IR (KBr): $\tilde{\nu} =$ 3064, 2928 (C–H), 2852, 1496, 1456, 1428, 1344, 1332, 1292, 1208, 1028, 848, 772, 756, 708, 696 cm^{-1}.[59]

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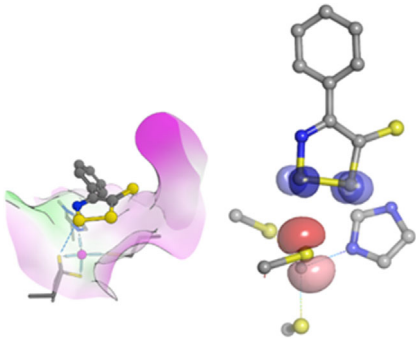
Antiviral 1,2,3! A diverse library of 1,2,3-dithiazole derivatives were synthesized and tested against feline immunodeficiency virus (FIV) in cells using density functional theory to determine a degree of suitability in a simplified nucleocapsid homology model. The compounds displayed nanomolar activity and relatively low toxicity and represent a new class of compounds for the treatment of FIV and HIV. This work demonstrates the versatility of the of 1,2,3-dithiazole scaffold, adding antiviral capacities to the portfolio of biological activities already known for this compound class.



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Evaluation of Substituted 1,2,3-Dithiazoles as Inhibitors of the Feline Immunodeficiency Virus (FIV) Nucleocapsid Protein via a Proposed Zinc Ejection Mechanism

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