CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Investigation of the pentathiepin functionality as an inhibitor of Feline Immunodeficiency Virus (FIV) via a potential zinc ejection mechanism, as a model for HIV infection

Authors: Christopher Robert Michael Asquith, Tuomo Laitinen, Lidia S Konstantinova, Graham Tizzard, Antti Poso, Oleg A Rakitin, Regina Hofmann-Lehmann, and Stephen T Hilton

To be cited as: *ChemMedChem* 10.1002/cmdc.201800718

Link to VoR: http://dx.doi.org/10.1002/cmdc.201800718

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

WILEY-VCH

COMMUNICATION

Investigation of the pentathiepin functionality as an inhibitor of Feline Immunodeficiency Virus (FIV) *via* a potential zinc ejection mechanism, as a model for HIV infection

Christopher R. M. Asquith[a][b]+*, Tuomo Laitinen[c], Lidia S. Konstantinova[d][e], Graham Tizzard[f], Antti Poso[c], Oleg A. Rakitin[d][e], Regina Hofmann-Lehmann[b], Stephen T. Hilton[a]*

Abstract: A small diverse library of pentathiepin derivatives were prepared to evaluate their efficacy against the nucleocapsid protein function of the Feline Immunodeficiency Virus (FIV) as a model for HIV, using an *in-vitro* cell culture approach. This approach led to the development of nanomolar active compounds with low toxicity.

Introduction

The Human Immunodeficiency Virus (HIV) presents a complex challenge for drug discovery with a constantly moving goal post for success. The ability of the virus to mutate and still function post-mutation, constantly erodes the armoury of drugs that exist to combat multiple points in the replication cycle in order to control the virus.[1]

HIV infection resulting in Acquired Immune Deficiency Syndrome (AIDS) has caused over 25 million deaths worldwide; with over 34 million people currently suffering from HIV.[2] The domestic cat provides an effective natural non-primate animal model for the study of lentivirus infections. Feline Immunodeficiency Virus (FIV) has significant homology across key protein domains with HIV-1/2. This model is also the only one that has central nervous system involvement and analogues AIDS-type progression, both seen in humans infected with HIV-1/2.[3,4]

There are multiple inhibition targets in the FIV/HIV, but the short basic nucleic acid binding nucleocapsid protein (NCp) provides an interesting anti-viral target with no clinically approved compounds, despite its involvement at multiple points of the viral replication cycle, which could mitigate emerging resistance.

[a] Dr. C. R. M. Asquith, Dr. S. T. Hilton
School of Pharmacy, Faculty of Life Sciences,
University College London, London, WC1N 1AX, United Kingdom
E-mail: ucnvcrm@ucl.ac.uk; s.hilton@ucl.ac.uk
[b] Dr. C. R. M. Asquith, Prof. R. Hofmann-Lehmann
Clinical Laboratory & Center for Clinical Studies, Vetsuisse Faculty,
University of Zurich, 8057 Zurich, Switzerland
[c] Prof. T. Laitinen, Prof. A. Poso
School of Pharmacy, Faculty of Health Sciences,
University of Eastern Finland, Kuopio, 70211, Finland
[d] Dr. L. S. Konstantinova, Prof. O. A. Rakitin
Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, 119991, Russian Federation
[e] Dr. L. S. Konstantinova, Prof. O. A. Rakitin
Nanotechnology Education and Research Center, South Ural State University, Lenina Ave. 76, Chelyabinsk, Russian Federation
[f] Dr Graham Tizzard
UK National Crystallography Service, School of Chemistry,
University of Southampton, Highfield Campus, Southampton, SO17 1BJ, United Kingdom.
+ Current address - Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, 120 Mason Farm Road, Genetic Medicine Building, Chapel Hill, NC 27599, USA

The NCp contains the 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.[5] These include HIV-1/2,[6,7] FIV,[8] Simian Immunodeficiency Virus (SIV),[9] Equine Infectious Anaemia Virus (EIAV)[10] and others.[11] Effective targeting of these zinc fingers would render the virus inert as deletion or modification of either zinc finger leads to virus inactivation.[12,13]

The NCp is involved in annealing of the cellular primer $^{t}RNA_3lys$ to the primer binding site in reverse transcrption,[14,15] promotion of packing, dimerization and organization of the protein-RNA complexes within the newly created virons.[16-18] The NCp is an attractive drug target as it has been demonstrated to be mutation resistant and inhibition leads to non-infectious virons.[19]

Two methods of NCp inhibition exist: one to compete with the nucleic acid binding site,[20,21] the other to interfere directly with the zinc finger structure *via* zinc ejection. Both approaches have shown promise, however, we choose to utilize the permanent irreversible to eject the structural Zn^{2+} ion from the viral protein. This route has previously created several lead compounds including 3-nitrosobenzamide (NOBA) - **1**, 2,2ô-di-thiobisbenzamide (DIBA) - **2**, azodicarbonamide (ADA) - **3**, Dithanes - **4**, benzisothiazolones (BITA) - **5**, pyridinioalkanoyl thiolesters (PATES) - **6** (Figure 1).[22-27]

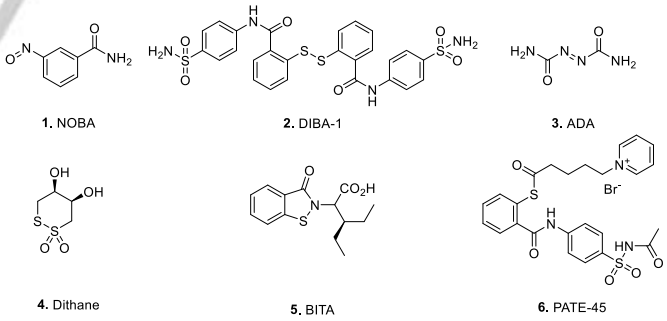


Figure 1. *Examples of some significant previously reported zinc ejectors*

As part of a wider medicinal chemistry program to find novel chemotypes for the NCp inhibitors we were investigating a set of dual functionality disulfide tetrathiocine derivatives. During this we also tested one of the pentathiepin starting materials (**14**), which showed good activity (EC_{50} = 169 nM) which justified further investigation of this core.[28]

The literature has a number of reports with varying levels of biological activities within the pentathiepin core sub-structure (Figure 2). Reports by Feher *et al*. dating back to the late 1960s detailed several synthetic routes to this unusual ring system.[29-31] These were used as the basis for the initial work in the area by

DuPont to explore the potent anti-fungal properties. The first compound developed was 7-methyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrazole - **11**, which provided near total control of several species of crop fungi and was part of a wider program.[32-37]

Following on from this work there was a renaissance of interest with the discovery of the natural product Varacin - **7** and it`s various structural relatives **8**-**10**.[38-39] Over the next few decades these would demonstrate a broad range of activities including anti-cancer, antibiotic, antifungal and an inhibitor of protein kinase C.[38,40-42]

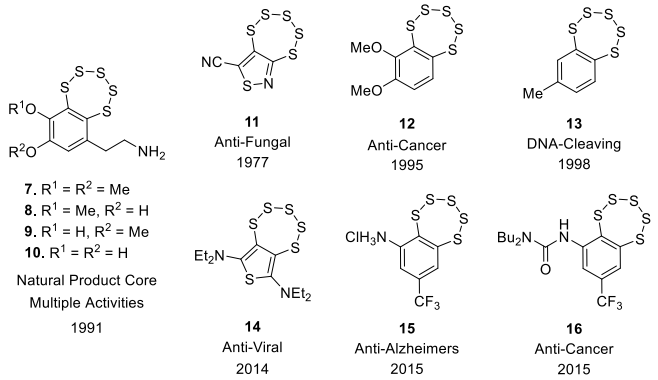


Figure 2. *Previous reported biologically active pentathiepin compounds.*

These activities brought interest to the pentathiepin with the development of **12**-**13**, but as yet there has been only limited research to identify the mechanisms of action or toxicity.[43-45] More recently there have been new developments with a number of new pentathiepin activities disclosed including the anti-viral potency against FIV (**14**) and novel anti-Alzheimer's/anti-cancer candidates, by high through-put screening and rational design respectively **15** and **16**.[46-48]

Results and Discussion

We investigated a number of heteroaromatic pentathiepin scaffolds by visualizing HOMO and LUMO orbitals calculated by Jaguar software using DFT theory and estimating relative reactivity from atomic fukui indices derived from frontier orbital theory to guide our design (Figure 3 and S1). This was instead an effort to moderate toxicity and increase cell permeability while still retaining the activity seen in **14**.

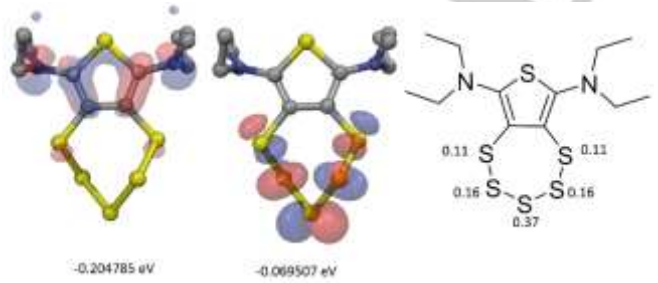


Figure 3. *HOMO (left) LUMO (centre) orbitals for compound **14**. On right f-NN-LUMO atomic fukui indices pointing out most susceptible atoms for nuleophilic reaction when acting as substrate.*

Modelling suggests that the HOMO-LUMO gap (0.13 eV) compound **14** is stable in its ground state, which is in agreement with the atomic fukui indices (f-NN-LUMO). The most susceptible bond for nucleophilic attack will be S2-S3 in the seven membered ring. The relatively low f-NN-HOMO values (max atomic value 0.11 for S3) indicate that compound is less electrophilic when acting as a substrate. This may partly explain the good toxicity profile of **14**. Using a model compound set up derived from our previously described homology model of HIV-1 and EIAV, we found that the cysteine and S2-S3 bond of the pentathiepin functionality were aligned so as the HOMO and LUMO were set up for a nucleophlic cascade.[28] This is due in part to the twist boat conformation adopted prior to reaction, evidenced by our model compound approach shown in Figure 4.

Comparison of f-NN-HOMO indices suggests that in the case of twisted boat conformation S2-S3 bond is slightly more favourable for nucleophilic attack. Our zinc finger model suggests that an even more attractive orbital overlap can be formed (Figure 4 right panel). The LUMO orbitals are oriented to the direction of S2-S3 bond, which enhances bond reactivity. Seven membered sulfur rich ring systems are able to accommodate many ring conformations separated by shallow energy barriers, which if controlled correctly could yield a viable lead compound.[49-50] This also supported our investigation of the 7-membered pentathiepin ring compounds for their anti-viral profile.

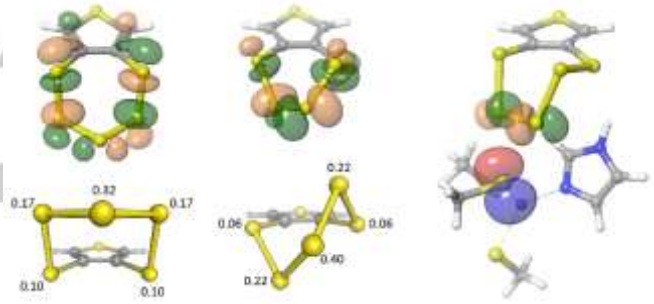


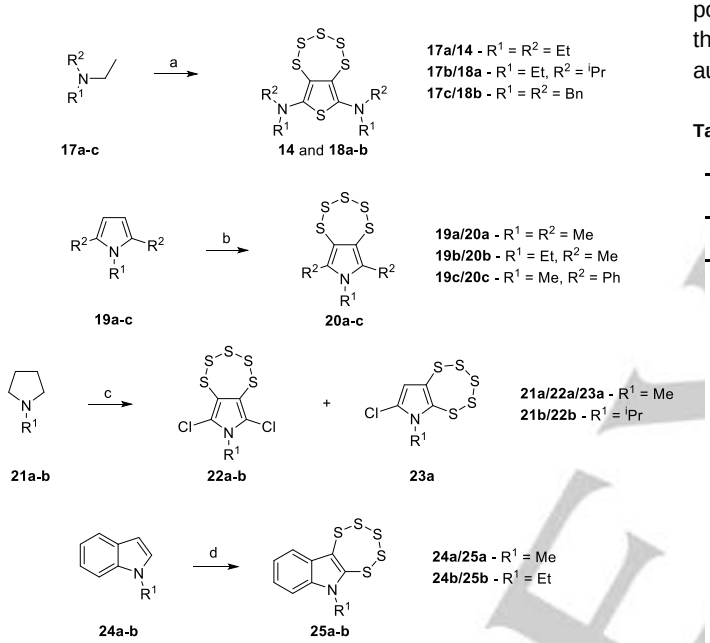
Figure 4. *Model compounds presenting LUMO orbitals of chair conformation (top left) compared with twisted boat conformation (top centre). Below each version respectively - f-NN-LUMO atomic fukui indices showing most susceptible atoms for nucleophilic reaction when compound of interest is acting as substrate. On right gas phase optimised to zinc finger model showing that favourable orbital interactions can be formed between frontier orbitals (HOMO=blue-red, LUMO green-orange).*

A number of synthetic methods exist for the preparation of pentathiepins.[51] The most common methods involve treating *ortho*-dithiols with an activated sulfur source such as S_2Cl_2 or S_3Cl_2 or directly with elemental sulfur (S_8) dissolved in ammonia, generally under harsh conditions.[29-31] More elaborate methods have also been employed such as the use of P_4S_{10} or a molybdenum oxo *bis*-tetrasulfide catalyst to access more decorated scaffolds from more complex starting materials.[52-54]

We chose to pursue a C-H activated route mediated by S_2Cl_2, utilising the properties of the 1,4-diaza-bicyclo[2.2.2]octane (DABCO) sulfur monochloride complex to form the pentathiepins in one step from commercially available reagents (Scheme 1).[55] We started by synthesizing direct analogues of the previously identified compound **14**. Our established method, treating the corresponding Hünig's base analogues **17a-c** with (DABCO) and sulfur monochloride for 48 hours followed by refluxing with triethylamine for a further 2 hours, to produce **14** and **18a-b** in good yield.[28,56]

Substitution on the pyrrole analogs was explored by synthesizing a small series of preformed substituted pyrrole rings,

first by converting **19a-c** to **20a-c** *via* treatment with DABCO and sulfur monochloride for 48 hours in good yield.[57] Then by treating *N*-methylpyrrolidine **21a** with analogous conditions allowed access to **22a** and **23a** in a one-pot reaction. However, under the same conditions *N-iso*propylpyrrolidine **21b** produced exclusively **22b** with the corresponding asymmetric product not observed. One explanation for this could be due to the steric interference of the *iso*-propyl group on the reaction mechanism.[58-60] Previous work has indicated that the corresponding tetrathiocine (7,14-dimethyl-7*H*,14*H*-[1,2,5,6]tetrathiocino[3,4-*b*:7,8-*b*']diindole/5,8-dimethyl-5*H*,8*H*-[1,2,5,6]tetrathiocino[3,4-*b*:8,7-*b*']diindole (1:1)) of **25a** was active against FIV.[28] Hence the simple methyl (**25a**) and ethyl (**25b**) were synthesized under analogous conditions to form **24a-b** in high yield to explore the indole subsitution in more detail.[58,61]



Scheme 1 *- Synthesis route to pentathiepin derivatives - reagents and conditions - a*= (DABCO), S_2Cl_2, $CHCl_3$, *0°C to rt; 48 h, reflux 2 h (**14** - 30%, **18a** - 21%, **18b** - 2%); b*= (DABCO), S_2Cl_2, $CHCl_3$, *0°C; 48 h (**20a** - 36%, **20b** - 40%, **20c** - 62%); c*= (DABCO), S_2Cl_2, $CHCl_3$, *20°C; 48 h (**22a** - 18%, **23a** - 38%, **22b** - 16%; d= S_2Cl_2 (0.8 eq), $CHCl_3$, 0°C; 48 h (**25a** - 70%, **25b** - 72%).*

The compounds were initially tested at three high concentrations (1 ε M - 100 ε M) in a short MTT cell viability assay[62] over 24 hours using Crandell Rees Feline Kidney (CrFK) cells.[63] This enabled us to have a view of the non-specific toxicity of each compound. The compounds were then tested against FIV using an IL-2 independent feline lymphoblastoid (FL-4) cell at six concentrations (1 nM - 100 ε M) over seven days.[64] The 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/ viral suppression. Viral RNA was then isolated from cell culture supernatants using the MagNA Pure LC System with the Total Nucleic Acid Isolation Kit (Roche Applied Science). The viral loads were determined by quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR).[65] The remaining cells were subjected to a MTT cell viability assay to rule out any toxicity effects and validate that the RT-qPCR result was not caused by non-specific toxicity and to provide a therapeutic index. The toxicity and activity of azidothymidine (AZT) - **26** and Raltegravir - **27** were consistent with previous reports on FIV/HIV and were used as an internal standard.[66-67]

The results (Table 1), support the rationale that the pentathiepin functionality can be optimized for anti-viral activity. Activity is not just nucleated around the core seven member rings as varying potencies and toxicities were observed across each series. The observed toxicity in the initial screening with CrFK cells with 24-hours exposure can be a good indication of the level of toxicity that would be observed if there was a clearance mechanism in the FL-4 assay. There was roughly a 1-2 log increase in toxicity when looking at the results for the longer-term FL-4 assay. The initial 24-hour assay result, taken in the context of a chronic exposure *in-vitro* assay, is quite encouraging. It means that with the potential clearance of *in-vivo* system these effects could be mitigated. Even with prolonged exposure to these potential zinc abstracting agents there is an acceptable therapeutic index, which indicts that zinc abstraction does not automatically equate to cytotoxicity.

Table 1. Results of FIV and cytotoxicity screening

Compound	CrFK[a]	CC_{50}[b]	EC_{50}[b]	TI	cLogP[c]
Number	%	(μM)	(μM)		
14	>100	4.2	0.17	25.1	5.89
18a	63.4	0.50	0.44	1.1	6.95
18b	>100	>100	75	1.3	10.65
20a	77.4	0.46	0.0044	105	3.63
20b	87.4	5.0	0.12	41.5	4.15
20c	>100	55	0.87	63.5	6.82
22a	90.9	0.048	0.0058	8.3	4.13
22b	>100	0.030	0.0019	15.8	4.97
23a	76.2	0.52	0.21	2.4	3.40
25a	80.1	0.61	0.55	1.1	4.01
25b	>100	0.54	0.50	1.1	4.54
26	>100	>100	5.3	>18.8	-0.16
27	>100	>100	0.010	>10000	1.16

[a]Cytotoxicity (% Viability) at 10 ε M in CrFK cells; [b]Geometric mean, each concentration tested in triplicate after 7 days as a difference of the untreated cells; [c]Calculated using *Chemdraw Ultra 16*

The original hit compound **14**, was consistent with previous reports, demonstrating good activity with an acceptable therapeutic index. However, two new analogs **18a** and **18b** failed to separate toxicity and activity. Compound **20a** while not the most active at EC_{50} = 4 nM did have the best therapeutic index at >100.

We wanted to investigate the conformation of pentathiepin ring system and found the small molecules crystal structure of compound **20a** crystallizes in the triclinic (Figure 5), with a *P*-1 space group forming two independent molecules in the asymmetric unit.[68] The conformations of both molecules are virtually identical, all bond lengths and angles conform to standard values in comparison with the Cambridge Crystallographic Data Centre (CCDC) and support our observations in figure 3 & 4.[69]

Figure 5. *Crystal structure of compound* ***20a*** *showing bond lengths (Å) and angles ( ) of 7-membered pentathiepin rings of the two independent molecules in the asymmetric unit. ADP ellipsoids are displayed at 50% probability.*

The molecular dynamics discussed in figure 3 and 4 support by the small molecules crystal structure (Figure 5) suggest a break between S-2 and S-3 is the most likely point of a nucleophilic attack; by a reactive cystine to the pentathiepin structure due to the electronics and sterics. This has also been suggested by the high iso-values and molecular dynamics DFT calculations looking at Varacin ï **7** and a generalized structure of **14** with **t**he overall results and overlays are demonstrated in figure 6.

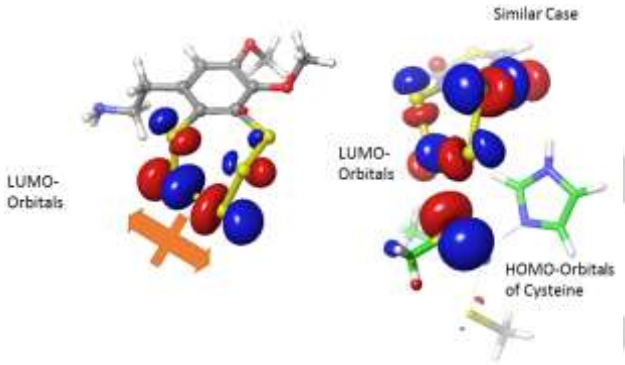


Figure 6. *Simulation of* ***7*** *and generalised structure of* ***14*** *interactions with standard CCHC zinc finger active site*

The mechanism of zinc ejection from NCp is proposed to involve a zinc-coordinating cysteine thiol(ate) reacting with the pentathiepin ring system at the S-2 S-3 bond to generate a transient protein-pentathiepin core inserted bound unit (Figure 7)

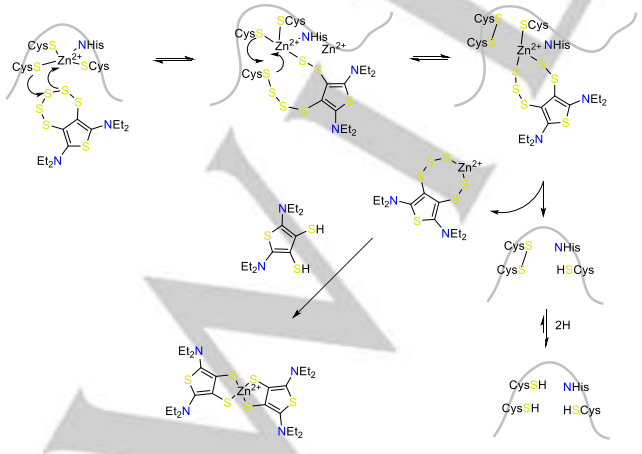


Figure 7. *Proposed mechanism of zinc ejections by compound* ***14***

This then undergoes a rearrangement to form an intramolecular protein disulfide with consequent reduction in zinc ion affinity. The ejected zinc ion (or zinc pentathiepin complex) can then complex with a second (reduced) pentathiepin or dithiol core to form a stable complex. This is supported by several previously reported ejection mechanisms and by our DFT simulations (Figure 8).[70]

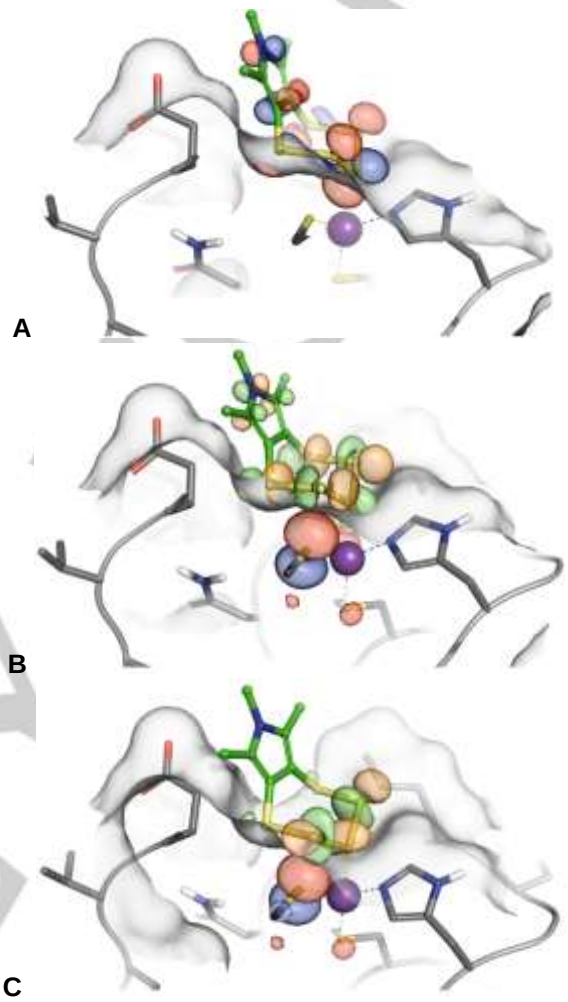


Figure 8. *A - HOMO-1 orbital of* ***20a*** *overlapping with FIV model; B - Beta-HOMO orbital of FIV model overlapping with* ***20a*** *LUMO+2 orbital; C - Beta-HOMO orbital of FIV model overlapping with* ***20a*** *LUMO+ orbital*

Interestingly with just the addition of one methyl group **20b**, a drop of 100 nM in potency against FIV was also matched with an increase in CC_{50} from just under 0.5 µM to around 5 µM. The most encouraging result of all was **20c** as it demonstrated good activity (<1 µM) while having a sharp reduction in toxicity (>50 µM). This series of compounds has potential for further optimization as the toxicity observed in this chemotype is likely due to the susceptibility of non-specific reactivity and possible ring opening. This is supported by the fact that this compound (**20c**) is more resilient to force dimerization with sodium cyanide to form the corresponding tetrathiocine.[71]

The switch in electronics toward **22a** and **22b** produced the most active compounds at EC_{50} = 5.8 nM and 1.9 nM respectively but there was also a matching increase in toxicity associated with the compounds. The asymmetric variant **23a** however showed lower activity (>200 nM) but also a drop off in toxicity (>0.5 µM). The substituted indoles **25a** and **25b** provided good activities with the ethyl substitution was preferred over the methyl but further examples would be needed to probe the potential of this result.

This could also include creation of a conjugate as recently exemplified by Greer *et al.* to increase cell penetration.[72]

During the course of our investigations we also demonstrated the validity of the FIV model for the use as a HIV-1 surrogate with a 53% homology in the minimised model (Figure 9), which is supported by a number of recent reviews.[73-75]

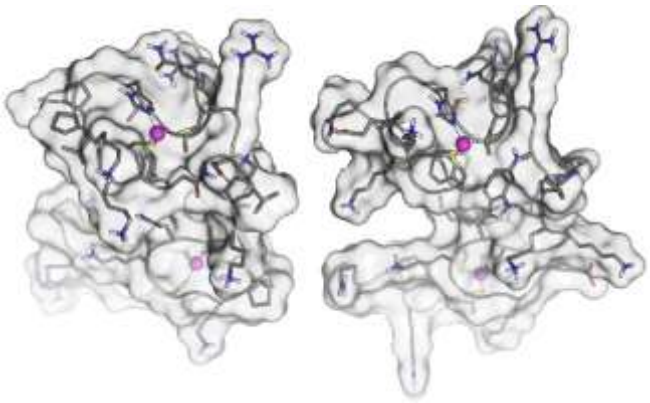


Figure 9. *Direct comparison of minimised nucleocapsid proteins of FIV and HIV-1*

Conclusions

The sub-micro molar potencies of these compounds coupled with moderate toxicities show an interesting avenue of investigation towards the development of a candidate compound for targeting the FIV/HIV NCp. The low toxicity and good activity of the initial thiophene hit **14** that precipitated our initial investigation was improved by substitution with an alkyl pyrrole scaffold. However, the simple trimethyl substituted compound **20a** was highly active albeit with some toxicity. **20c** was the most interesting result with slightly lower activity but significantly reduced toxicity. This suggests that there is potential to reduce any non-specific toxicity associated with this scaffold and it is not the pentathiepin functionality itself that is the toxic motif, rather the electronic contributions pendant arms. This can clearly be seen with the chlorinated derivatives **22a** and **22b**, but with a shift in electronics, such as asymmetric **23a,** there is slight reduction in activity but also bigger reduction of toxicity. The pentathiepin functionality while under-explored has the potential to generate a pre-clinical candidate that can treat both FIV and HIV in an *in-vivo* setting.

Experimental Section

Chemistry

Previously reported compounds repeated synthesis consistant with previous results: N^6,N^6,N^8,N^8-Tetraethylthieno[3,4-*f*][1,2,3,4,5]pentathiepine-6,8-diamine (**14**),[56] N^6,N^8-diethyl-N^6,N^8-dipropylthieno[3,4-*f*][1,2,3,4,5]pentathiepine-6,8-diamine (**18a**),[56] N^6,N^6,N^8,N^8-tetrabenzylthieno[3,4-*f*][1,2,3,4,5]pentathiepine-6,8-diamine (**18b**),[56] 6,7,8-trimethyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrrole (**20a**),[57] 7-ethyl-6,8-dimethyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrrole (**20b**),[57] 7-methyl-6,8-diphenyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrrole (**20c**),[57] 6,8-dichloro-7-methyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrrole (**22a**),[61] 6,8-dichloro-7-isopropyl-7*H*-[1,2,3,4,5]pentathiepino[6,7-c]pyrrole (**22b**),[61] 6-methyl-6*H*-[1,2,3,4,5]pentathiepino[6,7-*b*]indole (**25a**),[61] 6-ethyl-6*H*-[1,2,3,4,5]pentathiepino[6,7-*b*]indole (**25b**)[61] were prepared according to literature procedures. Synthesis, analytical, physicochemical and spectroscopic data for newly synthesized compound **23a** are reported below.

7-Chloro-6-methyl-6*H*-[1,2,3,4,5]pentathiepino[6,7-*b*]pyrrole (**23a**). Sulfur monochloride (1.6 ml, 20 mmol) was added dropwise at ï 30 - - 35 °C to a stirred solution of *N*-methylpyrrole (0.40 g, 5 mmol) and DABCO (2.24 g, 20 mmol) dissolved in chloroform (50 ml). Then the mixture was stirred for 15 min at -20 °C and at room temperature for 48 h. The solvent was removed under reduced pressure. The residue was separated by column chromatography (silica, light petroleum, and then light petroleum-DCM mixtures) to produce a yellow solid (0.518 g, 1.9 mmol, 38%) m.p. 68-69 °C. Anal. Calcd for $C_5H_4ClNS_5$ (%): C, 21.93; H, 1.47; N, 5.11. Found (%) C, 22.08; H, 1.56; N, 5.23%. ^{1}H NMR (300 MHz, $CDCl_3$) ŭ: 3.71 (s, 3H, CH_3), 6.43 (s, 1H, pyr); ^{13}C NMR (75.5 MHz, $CDCl_3$) 33.2 (CH_3), 113.7 (CH), 118.8, 128.2 and 132.1 (3 sp^2 tertiary C); m/z (EI) 275 (M^++ 2, 13%), 273 (M^+, 24%, 209 (M-S_2, 100). $C_5H_4ClNS_5$ requires [M^+] 272.8636, found [M^+] 272.8643.

Molecular Modelling

DFT studies. Homology model based on model compound composition was set up using Maestro software (Schrödinger Inc., New York, NY, USA). The geometry of zinc finger cysteines and histidines were constrained to their initial geometry using Cartesian constraints to connector carbon atoms. The density functional B3LYP of theory and MSV basis set were used for geometry optimizations in the gas phase using Jaguar module of Schrödinger suite. Furthermore, other than zinc-finger minimisation, the model structures were geometry optimised using unconstrained gas phase geometry DFT using B3LYP theory and 6-31+G** basis set to obtain atomic fukui indices and visulaized orbitals.

Acknowledgements

The authors are grateful to Bloomsbury Colleges - University of London; University College London; Biocenter Finland/DDCB & Russian Science Foundation (grant no. 15-13-10022) for financial support towards the goals of our work. We also thank the Center for Clinical Studies, Vetsuisse Faculty, University of Zurich for logistical support and the CSC-IT Center for Science Ltd. - Finland, for the allocation of computational resources. In addition we thank the EPSRC UK National Crystallography Service for funding and the collection of the crystallographic data of **20a**.

Keywords: 1,2,3,4,5-Pentathiepin ÅVaracin ÅNucleocapsid protein ÅFIV ÅHIV

References

[1] A. J. Leslie, K. J. Pfafferott, P. Chetty, R. Draenert, M. M. Addo, M. Feeney, Y. Tang, E. C. Holmes, T. Allen, J. G. Prado, M. Altfeld, C. Brander, C. Dixon, D. Ramduth, P. Jeena, S. A. Thomas, A. St John, T. A. Roach, B. Kupfer, G. Luzzi, A. Edwards, G. Taylor, H. Lyall, G. Tudor-Williams, V. Novelli, J. Martinez-Picado, P. Kiepiela, B. D. Walker, P. J. R. Goulder, *Nat Med.* **2004**, *10*, 282-289.

[2] Report on the Global AIDS Epidemic; UNAIDS: Geneva, Switzerland **2013**

[3] N. C. Pedersen, E. W. Ho, M. L. Brown, J. K. Yamamoto, *Science*. **1987**, *235*, 790-793.

[4] T. Hatziioannou, D. T. Evans, *Nat. Rev. Microbiol.* **2012**, *10*, 852-867.

[5] L. Rong, C. Liang, M. Hsu, L. Kleiman, P. Petitjean, H. de Rocquigny, B. P. Roques, M. A. Wainberg, *J. Virol.* **1998**, 72, 9353-9358.

[6] S. Ramboarina, Druillennec, N. Morellet, S. Bouaziz, B. P. Roques, *J. Virol.* **2004**, *78*, 6682-6687.

[7] T. Matsui, Y. Kodera, E. Miyauchi, H. Tanaka, H. Endoh, H. Komatsu, T. Tanaka, T. Kohno, T. Maeda, *Biochem. Biophys. Res. Commun.* **2007**, *358*, 673-678.

[8] M. L. Manrique, M. L. Rauddi, S. A. González, J. L. Affranchino, *J. Virol.* **2004**, *327*, 83-92.

[9] N. Morellet, H. Meudal, S. Bouaziz, B. P. Roques, *Biochem. J.* **2006**, *393*, 725-732.

[10] P. Amodeo, M. A. Castiglione-Morelli, A. Ostuni, G. Battistuzzi, A. Bavoso, *Biochemistry.* **2006**, *45*, 5517-5526.

[11] J. L. Darlix, M. Lapadat-Tapolsky, H. de Rocquigny, B. P. Roques, *J. Mol. Biol.* **1995**, *254*, 523-537.

[12] H. Demene, C. Z. Dong, M. Ottmann, M. C. Rouyez, N. Jullian, N. Morellet, Y. Mely, J. L. Darlix, M. C. Fournie-Zaluski, S. Saragosti, B. P. Roques, *Biochemistry.* **1994**, *33*, 11707-11716.

[13] R. J. Gorelick, T. D. Gagliardi, W. J. Bosche, T. A. Wiltrout, L. V. Coren, D. J. Chabot, J. D. Lifson, L. E. Henderson, L. O. Arthur, *Virology.* **1999**, *256*, 92-104.

[14] F. Brulé, R. Marquet, L. Rong, M. A. Wainberg, B. P. Roques, S. F. Le Grice, B. Ehresmann, C. Ehresmann, *RNA.* **2002**, *8*, 8-15.

[15] C. Isel, C. Ehresmann, R. Marquet, *Viruses.* **2010**, *2*, 213-43.

[16] Y. Zhang, H. Qian, Z. Love, E. J. Barklis, *J. Virol.* **1998**, 72, 1782-1789.

[17] S. Carteau, S. C. Batson, L. Poljak, J. F. Mouscadet, H. de Rocquigny, J. L. Darlix, B. P. Roques, E. Käs, C. Auclair. *J. Virol.* **1997**, 71, 6225-6229.

[18] G. Mirambeau, S. Lyonnais, D. Coulaud, L. Hameau, S. Lafosse, J. Jeusset, I. Borde, M. Reboud-Ravaux, T. Restle, R. J. Gorelick, E. Le Cam, *PLoS One.* **2007**, *7*, e669.

[19] V. Tanchou, D. Decimo, C. Péchoux, D. Lener, V. Rogemond, L. Berthoux, M. Ottmann, J. L. Darlix, *J. Virol.* **1998**, *72*, 4442-4447.

[20] L. Sancineto, N. Iraci, O. Tabarrini, C. Santi, *Drug Discov Today.* **2018**, *23*, 260-271.

[21] N. Iraci, O. Tabarrini, C. Santi, L. Sancineto, *Drug Discov Today.* **2018**, *23*, 687-695.

[22] W. G. Rice, C. A. Schaeffer, L. Graham, M. Bu, J. S. McDougal, S. L. Orloff, F. Villinger, M. Young, S. Oroszlan, M. R. Fesen, Y. Pommier, J. Mendeleyev, E. Kun, E. *Proc. Natl. Acad. Sci. USA.* **1993**, *90*, 9721-9724.

[23] W. G. Rice, J. G. Supko, L. Malspeis, R. W. Jr. Buckheit, D. Clanton, M. Bu, L. Graham, C. A. Schaeffer, J. A. Turpin, J. Domagala, R. Gogliotti, J. P. Bader, S. M. Halliday, L. Coren, R. C. 2nd. Sowder, L. O. Arthur, L. E. Henderson, *Science.* **1995**, *270*, 1194-1197.

[24] W. G. Rice, J. A. Turpin, M. Huang, D. Clanton, R. W. Jr. Buckheit, D. G. Covell, A. Wallqvist, N. B. McDonnell, R. N. DeGuzman, M. F. Summers, L. Zalkow, J. P. Bader, R. D. Haugwitz, E. A. Sausville, *Nat Med.* **1997**, *3*, 341-345.

[25] J. A. Loo, T. P. Holler, J. Sanchez, R. Gogliotti, L. Maloney, M. D. Reily, *J. Med. Chem.* **1996**, *39*, 4313-4320.

[26] J. A. Turpin, Y. Song, J. K. Inman, M. Huang, A. Wallqvist, A. Maynard, D. G. Covell, W. G. Rice, E. Appella. *J. Med. Chem.* **1999**, *42*, 67-86.

[27] Mayasundari, A.; Rice, W. G.; Diminnie, J. B.; Baker, D. C. Bioorg Med Chem. 2003, 11, 3215-3219.

[28] C. R. M. Asquith, M. L. Meli, L. S. Konstantinova, T. Laitinen, A. Poso, O. A. Rakitin, K. Allenspach, R. Hofmann-Lehmann, S. T. Hilton. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1352-1355.

[29] F. Fehér, B. Degen, *Angew. Chem. Int. Ed.* **1967**, *6*, 703-704.

[30] F. Fehér, M. Langer, *Tetrahedron Lett.* **1971**, *12*, 2125-2126.

[31] F. Fehér, Langer, R. Volkert, *Z. Naturforsch. B* **1972**, *27*, 1006-1007.

[32] S. A. Vladuchick, **1977**, US 4094985 A

[33] K. Moberg, **1981**, US 4275073 A

[34] B. L. Chenard, **1984**, US 4571404 A

[35] B. L. Chenard, **1985**, EP 0138622 A3

[36] S. A. Vladuchick, T. Fukunaga, H. E. Simmons, O. W. Webster, *J. Org. Chem.* **1980**, *45*, 5122-5130.

[37] B. L. Chenard, T. J. Miller, *J. Org. Chem.* **1984**, *49*, 1221-1224.

[38] B. S. Davidson, T. F. Molinski, L. R. Barrows, C. M. Ireland, *J. Am. Chem. Soc.* **1991,** *113*, 4709-4710.

[39] M. Litaudon, F. Trigalo, M. Martin, F. Frappier, M. Guyot, *Tetrahedron* **1994**, *50*, 5323-5334.

[40] A. H. Lee, A. S. Chan, T. Li, *Chem. Commun. (Camb).* **2002**, *18*, 2112-2113.

[41] D. Barton, K. Nakanishi, O. Meth-Chon, Comprehensive Natural Products Chemistry, **1999**.

[42] R. S. Compagnone, D. J. Faulkner, B. K. Carté, G. Chan, A. Freyer, M. E. Hemling, G. A. Hofmann, M. R. Mattern. *Tetrahedron* **1994**, *50*, 12785-12792.

[43] R. Sato, T. Ohyama, S. Ogawa, *Heterocycles* **1995**, *41*, 893-896.

[44] T. Chatterji, K. S. Gates, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 535-538.

[45] T. Chatterji, K. S. Gates, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1349-1352.

[46] T. D. Baguley, A. C. Nairn, P. J. Lombroso, J. A. Ellman, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1044-1046.

[47] J. Xu, M. Chatterjee, T. D. Baguley, J. Brouillette, P. Kurup, D. Ghosh, J. Kanyo, Y. Zhang, K. Seyb, C. Ononenyi, E. Foscue, G. M. Anderson, J. Gresack, G. D. Cuny, M. A. Glicksman, P. Greengard, T. T. Lam, L. Tautz, A. C. Nairn, J. A. Ellman, P. J. Lombroso, *PLoS Biol.* **2014**, *12*, e1001923.

[48] A. Zakharenko, T. Khomenko, S. Zhukova, O. Koval, O. Zakharova, R. Anarbaev, N. Lebedeva, D. Korchagina, N. Komarova, V. Vasiliev, J. Reynisson, K. Volcho, N. Salakhutdinov, O. Lavrik, *Bioorg. Med. Chem.* **2015**, *23*, 2044-2052.

[49] G. Buemi, F. Zuccarello, A. Raudino, *J. Mol. Struct.* **1988,** *167*, 245-252.

[50] B. Meyer. *Chem Rev.* **1976**, *76*, 367-388.

[51] (a) L. S. Konstantinova, O. A. Rakitin, C. W. Rees, *Chem Rev.* **2004**, *104*, 2617-2630; (b) L. S. Konstantinova, S. A. Amelichev, O. A. Rakitin, *Russ. Chem. Rev.* **2007**, *76*, 195-212.

[52] T. Janosik, B. Stensland, J. Bergman, *J. Org. Chem.* **2002**, *67*, 6220-6223.

[53] G. W. Rewcastle, T. Janosik, J. Bergman, *Tetrahedron* **2001**, *57*, 7185-7189.

[54] M. Zubair, A. C. Ghosh, C. Schulzke, *Chem. Commun.* **2013**, *49*, 4343-4345.

[55] (a) L. S. Konstantinova, O. A. Rakitin, *Mendeleev Commun*., **2009**, *19*, 55-61; (b) O. A. Rakitin, L. S. Konstantinova, *Adv. Heterocycl. Chem*., **2008**, *96*, 175-229**.**

[56] L. S. Konstantinova, O. A. Rakitin, C. W. Rees, L. I. Souvorova, D. G. Golovanov, K. A. Lyssenko, *Org. Lett.* **2003**, *5*, 1939-1942.

[57] S. A. Amelichev, R. R. Aysin, L. S. Konstantinova, N. V. Obruchnikova, O. A. Rakitin, C. W. Rees, *Org. Lett.* **2005**, *7*, 5725-5727.

[58] L. S. Konstantinova, O. A. Rakitin, C. W. Rees, *Chem. Comm.* **2002**, *11*, 1204-1205.

[59] L. S. Konstantinova, S. A. Amelichev, O. A. Rakitin, *Russ. Chem. Bull.* **2006**, *55*, 2081-2084.

[60] L. S. Konstantinova, O. A. Rakitin, C. W. Rees, S. A. Amelichev, *Mendeleev Commun.* **2004**, *14*, 91-92.

[61] S. A. Amelichev, L. S. Konstantinova, K. A. Lyssenko, O. A. Rakitin, C. W. Rees, *Org. Biomol. Chem.* **2005**, *3*, 3496-3501.

[62] P. W. Sylvester, *Methods Mol. Biol.* **2011**, *716*, 157-168.

[63] R. A. Crandell, C. G. Fabricant, W A. Nelson-Rees, *In Vitro.* **1973**, *9*, 176-185.

[64] J. K. Yamamoto, C. D. Ackley, H. Zochlinski, H. Louie, E. Pembroke, M. , H. Hansen, R. Munn, T. Okuda, *Intervirology.* **1991**, *32*, 361-375.

[65] D. Klein, C. M. Leutenegger, C. Bahula, P. Gold, R. Hofmann-Lehmann, B. Salmons, H. Lutz, W. H. Gunzburg, *J. Acquir. Immune. Defic. Syndr.* **2001**, *26*, 8-20.

[66] L. R. Bisset, H. Lutz, J. Böni, R. Hofmann-Lehmann, R. Lüthy, J. Schüpbach, *J. Antiviral. Res.* **2002**, *53*, 35-45.

[67] V. Summa, A. Petrocchi, F. Bonelli, B. Crescenzi, M. Donghi, M. Ferrara, F. Fiore, C. Gardelli, O. Gonzalez Paz, D. J. Hazuda, P. Jones, O. Kinzel, R. Laufer, E. Monteagudo, E. Muraglia, E. Nizi, F. Orvieto, P. Pace, G. Pescatore, R. Scarpelli, K. Stillmock, M. V. Witmer, M. Rowley, *J. Med. Chem.* **2008**, *51*, 5843-5855.

[68] CCDC depostion number for **20a** crystal structure 1582904

[69] The Cambridge Structural Database, C. R. Groom, I. J. Bruno, M. P. Lightfoot, S. C. Ward, *Acta Cryst.,* **2016**, B72, 171-179

[70] (a) J. C. Woodcock, W. Henderson, C. O. Miles *J. Inorg. Biochem.* **2001**, *85*, 187-199 (b) J. C. Woodcock, W. Henderson, C. O. Miles, B. K. Nicholson *J. Inorg. Biochem.* **2001**, *84*, 225-232 (c) K. M. Cook, S. T. Hilton, J. Mecinoví , W. B. Motherwell, W. D. Figg, C. J. Schofield *J Biol Chem.* **2009**, *284*, 26831-26838 (d) C. R. M. Asquith, L. S. Konstantinova, T . Laitinen, M. L. Meli, A. Poso, O. A. Rakitin, R. Hofmann-Lehmann, S. T. Hilton *ChemMedChem.* **2016**, *11*, 2119-2126.

[71] L. S. Konstantinova, S. A. Amelichev, O. A. Rakitin, *Russ. Chem. Bull.* **2007**, *56*, 1540-1543.

[72] A. Mahendran, A. A. Ghogare, R. Bittman, G. Arthur, A. Greer, *Chem Phys Lipids.* **2016**, *194*, 165-170.

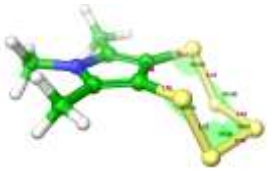
[73] R. B. Meeker, L. Hudson, *Vet Sci.* **2017**, *4*, pii: E14.

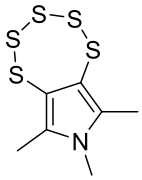
[74] K. Sliva *Expert Opin Drug Discov*. **2015**, *10*, 111-123.

[75] C. Miller, Z. Abdo, A. Ericsson, J. Elder, S. VandeWoude, *Viruses.* **2018**, 10, pii: E206

10.1002/cmdc.201800718

Summary





Compound **20a**
FIV profile
CC_{50} = 460 nM
EC_{50} = 4.4 nM
Theraputic Index = 105
cLogP = 3.63

A diverse library of pentathiepin derivatives were prepared which showed nanomolar efficacy against FIV in cells. The low molecular weight and toxicity, make these compounds interesting starting points for future development of a nucleocapsid inhibitor. The structure of these compounds and potential mechanism of action was also probed resulting in an identification of the S_2-S_3 position as the likely initial step of the zinc ejection mechanism.