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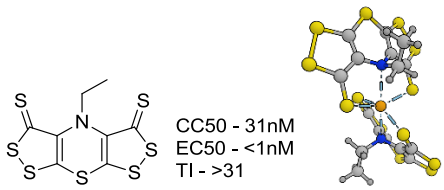

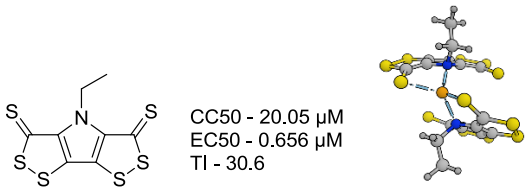

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Evaluation of the antiviral efficacy of *bis*[1,2]dithiolo[1,4]thiazines and *bis*[1,2]dithiolopyrrole derivatives against the nucelocapsid protein of the Feline Immunodeficiency Virus (FIV) as a model for HIV infection

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ABSTRACT

A diverse library of *bis*[1,2]dithiolo[1,4]thiazines and *bis*[1,2]dithiolopyrrole derivatives were prepared for evaluation of activity against the nucleocapsid protein of the Feline Immunodeficiency Virus (FIV) as a model for HIV, using an *in-vitro* cell culture approach, yielding nanomolar active compounds with low toxicity.

Therapeutic resistance to anti-viral drugs *via* mutation is a major challenge affecting both human and veterinary medicine. Following the discovery of the human immunodeficiency virus (HIV), this single disease alone has caused over 25 million deaths worldwide, with over 34 million people currently infected,[1] highlighting the unique challenge in this research area. Amongst non-human vertebrates, feline immunodeficiency virus (FIV) infection in cats is perhaps the closest biological model of HIV infection with an analogous AIDS-like progression,[2] although infection primarily occurs through biting and reproduction with about 11% of cats currently infected worldwide.[3] FIV and HIV are closely related to other lentiviruses including the simian (SIV) and equine (EIAV) variants,[4,5] each of which is a species-specific virus using analogous proteins in the viral life cycle.

The nucleocapsid protein of FIV is a short basic nucleic acid binding protein, which contains two key zinc finger domains based on the peptide unit C-X_2-C-X_4-H-X_4-C (CCHC) that is found in nearly all retroviruses.[6-10] The nucleocapsid protein is implicated in both early (DNA synthesis and integration) and late phases (genomic RNA selection, dimerisation, packaging and budding) of viral replication.[10-12] Mutagenesis studies have clearly demonstrated that transcription errors in this key protein are not tolerated and deletion or modification of either zinc finger leads to virus inactivation.[13,14] Current approaches towards the development of compounds that target the nucleocapsid protein have focused on small molecules that sequester the Zn^{2+} ion from within the zinc finger core. These compounds are able to achieve this with high affinity in protein based assays (Fig. 1).[15-20]

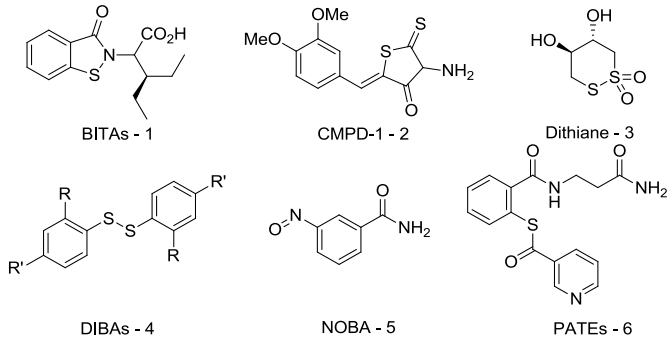

Figure 1 - Previously reported NCp7 targeting small molecules.

Despite the promising activities displayed by a number of these compounds, several have shown unacceptable levels of

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toxicity and as such, the search for new chemical entities against this mutation resistant target continues.[15-20] Herein we describe the development of a novel series of compounds with low toxicity and high potency that were selected for their potential to target the nucleocapsid protein in FIV, as part of our longer-term goal of developing mutation-resistant therapeutics against HIV.[15]

Previous reported agents against the nucleocapsid protein have been shown to cause inhibition, by covalent modification of the zinc fingers in NCp7, leading to zinc ejection and ultimately, inactivation of protein function.[15-20] The electrophilic center plays a key role in compound activity and we were intrigued by the possibility of developing novel agents based around scorpionine derivatives **8a** and **9** (Fig. 2); which contain two disulfide moieties that could potentially target the nucleocapsid of FIV in the first instance and ultimately lead to novel mutation resistant drugs against HIV.

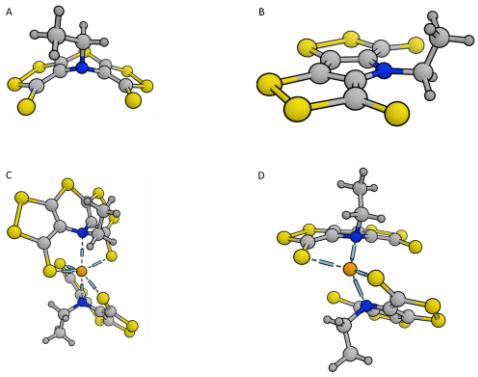


Figure 2 - Comparison of molecules **8a** (A) with octahedral binding (C) and **9** (B) with tetrahedral binding (D) upon Zinc (Zn^{2+}) binding. Optimized gas phase geometries using Gaussian 09 software.[21]

We initially developed a homology model of the nucleocapsid protein of FIV, based on the published structure of the NCp7 protein of HIV, to enable us to investigate whether we could treat FIV via this protein target, by Zn^{2+} ejection (Fig. 3).[22]

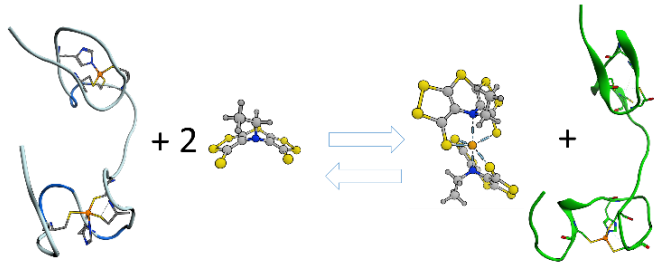
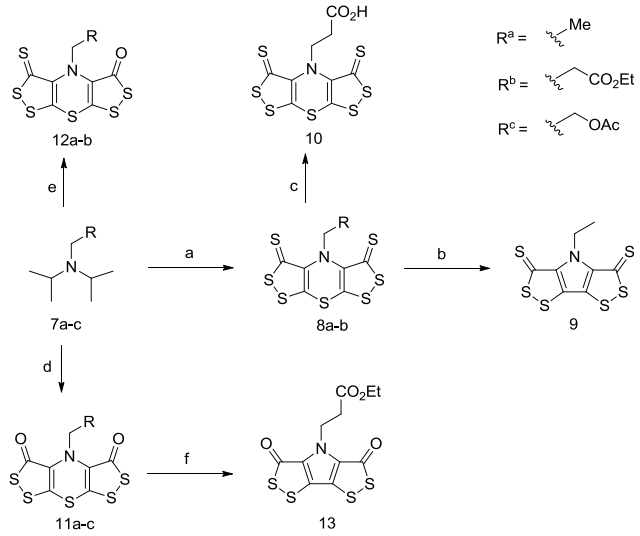


Figure 3 - Putative Zinc abstraction from a homology model of NCp7 by compound **8a**.[21]

Our proposed mechanism of action is based on nucleophilic attack on the Zn-S bond, by a form of redox reaction between the cysteine groups followed by successful chelation and ejection leading to protein dysfunction as outlined above (Fig. 3). The scorpionine core **8a** is ideal, as it potentially obviates the need for a second molecule in our proposed mechanism as it has the required number of functionalities for tetrahedral coordination around the Zn^{2+} atom. This would potentially lead to a lower dose requirement and a higher potency compound.[2,23]

The synthesis of the scorpionine core **8a**, involves a multi-step process and poses a synthetic challenge. Reacting *N*-ethyldiisopropylamine derivatives such as **7a** with a disulfur dichloride/DABCO complex is one way to achieve this under mild conditions. The reaction can produce scorpionine **8a** in good yield (40%), with surprising control.[24-27] The one pot conversion is surprisingly scalable with the correct conditions, especially considering the proposed eleven conversion step process involved. Disulfur dichloride was added dropwise to a solution of *N*-ethyldiisopropylamine dervatives **7a-b** and DABCO at -40 °C in 1,2-dichloroethane/chloroform and stirred for 15 min at -40 °C, then at room temperature for 3 days and a further 2 hours at reflux to produce **8a-b**. The product **8a** can then be further manipulated by suspension in xylene at reflux for 30 minutes to extrude the central sulfur atom and produce **9**.[28] Considering the polar nature of the assay medium involved we converted compound **8b** into the corresponding carboxylic acid **10** with the aim of increasing solubility.[27]

The original disulfur dichloride/DABCO reaction can be manipulated to produce different products by a change of solvent[26] and the addition of formic acid or cyclopenten-1-ylacetic acid.[29] Switching from 1,2-dichloroethane to THF with the addition of formic acid or cyclopenten-1-ylacetic acid allowed the furnishing of compounds containing two oxygen atoms **11a-c**[27-30] and one oxygen atom and one sulfur atom **12a-b** with reasonable selectivity, from the corresponding reactants **7a-c**. Compound **11b** was then also suspended in xylene at reflux for 18 hours to extrude the central sulfur and produce **13**. This synthetic strategy allowed us to synthesize a number of derivatives as outlined in Scheme 1 exploring the core of scorpionine **8a**.



Scheme 1 - Formation of *bis*[1,2]dithiolo[1,4]thiazine and *bis*[1,2]dithiolopyrrole derivatives. *Reagents and conditions:* (a) S_2Cl_2/DABCO - 40 °C to reflux, **8a** - 1,2-dichloroethane, 40%, **8b** -$CHCl_3$, 13%: (b) xylene, reflux, 1 h, >99%; (c) H_2SO_4, 60°C, 1 h, 93%; (d) S_2Cl_2/DABCO -40 °C to reflux, formic acid**, 11a** - CH_2Cl_2, 42%, **11b** - $CHCl_3$, 30%, **11c** - $CHCl_3$, 29%;[30] (e) S_2Cl_2/DABCO -40 °C to reflux, **12a**-THF, cyclopenten-1-ylacetic acid, 42% **12b** - $CHCl_3$, formic acid**,** 13%; (f) xylene, reflux, 18 h, >99%.

Biological testing was split into two stages, the first assay ruling out medium compound toxicity effects, with a short screen over 24 hours at three higher concentrations (100 μM - 1 μM) exposing the compounds to the Crandell Rees Feline Kidney cell line (CrFK), with the second assay giving an enhanced toxicity and anti-FIV profile. This assay was carried out over seven days at six concentrations ranging between 100 μM and 1 nM, exposing the compounds to an IL-2 independent feline lymphoblastoid cell line (FL-4). The different toxicity profiles between cell lines can be accounted for by the different exposure intervals and the susceptibility of the cell line to the compounds.

The 24-hour assay rules out any major toxicity issues, which is key, as cytotoxic compounds removed at an early stage can prevent false positives and later development of toxic core structures. Compounds that passed the initial 24-hour exposure

test with reasonable viabilities in CrFK cells of >75% at 10 μM were then screened against FIV, with an MTT assay used to quantify the level of cell viability.[31,32]

The second phase of testing exposed the compounds to FL-4 cells over a period of seven days, with sampling every day and at each concentration (100 μM-1 nM serial dilution (x10)). Viral RNA was isolated from supernatants in a MagNA Pure LC System using the Total Nucleic Acid Isolation Kit (Roche Applied Science) and was subsequently used to determine the viral load by a quantitative real-time reverse-transcription polymerase chain reaction (RT-qPCR) for FIV RNA.[33] The screening results were then checked for their viability to rule out any toxicity effects validating the RT-qPCR result.

Table 1 - Results of cytotoxic screening and FIV viral loading assay of the *bis*[1,2]dithiolo[1,4]thiazines and *bis*[1,2]dithiolopyrroles derivatives.

Compound				Residue	CrFK[a]	CC50[b]	EC50[b]	TI	clogP[c]
Number	X	X	CRS		% Viability	μM	nM		
8a	S	S	6		89.8	0.031	<1	>31	3.59
9	S	S	5		>100	20.060	655.8	30.6	2.29
10	S	S	6	CO_2H	97.2	1.720	379.0	4.5	2.63
11a	O	O	6		40.2	n/d	n/d	n/d	2.20
11b	O	O	6	CO_2Et	76.8	0.056	16.2	3.5	1.67
11c	O	O	6	OAc	39.2	n/d	n/d	n/d	3.50
12a	O	S	6		>100	>10	344.9	>29	3.86
12b	O	S	6	CO_2Et	91.2	2.270	641.9	3.5	2.94
13	O	O	5	CO_2Et	78.2[d]	n/d	n/d	n/d	1.92
AZT	-	-	-	-	>100	>100	5310.0	>18.8	-0.16
Raltegravir	-	-	-	-	>100	>100	9.98	>100000	1.16

[a]Sample concentration of compound in CrFK cells at 10μM; [b]Geometric mean, each concentration tested in triplicate after 7 days as a difference of the untreated cells; [c]Calculated using *Chembiodraw ultra 12*; [d]not done due lack of improvement to toxicity profile compared to **11b** [e]n/d - Not done due to toxicity.

The results in Table 1 clearly show that the scorpionine core displayed a range of activity, with compound **8a** demonstrating excellent activity below 1 nM, with a reasonable ratio between the CC50 and EC50 resulting in an acceptable therapeutic index. Development of this initial compound led us to different ring systems such as **9** and **13** with differing electron densities in dioxothiones **11a-c** and mono-substituted oxothiones **12a-b**, in the hope that some toxicity issues could be overcome whilst maintaining potency.

Known anti-retroviral drugs were also tested in our assay, with Raltegravir displaying similar results to the reported values for HIV.[34] AZT whilst consistent with the literature for FIV,[35,36] displayed a surprising lack of toxicity *in-vitro* than that which is known *in-vivo*. This could be due to the lack of available enzymes *in-vitro* required for phosphorylation and activation *in-vivo*.[36]

Modification of the core was designed to improve potency while reducing the toxicity seen with **8a**. The introduction of different heteroatoms to the thione functionality proved key - changing from sulphur to oxygen atoms appears to be more toxic in **8a** to **11a**, but mono-substitution as in **12a** gave some improvement in toxicity. This was broadly true for the longer toxicity screen with two oxygen atom substitutions, proving too toxic to be worth screening further than the initial assay.

Despite modification, the original scorpinine compound **8a** proved the most potent compound, but exploration of the toxicity profile via **11a-11c** and **13** showed that double oxygen atom substitution proved unworthy of further investigation. **11b** was slightly less toxic than **8a**, but the potency also dropped, whilst the equivalent mono substituted compound **12b** had better toxicity with a CC50 of 2.27 μM, but had the same therapeutic index of 3.5, so had actually made little improvement. Reducing the ring size of **11b** to **13** also showed no improvement in the initial toxicity screen and so was not tested further. A surprising result was that **12a** showed similar potency to **10**, as it was anticipated that a carboxylic acid would be superior to an ester due to the enhanced solubility and reduced cLogP. The compound with the most promise was **9**; the decreased ring size clearly had an effect on the toxicity unlike **13**. The activity of **9** was lower than **8a**, but the increased therapeutic window partially compensated for this. This could also be attributed to the gas phase data that shows octahedral binding for **8a** and tetrahedral for **9**.

The progression from day one to day seven showed a marked decrease in viral loads in the supernatant and hence less virion shedding, as exposure of the cells continued. We observed a full spectrum of results from inactive/toxic at high concentration to highly active/non-toxic at low concentration. False positives were ruled out *via* toxicity screening, which validated the idea that the compounds can actively reduce the amount of virus shed from cells. This means application of the compounds to cells, slows down viral replication and decreases viral load. In the seven-day screening we saw significant viral reduction that was independent of toxicity.

Overall, the screening has shown that the scorpionine core is able to reduce viral loading and that it is a viable therapeutic target against the nucleocapsid protein of FIV that warrants further development. We have also shown that the diketothione was far more potent than the oxothione as it mitigates the toxicity concerns. We have also demonstrated that moving to a 5+5+5 scaffold over the initial 5+6+5 heterocyclic system appears to be favorable with increased potency and ligand efficiency while reducing toxicity. Compound **9** clearly show that this compound class has potential, with high activity and a reasonable therapeutic window. Investigations into this class continue with the aim of generating a pre-clinical candidate that can treat both FIV and HIV and will be reported in due course.

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References and notes

1. UNAIDS **2009**, AIDS Epidemic Update.
2. Goldschmidt, V.; Jenkins, L. M.; de Rocquigny, H.; Darlix, J-L.; Mély, Y. *HIV Therapy* **2010**, *4*, 179.
3. Richards, J. R. *Biologicals* **2005**, *33*, 215.
4. Letvin, N. L.; Eaton, K. A.; Aldrich, W. R.; Sehgal, P. K.; Blake, B. J.; Schlossman, S. F.; King, N. W.; Hunt, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2718.
5. Vallee, H.; Carré, H. *C. R. Hebd. Acad. Sci.* **1904**, *139*, 331.

6. Dietz, J.; Koch, J.; Kaur, A.; Raja, C.; Stein, S.; Grez, M.; Pustowka, A.; Mensch, S.; Möller, L.; Bannert, N.; Tampé, R.; Divita, Y.; Schwalbe, H.; Dietrich, U. *Chem. Med. Chem*. **2008**, *3*, 749.
7. Houzet, L.; Morichard, Z.; Didierlaurent, L.; Muriaux, D.; Darlix, J.; Mougel, M. *Nucleic Acids Res.*, **2008**, *36*, 2311.
8. Tummino, P. J.; Harvey, P. J.; McQuade, T.; Domagala, J.; Gogliotti, R.; Sanchez, J.; Song, Y.; Hupe, D. *Antimicrob. Agents Chemother*. **1997**, *41*, 394.
9. Von Schwedler, U. K.; Stemmler, T. L.; Klishko, V. Y.; Li, S.; Albertine, K. H.; Davis, D. R.; Sundquist, W. I. *EMBO* **1998**, *17*, 1555.
10. Rice, W. G.; Supko, J. G.; Malspeis, L.; Buckheit Jr., R. W.; Clanton, D.; Bu, M.; Graham, L.; Schaeffer, C. A.; Turpin, J. A.; Domagala, J.; Gogliotti, R.; Bader, J. P.; Halliday, S. M.; Coren, L.; Sowder II, R. C.; Arthur, L. O.; Henderson, L. E. *Science*, **1995**, *270*, 1194.
11. Wapling, J.; Srivastava, S.; Shehu-Xhilaga, M. Tachedjian, G. *Drug Target Insights* **2007**, *2*, 159.
12. Greene, W. C.; Debyser, Z.; Ikeda, Y.; Freed, E. O.; Stephens, E.; Yonemoto, W.; Buckheit, R. W.; Este, J. A.; Cihlar, T. *Antiviral Res.* **2008**, *80*, 251.
13. Schmalzbauer, E.; Strack, B.; Dannull, J.; Guehmann, S.; Moelling, K. *J. Virology* **1996**, *70*, 771.
14. Ramboarina, S.; Moreller, N.; Fournié-Zaluski, M.; Roques, B. P. *Biochemistry* **1999**, *38*, 9600.
15. Elder, J. H.; Lin, Y-C.; Fink, E.; Grant, C. K, *Current HIV Research* **2010**, *8*, 73.
16. Maynard, A. T.; Huang, M.; Rice, W. G.; Covell, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11578.
17. Breuer, S.; Chang, M. W., Yuan, J.; Torbett, B. E. *J. Med. Chem.* **2012**, *55*, 4968.
18. Mayasundari, A.; Rice, W. G.; Diminnie, J. B.; Baker, D. C. *Bioorg. Med. Chem.* **2003,** *11*, 3215.
19. Turpin, J. A.; Song, Y.; Inman, J. K.; Huang, M.; Wallqvist, A.; Maynard, A.; Covell, D. G.; Rice, W. G.; Appella, E. *J. Med. Chem.* **1999**, *42*, 67.
20. Warui, D. M.; Baranger, A. M. *J. Med. Chem.* **2012**, *55*, 4132.
21. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Cliff ord, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.Gaussian 03, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
22. Manrique, M. L.; Rauddi, M. L.; González, S. A.; Affranchino, J. L. *J. Virology* **2004**, *327*, 83.
23. Loo, J. A.; Holler, T. P.; Sanchez, J.; Gogliotti, R.; Maloney, L.; Reily, M. D. *J. Med. Chem.* **1996**, *39*, 4313.
24. Rakitin, O. A.; Konstantinova, L. S. *Adv. Heterocycl. Chem*. **2008**, *96*, 175.
25. Konstantinova, L. S.; Rakitin, O. A. *Mendeleev Commun*. **2009**, *19*, 55.
26. Rees, C. W.; Marcos, C. F.; Polo, C.; Torroba, T.; Rakitin O. A. *Angew. Chem. Int. Ed*. **1997**, *36*, 281.
27. Konstantinova, L. S.; Obruchnikova, N. V.; Rakitin, O. A.; Rees, C. W. Torroba. T. *J. Chem. Soc*., *Perkin Trans. 1* **2000**, 3421.
28. Marcos, C. F.; Polo, C.; Rakitin, O. A.; Rees, C. W.; Torroba, T. *Chem. Commun.* **1997**, 879.
29. Rees, C. W.; White, A. J. P.; Williams, D. J.; Rakitin, O. A.; Marcos, C. F.; Polo, C.; Torroba, T. *J. Org. Chem.* **1998**, *63*, 2189.
30. **2-(3,5-Dioxo-3*H*-bis([1,2]dithiolo)[3,4-*b*:4',3'-*e*][1,4]thiazin-4(5*H*)-yl)ethyl acetate (11c)**. Disulfur dichloride (0.80 ml, 10.0 mmol) was added dropwise at -35 to -30 ºC to a stirred solution of 2-(diisopropylamino)ethyl acetate (0.19 g, 1.00 mmol) and DABCO (0.78 g, 7.00 mmol) in chloroform (20 ml). The resulting mixture was stirred for 15 min at -30 ºC and then at room temperature for 72 h. Formic acid (3.75 ml, 100 mmol) was added dropwise at 0 ºC and the mixture was heated at reflux for 1 h, filtered and solvent was removed *in vacuo*. The residue was separated by column chromatography (silica, light petroleum, and then light petroleum–DCM mixtures) to give the *title compound **11c*** (105 mg, 29%) as yellow crystals, mp 149-151 ºC; Anal. Calcd for $C_{10}H_7NO_4S_5$: C, 32.86; H, 1.93; N, 3.83. Found: C, 33.06; H, 1.88; N, 3.80; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.02 (3H, s, Me), 4.09 (2H, t, CH_2, J = 5.1 Hz), 4.30 (2H, t, CH_2, J = 5.1 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 20.7 (Me), 44.5 (CH_2), 63.5 (CH_2), 136.7 (C), 146.2 (C), 170.7(C), 182.0 (C); MS (EI, 70 eV), m/z (%): 365 (M+, 16), 305 (5), 292 (16), 279 (10), 262 (6), 87 (100). HMRS: found *m/z* 364.8964; calc. for $C_{10}H_7NO_4S_5$ [M]+ 364.8979.
31. Sylvester, P. W. *Methods Mol. Biol*. **2011**, *716*, 157.
32. Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader. J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 577.
33. Klein, D.; Leutenegger, C. M.; Bahula, C.; Gold, P.; Hofmann-Lehmann, R.; Salmons, B.; Lutz, H.; Gunzburg, W. H. *J. Acquir. Immune Defic. Syndr.* **2001**, *26*, 8.
34. Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P. Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich R, Grobler, J.; Miller, M.D.; Espeseth, A.S.; Jin, L.; Chen, I. W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11233.
35. Bisset, L. R.: Lutz, H.: Böni, J.: Hofmann-Lehmann, R.; Lüthy, R.; Schüpbach, *J. Antiviral. Res.* **2002**, *53*, 35.
36. Vahlenkamp, T. W.; De Ronde, A.; Balzarini, J.; Naesens, L.; De Clercq, E.; van Eijk, M. J.; Horzinek, M. C.; Egberink, H. F. *Antimicrob. Agents Chemother*. **1995**, *39*,