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Synthesis of non-nucleoside anti-viral cyclopropylcarboxacyl hydrazones and initial anti-HSV-1 structure-activity relationship studies

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ABSTRACT

The synthesis of a lead anti-viral cyclopropyl carboxy acyl hydrazone 4F17 (**5**) and three sequential arrays of structural analogues along with the initial assessment and optimization of the antiviral pharmacophore against the herpes simplex virus type 1 (HSV-1) are reported.

The discovery of novel anti-viral agents targeting herpesviruses such as herpes simplex virus (HSV-1), varicella zoster virus (VZV) and human cytomegalovirus (HCMV) is of increasing concern given their prevalence (global infection rates 60–95%) and the severity of effects on the central nervous system.[1] Current treatment options are limited to nucleoside analogues acyclovir (ACV) **1** and the pro-drug valacyclovir **2**.[2] Both molecules cause serious side effects, including nephrotoxicity,[3] and drug-resistant viral strains continue to be reported.[4] These factors have provided a strong impetus for the discovery of molecules that exhibit alternative modes of action. We recently investigated the anti-viral activity of a collection non-nucleoside library-array compounds resulting in the discovery of several lead molecules that demonstrated broad-spectrum inhibition of human herpesviruses.[5a] Screening this collection of compounds[5b] revealed that the three derivatives 30 N12 (**3**), 16F19 (**4**) and 4F17 (**5**) (Fig. 1) demonstrated broad-spectrum activity against human herpesviruses.[6] While all three compounds demonstrated potent anti-viral activity to HSV-1 and varicella zoster virus (VZV), 4F17 (**5**) was distinguished by demonstrating low cytotoxicity in the respective neuronal or ARPE-19 host cells.

Most importantly, compound **5** was shown to significantly inhibit reactivation of quiescent HSV-1 infection in induced pluripotent stem cell (iPSC)-derived neurons, conferring a privileged status to this molecule toward the discovery of selective anti-viral therapeutics that target viral reactivation. While acyclovir **1** and valacyclovir **2** target viral replication, the mechanism of action of the non-nucleoside compound 4F17 is uncertain at present. The compound may act as a lysomotropic agent inhibiting viral packaging and maturation through modulating local pH.[7] Structurally similar acylhydrazones **6** have been shown to inhibit Dengue viral entry through similar pH modulation of endosomes,[7] while another set of analogues **7**, developed as alternatives to the lysomotropic agent chloroquine, have been shown to possess antimalarial activity.[8] Lastly, NMR-investigation of the library compound 4F17 was complicated by the presence of two sets of overlapping signals indicative of either decomposition or the existence of rotamers. In view of the valuable biological activity and chemical issue described, compound 4F17 became a high priority target in our anti-viral drug discovery program.[9] In this communication, we report the synthesis, and confirmation of the structure and stability of 4F17 (**5**) as the rotamers **5** and **8**. The synthesis and optimization of anti-viral activity through three consecutive rounds of structure–activity (HSV-1) investigations is described.

The synthesis of 4F17 (**5**) is outlined in Scheme 1. Cyclopropanation of commercially available 1,1,-diphenylethylene **9** with ethyl diazoacetate **10** was initially attempted under heterogeneous conditions with copper (II) salts[10] with limited success. In order to develop an inexpensive initially racemic version of the synthesis, we prepared a homogeneous copper (II) complex from racemic *N*-tosyltryptophan **18**, reacting with copper sulfate monohydrate **19**.

This method is based upon earlier unpublished observations from our group and results in the formation of a complex that is highly soluble in organic media, tentatively described as **20**. This homogeneous complex proved extremely efficient and the cyclopropane derivative **11** was isolated in good yield. Reaction with hydrazine hydrate **12** in refluxing ethanol provided the acylhydrazide **13** that was condensed with

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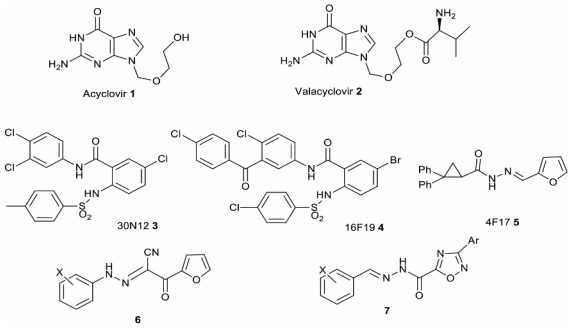
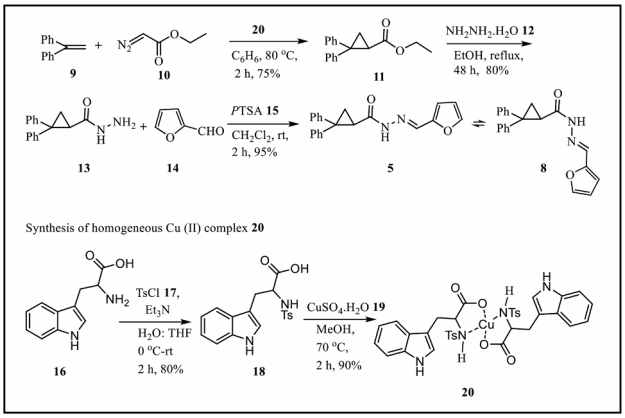


Fig. 1. Structure of current approved anti-herpesvirus drugs acyclovir 1, valacyclovir 2 and novel non-nucleoside hits **3–5**, anti-Dengue **6** and antimalarial **7** acylhydrazones.



Scheme 1. Synthesis of library target 4F17 (**5**) proceeding via homogeneous copper (II) catalysed cyclopropanation with **20**, hydrazinolysis and hydrazone condensation with furfural.

furfural **14** under acid catalysis to give synthetic 4F17, compound **5**. Analysis of synthetic **5** by NMR showed the pure molecule to exist as a **3:1** mixture of rotamers **5**:**8**, identical to that observed for the commercial library product 4F17, thus confirming the structure and stability and validating the initial anti-viral activity described for this compound. Synthetic **5** also exhibited anti-HSV1 activity indistinguishable from the library compound 4F17.

The synthetic route developed toward **5** outlined in Scheme 1 was designed to allow the preparation of multiple analogues for structure-antiviral activity investigations following our late-stage (last step) diversity-oriented approach that has proven successful elsewhere.[11] The first array of hydrazone derivatives was readily accessed from the penultimate intermediate **13** through condensation with a range of aldehydes and ketones to generate the acyl hydrazones **21–31** shown in Fig. 2. The anti-HSV-1 activity of these analogues was investigated in iPSC-neurons following the previously developed protocol.[5] The assay read-out is based on a recombinant HSV-1 strain derived from the KOS virus expressing the enhanced green fluorescent protein (EGFP) from the early ICP0 promoter and a monomeric red fluorescent protein (RFP) from the glycoprotein C promoter.[6] Uninfected cells as well as those treated with the recombinant HSV-1 in the presence of the positive control acyclovir (ACV) or synthetic 4F17 exhibited a low proportion of fluorescent cells, while those infected with HSV-1 alone or any of the initial compound screening set **21–31**, demonstrated relatively low inhibition of viral infection, with compounds **25**, **27** and **29–31** showing slight inhibition.[6]

Overall, the structure–activity correlation of the variously substituted benzaldehyde derived hydrazones disclosed no linear electronic effect, however, on the whole, electron poor analogues were seen to be somewhat less active (e.g. **23**, **28**). Conversely, electron rich

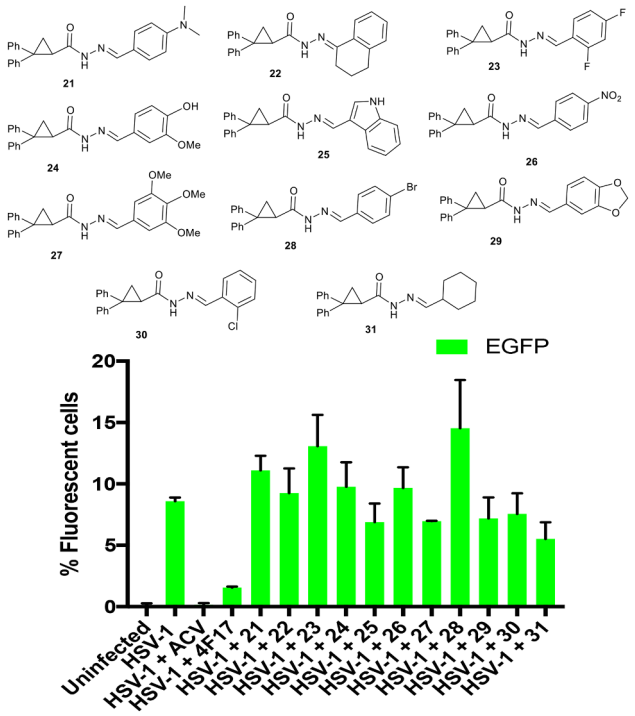


Fig. 2. Anti-viral assessment of the initial set of analogues **21–31** in HSV-1 infected iPSC neurons.

compounds, such as the indole derivative **25** and the highly substituted trimethoxy-derivative **27** proved more active. Nonetheless, the conclusion derived from the SAR on the initial array was that no derivative exhibited potency close to 4F17, indicating the requirement of a small, electron-rich heterocycle on the hydrazone portion of the molecule. These results with the array of aryl derivatives in comparison to the furano-substituted parent compound **5**, focussed our attention on the preparation of a second generation of analogues containing small, electron-rich heterocyclic rings. This second-generation compound array was readily accessed from the penultimate hydrazide **13** condensing with a range of commercially available heterocyclic aldehydes, as shown in Fig. 3.

This second-generation array compounds **32–38**, was also screened in the same assay as described above and the overall results presented in Fig. 3. The 2-thiofurano analogue **32** showed substantial activity, similar to the positive control acyclovir. To our delight, the corresponding 2-pyrrolo analogue **33** proved highly potent. Interestingly, the two constitutionally isomeric imidazoles **34** and **35** proved significantly less active, indicating that a basic nitrogen atom is detrimental, perhaps due to solubility and/or basicity of the side chain. In order to probe basicity further, we prepared the corresponding 2-pyridyl analogue **36** which proved highly potent, and the isomeric 3-pyridyl derivative **37** which was less active. The bromofurano analogue of **5**, compound **38**, proved less active than **5**. Overall, the second-generation structure-antiviral assessment allowed identification of the 2-pyrrolo- and 2-pyridinyl- substituted compounds **33** and **36** as being significantly and slightly more potent that acyclovir and the parent 2-furano derivative **5** itself.

These results set the stage for the preparation of the third generation of compounds that would allow probing the contribution of the 1,1-diarylmethano fragment to the anti-viral activity. Strategically, the synthetic approach followed the original synthetic protocol, allowing access to arrays through late-stage incorporation of 2-furanyl, 2-pyridyl and 2-pyrrolyl substituents. The synthesis of the required substituted 1,1-diarylethylenes required is shown in Scheme 2. The reaction of a 4-substituted benzaldehyde **39** or **40** with the corresponding 4-

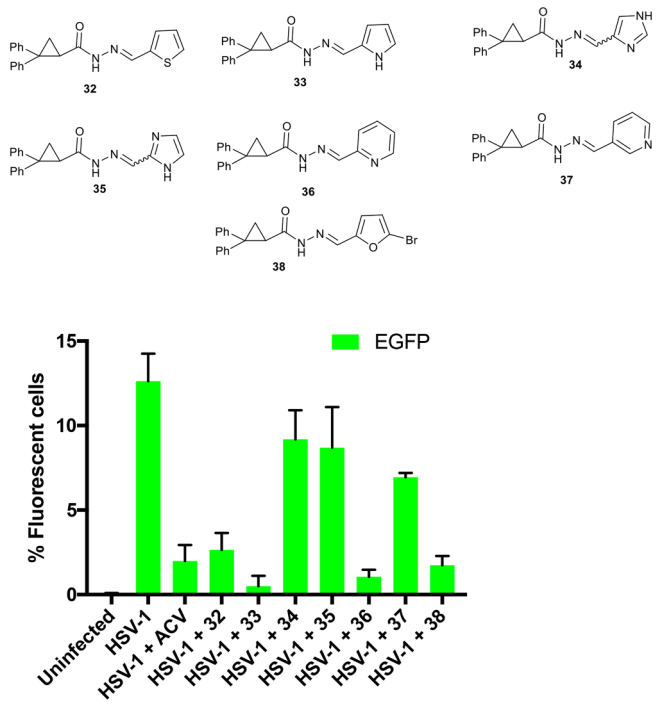
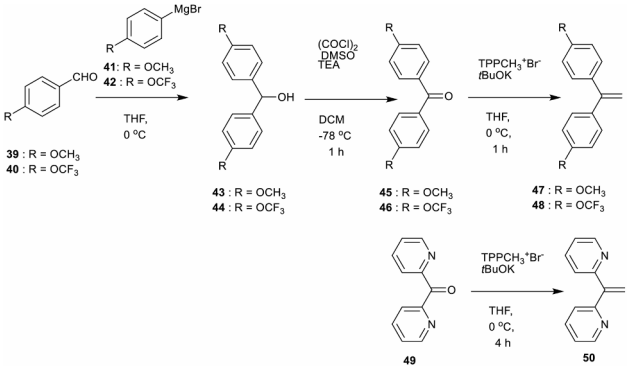
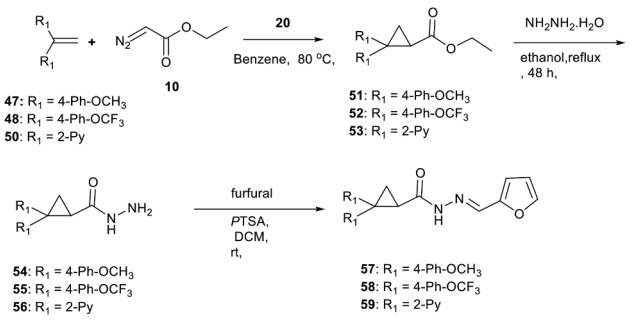


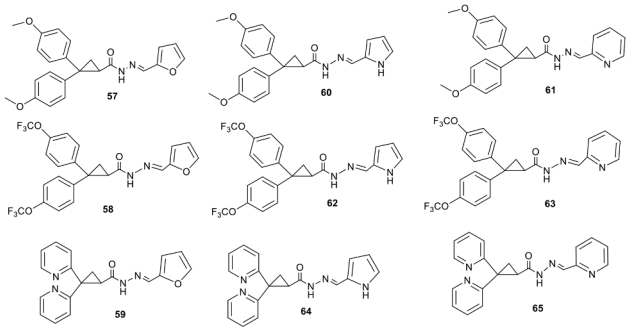
Fig. 3. Second generation heterocyclic hydrazones **32–38** containing modified heterocycles and their anti-viral assessment.



Scheme 2. Reparation of 1,1 diaryl substituted ethylenes.



Scheme 3. Synthesis of the substituted hydrazides **54-56** and the three furano hydrazides **57–59**. The reaction of **54–56** with 2-pyrrolocarboxaldehyde and separately 2-pyridylcarbox-aldehyde similarly yielded array compounds **60–65**.



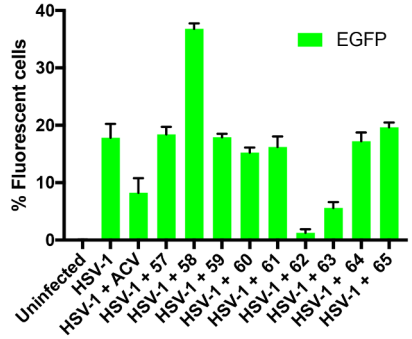


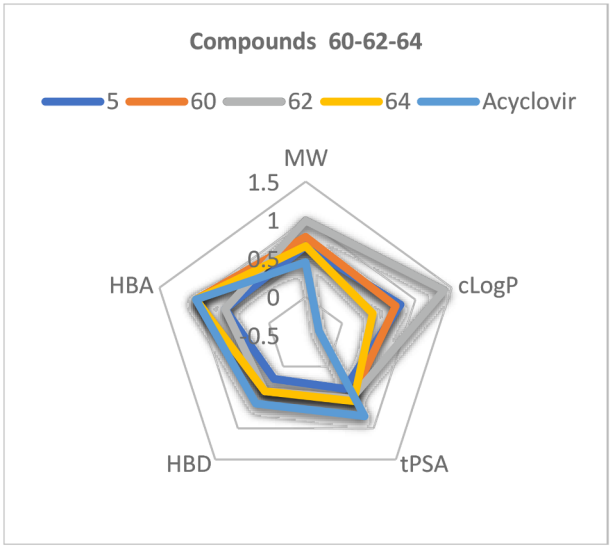
Fig. 4. Third generation array compounds **57–65** containing modified 1,1-diarylmethano-substituents and their anti-viral activity.

substituted Grignard reagent **41** and **42** yielded the diarylmethanols **43** and **44**. These were oxidised to the corresponding benzophenone derivatives **45** and **46**, along with the commercially available 1,1-bis-pyridyl-benzophenone analogue **49**, were converted to the 1,1-diaryl ethylenes **47**, **48** and **50** respectively, through Wittig olefination with the ylide derived from triphenylmethyl phosphonium bromide.

Conversion of each of these three substituted ethylene derivatives to their corresponding hydrazides was accomplished following the methodology developed earlier. Thus, the reaction of the 1,1-diarylethylene with ethyl diazoacetate in the presence of catalyst **20**, yielded the corresponding cyclopropanes **51–53** in good yield, as shown in Scheme 3. Interestingly, we observed that the cyclopropanation of the 1,1-bis-pyridyl ethylene **50** with ethyl diazoacetate did not require any catalyst. The reaction of this substrate ,alone of all derivatives investigated - was serendipitously found to proceed rapidly without the addition of any catalyst, indicating that the pyridyl substituent may be playing a role in an autocatalytic or organocatalytic cyclopropanation process.[12] Hydrazinolysis converted these to the three penultimate hydrazides **54**, **55** and **56** were then independently reacted with furfural to produce the array of hydrazones **57**, **58** and **59** as shown (Sch. **3**). Similarly, the reaction of the three hydrazides **54**, **55** and **56** with pyrrole-2-carboxaldehyde independently yielded the hydrazones **60**, **62** and **64**, and reaction with pyridine-2-carboxaldehyde yielded analogues **61**, **63** and **65** completing the third generation of array summarized in Fig. 4.

The third-generation array compounds **57–65**, was screened in the same assay as described above and the overall results presented in Fig. 4. Once again, the anti-viral activity of the 2-pyrrolyl compounds stood out with compounds **62** and **63** proving highly active, and especially **62** being significantly more potent than acyclovir.

In order to interpret the structure–activity relationships within the third set of compounds further we employed a Radar Plot analysis of the major pharmacological parameters as depicted in Fig. 5.[13] Normalised values for molecular weight (MW), calculated cLogP, tPSA and number of H-bond donors (HBD) and acceptors (HBA) were plotted with acyclovir and the initial hit compound **5**. The overall structural parameters of the cyclopropylcarboxacylhydrazone derivatives differ considerably from acyclovir as expected. The most revealing insights were gained from the analysis of the central horizontal and vertical analogues as shown in Fig. 4. Anti HSV-1 activity of the pyrrole derivatives **60**, **62** and **64** correlated strongly with cLogP, the major factor being contributed by the left-hand lipophilic trifluoromethoxy-containing diarylmethano-fragment. Lipophilicity alone is not sufficient for potent



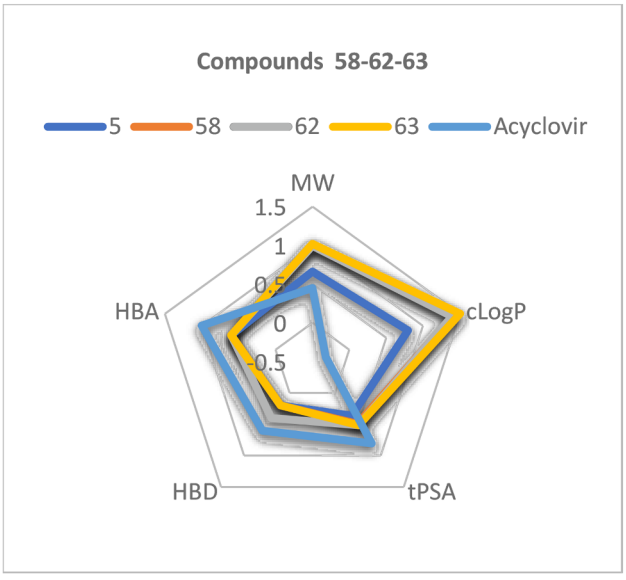


Fig. 5. Radar Plot analyses of the main pharmacological parameters along the vertical (**60-62-64**) and horizontal (**58-62-63**) sets of analogues as defined in Fig. 4.

HSV-1 inhibition, however, as the series **58**, **62** and **63** demonstrates. A strong synergistic effect is seen with the small H-bond donor pyrrole **62**, as the right-hand fragment, and to a lesser extent with the pyridine **63**. This data is also consistent with the activity of the pyrrole **33**, in comparison to imidazole substituted derivatives **34** and **35** (Fig. 3). Overall, this analysis indicates that potent anti HSV-1 activity correlates with an enhanced lipophilic substituent on the left–hand diaryl fragment and small electron-rich H-bond donor on the right-hand heteroaryl fragment of the molecule.

Lastly, the antiviral potency of compound **62** was determined in HSV-1-infected neuronal progenitor cells (NPCs) in comparison to ACV (Fig. 6). Compound **62** exhibited an IC_{50} of 14.1 μM, whilst the IC_{50} of ACV was estimated to be 27.4 μM. In terms of host cell viability, compound **62** demonstrated no toxicity to NPCs at a concentration of 25 μM.

In conclusion, we report the total synthesis of the cyclopropyl -carboxyacylhydrazone **5**, confirmation of its structure (rotamers) and anti HSV-1 activity.[5a] A new homogeneous copper (II) catalyst has been developed that is highly efficient in promoting the [2 + 1]-cycloaddition leading to the cyclopropyl core. Attempts to confirm the structure of catalyst **20** and investigate its potential in copper-catalyzed [2 + 1]-

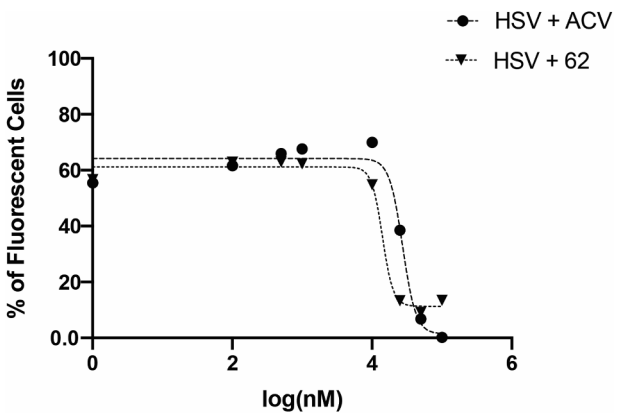


Fig. 6. Antiviral IC_{50} determination of compound **62**, 14.1 μM, R^2 = 0.9675, in comparison to acyclovir (ACV), 27.4 μM, R^2 = 0.9648 in HSV-1 infected neuronal progenitor cells.

cycloaddition reactions are underway. The synthetic route allowed for the rapid preparation of series of analogues and three successive rounds of structure-antiviral activity assessment identified compound **62** as an optimised anti HSV-1 derivative, about twice as potent as the standard therapeutic agent ACV. The SAR analysis demonstrates that anti-viral activity correlates with a small H-bond donor, such as pyrrole, as the right-hand fragment and liphophilic substituent on the diarylmethano-fragment of the cyclopropyl core. Further investigations to determine the target and mechanism of action of these potent HSV-1 derivatives and investigation of their anti-viral activity to emerging viruses is underway in our laboratories.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Correspondence concerning chemistry should be addressed to jmcnult@mcmaster.ca and anti-viral activity to nimga@pitt.edu. All compounds were synthesized by CBD. LD developed the iPSC neuronal HSV-1 assay and LM, MD, PP, KW and WZ conducted the anti-viral assessments. The viral construct was donated by Paul Kinchington, Ph.D., University of Pittsburgh. This work was supported by funding from the Stanley Foundation grant 13R-002 (JMcN), NIH R01 MH063480 and Stanley Foundation grant 07R-1712 (VLN) and R21 NS096405 (LD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127559.

Reference

1. (a) Schmutzhard E. *J Neurol.* 2001;248:469
   (b) Steiner I, Kennedy PG, Pachner AR. *Lancet Neurol.* 2007;6:1015
   (b) Treml J, Gazdová M, Šmejkal K, Šudomová M, Kubatka P, Hassan STS. *Viruses.* 2020;12:154.
2. (a) Kanai R, Wakimoto H, Cheema T, Rabkin SD. *Future Oncol.* 2010;6:619
   (b) Flint SJ, Enquist LW, Racaniello VR, Skalka AM. *Principles of Virology.* Third Ed. Washington DC: ASM Press; 2009:278–308.
3. Izedine H, Launay-Vacher V, Deray G. *Am J Kidney Dis.* 2005;45:804.
4. (a) Strasfield L, Chou S. *Infect Dis Clin North Am.* 2010;24:809–833
   (b) Burrel S, Aime C, Hermet L, Ait-Arkoub Z, Agut H, Boutolleau D. *Antiviral Res.* 2013;100:365
   (c) Stranska R, Schuurman R, Nienhuis E, et al. M. *J Clin Virol.* 2005;32:7

(d) Malvy D, Treilhaud M, Bouee S, et al. *Clin Infect Dis.* 2005;41:320.
5. (a) McClain, L.; Zhi, Y.; Cheng, H.; Gosh, A.; Piazza P.; Yee, M. B.; Kumar, S.; Milosevic, J.; Bloom, D. C.; Arav-Boger, R.; Kinchington, P. R.; Yolken, R.; Nimgaonkar, V.; Aiuto, L. D'. Anti-viral Res. 2015, 121, 16. (b) Library compounds were purchased from ChemBridge Corporation, San Diego, CA 92121, USA.
6. D'Aiuto L, Williamson K, Dimitrion P, et al. *Antiviral Res.* 2017;142:136.
7. Schmidt AG, Lee K, Yang PL, Harrison SC. *PLoS Pathog.* 2012;8:1002627.
8. Filho, J. M. D. S.; Silva, D. M. A. D. Q.; Macedo, T. S.; P. Teixeira, H. M.; Moreira, D. R. M.; Challal, S.; Wolfender, J.-L.; Queiroz, E. F.; Soares, M. B. P. Bioorg. & Med. Chem. 2016, 24, 5693.
9. (a) McNulty J, D'Aiuto L, Zhi Y, et al. *ACS Med Chem Lett.* 2016;7:46
(b) Revu O, Zepeda-Velazquez C, Nielsen AJ, McNulty J, Yolken RH, Jones-Brando L. *Chem Select.* 2016;1:5895
(c) Brown CE, Kong T, McNulty J, et al. *Bioorg Med Chem Lett.* 2017;27:4601–4605
(d) Brown CE, Kong T, Britten JF, et al. *ACS Omega.* 2018;3:11469
(e) D'Aiuto L, McNulty J, Hartline C, et al. *Sci Rep.* 2018;8:16662.
10. (a) Tayama E, Saito S. *Synlett.* 1880;2015:26
(b) Nozaki H, Takaya H, Moriuti S, Noyori R. *Tetrahedron.* 1968;24:3655
(c) Yates P. *J Am Chem Soc.* 1952;74:5376.
11. McNulty, J., Keskar, K.; Bordon, C.; Yolken, R.; Jones-Brando, L. Chem. Comm. 2014, 50, 8904. (b) McNulty, J. Keskar, K.; Jenkins, H. A.; Werstiuk, N. H.; Bordon, C.; Yolken, R.; Jones-Brando, L. Org. & Biomolec. Chem. 2015, 13, 10015.
12. (a) For auto catalysis and organocatalysis of related cyclopropanations see: A.P. Gray H. Kraus J. Org. Chem. 1966, 31, 399.
(b) Shim SY, Kim JY, Nam M, Hwang G-S, Ryu DH. *Org Lett.* 2016;18:160
13. Ritchie TR, Ertl P, Lewis R. *Drug Disc Today.* 2011;16:65.