MedChemComm

RESEARCH ARTICLE

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Cite this: *Med. Chem. Commun.*, 2019, **10**, 1173

Importance of lipophilicity for potent anti-herpes simplex virus-1 activity of α-hydroxytropolones†

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Received 16th April 2019,
Accepted 24th May 2019

DOI: 10.1039/c9md00225a

rsc.li/medchemcomm

We previously reported that troponoid compounds profoundly inhibit replication of herpes simplex virus (HSV)-1 and HSV-2 in cell culture, including acyclovir-resistant mutants. Synthesis of 26 alpha-hydroxylated tropolones (αHTs) led to a preliminary structure–activity relationship highlighting the potency of bi-phenyl side chains. Here, we explore the structure–activity relationship in more detail, with a focus on various biaryl and other lipophilic molecules. Along with our prior structure–function analysis, we present a refined structure–activity relationship that reveals the importance of the lipophilicity and nature of the side chain for potent anti-HSV-1 activity in cells. We expect this new information will help guide future optimization of αHTs as HSV antivirals.

Published on 30 May 2019. Downloaded by RSC Internal on 7/19/2019 2:58:23 PM.

Herpes simplex virus (HSV)-1 is a large double-stranded DNA virus that infects more than half of the U.S. population.[1] Virus replication in the oral or corneal epithelium causes painful ulcerative lesions. The virus enters sensory nerve endings innervating the epithelium and is transported to ganglia where it establishes life-long latency. Periodic reactivations lead to reappearance of disease in the epithelium, manifesting as cold sores, sight-threatening keratitis or, rarely, potentially lethal encephalitis if the virus spreads into the central nervous system. Along with HSV-2, HSV-1 also causes an increasing proportion of genital ulcerative disease,[2] and when transmitted to babies during birth the resulting disseminated infection can have serious health consequences.[1] HSV-1 and HSV-2 reactivate periodically from latency to cause recurrent bouts of disease throughout the life of infected individuals.

HSV replicates by a rolling circle mechanism.[3] A number of steps in the replication cycle require DNA excision repair and ligase activities. The linear genome circularizes upon entry into a cell, and its ends annealed by an unknown mechanism. Viral DNA replication occurs by conservative mechanisms involving activities of several viral proteins. Among these, the ICP8 single-stranded DNA binding protein holds the DNA strands open for polymerase to bind. The polymerase, pUL30, has 3′ to 5′ exonuclease activity that may assist with removal of Okazaki fragments. pUL12 exonuclease teams with ICP8 to generate branched concatemers late in the infection cycle. Lastly, the pUL15 terminase cleaves genome concatemers into unit length for packaging into nascent virions. These four viral proteins have activities or structural domains consistent with enzymes in the nucleotidyl transferase superfamily (NTS).[3,4]

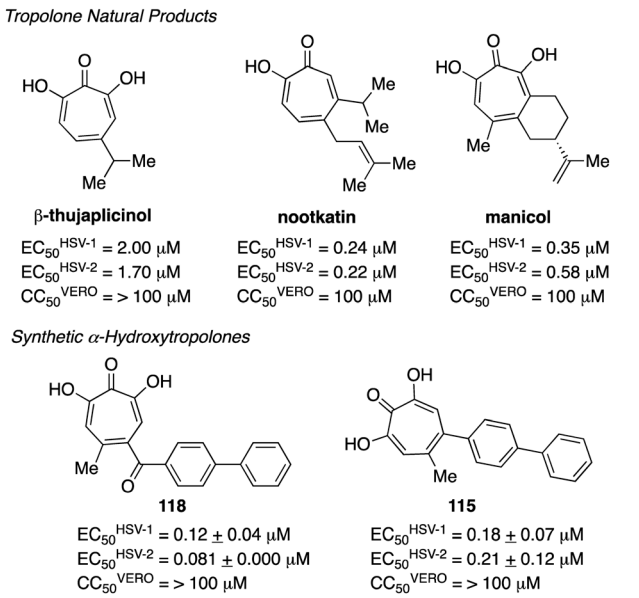

Fig. 1 Natural and synthetic tropolones and corresponding anti-herpesvirus activity determined previously.[5,8]

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† Electronic supplementary information (ESI) available: Synthetic procedures and characterization, including ^{1}H and ^{13}C NMR spectra of all new molecules. See DOI: 10.1039/c9md00225a

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We have found that hydroxylated tropolones known to inhibit NTS or closely related enzymes display powerful activity against HSV-1 and HSV-2, with several natural product tropolones able to suppress viral replication up to 1 million-fold at 5 μM (Fig. 1).[5] This finding led to an initial screen of 26 synthetic α-hydroxytropolones (αHTs),[6] 19 of which were made through an oxidopyrylium cycloaddition/ring-opening technique developed in our lab.[7] Virtually all of the molecules inhibit viral replication at 50 μM (ref. 8) indicating the advantage of this moiety for HSV inhibition. The most potent of these molecules is **118**, which has a 50% effective concentration (EC_{50}) value of 80 nM, and acts synergistically with acyclovir.[8] A subset of αHTs, 3,7-dihydroxytropolones, also demonstrate high potency for inhibiting HSV replication, but have higher toxicity.[9] In addition to the importance of the tropolone moiety revealed by our prior studies, the highly potent activity of another biaryl tropolone, **115**,[5] suggested advantages to a biaryl side chain (Fig. 1).

In our continued pursuit of therapeutically viable anti-HSV-1 tropolones, and to provide better structure–function insight, we analysed an additional 23 synthetic αHTs that were related to **118** and **115**, along with 6 previously reported αHTs with a range of activity as controls (**118**, **115**, **120**, **111**, **143**, **120**). Given the superior activity of **118**, our library screen was biased towards aromatic ketones, but also included aliphatic ketones, as well as biaryl- and sulfonyl-appended molecules (Fig. 2). The molecules were all assessed in plaque-reduction assays at 5 μM, 1 μM, and 0.2 μM unless otherwise noted, and are reported as the average of duplicate cultures ± standard deviation. Most experiments were run along with **118** as a control, which uniformly showed strong suppression at 1 μM, but low or no suppression at 0.2 μM. The data is compiled in Table 1, and is sorted based upon calculated octanol–water partition coefficient (clog *P*) values,[10] which are calculated in the molecules' monoanionic form to mimic their expected physiological protonation state.[11]

While no molecules suppressed HSV replication greater than 10-fold at a 0.2 μM concentration, 11 out of the 29 molecules (9 out of the 23 newly tested molecules) suppressed replication by more than 10-fold at 1 μM. Informatively, clog *P* values were highly predictive of likelihood of this suppression. Of the 12 molecules with clog *P* values equal to or greater than **118** (≥−0.2), 9 showed at least 10-fold-replication inhibition (8/11 new molecules). On the other hand, of the 16 molecules with clog *P* values less than that of **118** (≤0.2), only **315** showed greater than 10-fold-replication inhibition at 1 μM. Furthermore, not one of the 14 molecules with a clog *P* value less than −1 showed this level of suppression. Informatively, molecules with the longer aliphatic lipophilic side chains (**351**, **381**) were capable of over 10-fold viral suppression at 1 μM, while those of shorter lengths (**380**, **792**) were not. Meanwhile, biphenyl sulfonyl αHT **337**, which would seem closely homologous spatially to **118**, was inactive at 1 μM. Thus, a biaryl side chain was not the major, specific determinant for high potency initially suspected, but rather appeared to be a means to add beneficial lipophilicity.

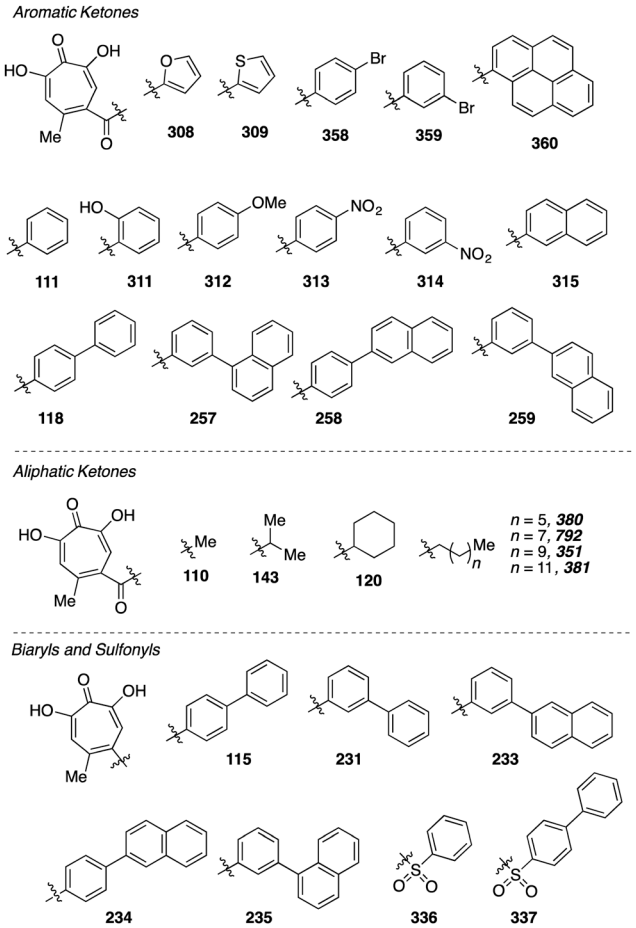

Fig. 2 αHTs tested in the current manuscript.

In order to obtain a more quantitative measure of the differences in potency observed, EC_{50} values were determined for a subset of molecules (Table 2). We specifically chose molecules in the aliphatic ketone series for EC_{50} analyses because these had a broad spread in both clog *P* values and activity. **118** was also run as a positive control, as well as compound **360**, which had the highest replication suppression at 0.2 μM of the molecules tested. The EC_{50} value of **118** was calculated to be 0.17 μM, which was in line with our prior measurements. The most potent molecule tested was **360**, which had an EC_{50} value of 55 nM. Of the aliphatic molecules tested, the most lipophilic of the series, **381**, was the most potent (EC_{50} = 0.14 μM), similar to **118**, followed by the second most lipophilic, **351** (EC_{50} = 0.35 μM). Compound **380**, with a shorter heptyl side-chain, was still fairly potent (EC_{50} = 0.44 μM), and was consistent with a cyclohexyl side chain of **120** (EC_{50} = 0.63 μM). **792** was comparable in potency to **110**, despite fairly different lipophilicity (EC_{50} = 1.1–1.2 μM). One hypothesis to explain this trend is that a balance exists between favourable lipophilicity and unfavourable high number of rotatable bonds that could impose entropic penalties. A similar phenomenon might also explain the decreased activity of **143** (EC_{50} = 26 μM), and the high potency of **360**.

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Table 1 HSV-1 replication inhibition by synthetic α-hydroxytropolones, along with their lipophilicity. Replication inhibition reported as the average of a duplicate run, ± standard deviation

		Replication inhibition ($\log_{10}$ ± SD)			Lipophilicity (monoanionic)
Entry	Cmpd	5 μM	1 μM	0.2 μM	clog *P*
1	**381**	4.31 ± 0.12	1.58 ± 0.60	0.36 ± 0.11	2.6
2	**351**	4.84 ± 0.08	3.23 ± 0.08	0.29 ± 0.23	1.6
3	**233**	4.10 ± 0.01	0.66 ± 0.20	0.06 ± 0.09	1.5
4	**234**	4.15 ± 0.03	0.57 ± 0.08	−0.03 ± 0.01	1.5
5	**235**	4.02 ± 0.35	4.00 ± 0.28	0.04 ± 0.29	1.5
6	**792**	3.78 ± 0.11	0.07 ± 0.14	n/a	1.3
7	**257**	4.60 ± 0.08	3.15 ± 0.01	0.15 ± 0.43	1
8	**258**	4.28 ± 0.14	3.54 ± 0.02	1.34 ± 0.43	1
9	**259**	4.11 ± 0.17	3.03 ± 0.06	0.14 ± 0.03	1
10	**360**	5.76 ± 0.03	5.01 ± 0.08	0.93 ± 0.09	0.7
11	**115**	4.08 ± 0.09	4.09 ± 0.29	−0.05 ± 0.07	0.3
12	**231**	4.28 ± 0.25	4.26 ± 0.10	0.02 ± 0.03	0.3
13	**118**	4.72 ± 0.23	4.86 ± 0.07	0.43 ± 0.07	−0.2
14	**380**	4.84 ± 0.08	0.20 ± 0.15	0.13 ± 0.17	−0.5
15	**315**	4.08 ± 0.28	3.82 ± 0.46	0.28 ± 0.19	−0.9
16	**337**	4.44 ± 0.12	−0.34 ± 0.07	n/a	−1
17	**358**	5.40 ± 0.20	0.42 ± 0.04	0.09 ± 0.06	−1.2
18	**359**	5.46 ± 0.03	0.21 ± 0.11	0.09 ± 0.12	−1.2
19	**120**	5.14 ± 0.17	0.76 ± 0.22	0.62 ± 0.51	−1.6
20	**111**	4.21 ± 0.16	0.11 ± 0.02	n/a	−2.1
21	**312**	3.94 ± 0.02	0.92 ± 0.20	0.02 ± 0.03	−2.2
22	**311**	3.86 ± 0.20	0.86 ± 0.01	0.10 ± 0.06	−2.3
23	**309**	0.92 ± 0.10	−0.06 ± 0.06	0.16 ± 0.06	−2.4
24	**313**	3.91 ± 0.90	0.60 ± 0.03	0.03 ± 0.17	−2.4
25	**314**	4.20 ± 0.02	0.90 ± 0.17	0.23 ± 0.19	−2.4
26	**143**	−0.01 ± 0.10	n/a	n/a	−2.8
27	**336**	0.34 ± 0.19	0.81 ± 0.69	n/a	−2.8
28	**308**	4.52 ± 0.17	0.41 ± 0.19	−0.10 ± 0.05	−2.9
29	**110**	0.48 ± 0.52	n/a	n/a	−3.6

It is at present unclear why lipophilicity correlates with efficacy. Not all molecules that had high clog *P* values were highly potent, yet all the molecules that had low clog *P* values (≤1) were less active. One possible explanation is that the lower activity of some of the less lipophilic molecules could be due to low cell permeability, whereas the lower activity of some of the more lipophilic molecules could be due to poor fit with the target enzyme(s). While at least two HSV enzymes are known to be inhibited by αHTs,[12] it remains unclear at present what the primary enzymatic target(s) of the HSV-specific tropolones is(are), and efforts in this regard remain ongoing. Until more structural information is obtained, however, considerations should be given to lipophilicity in the design of new anti-HSV tropolones. Furthermore, in addition to human HSV-1 and -2 antiviral activity, α-hydroxytropolones have demonstrated activity against herpesviruses associated with cattle (BoHV-1), horses (EHV-1) and cats (FHV-1),[13] as well as other human herpesviruses such as cytomegalovirus[5] and Kaposi's sarcoma-associated virus.[14] Understanding how similar trends in lipophilicity relate to these activities will also be important.

Table 2 Anti-HSV-1 effective concentration at 50% inhibition (EC_{50}) of select α-hydroxytropolones, along with 50% cytotoxic concentration (CC_{50}) in Vero cells. EC_{50} and CC_{50} values as calculated from average of 3 runs ± standard error. TI = therapeutic index, or CC_{50}/EC_{50}

Cmpd	EC_{50} ± SEM (μM)	CC_{50} ± SEM (μM)	TI
110	1.2 ± 0.1	>100	>83
118	0.17 ± 0.01	>100	>600
120	0.63 ± 0.51	73 ± 14	116
143	26 ± 6	91 ± 9	3.6
351	0.35 ± 0.09	95 ± 5	255
360	0.06 ± 0.04	>100	>1800
380	0.44 ± 0.29	81 ± 6	185
381	0.14 ± 0.05	91 ± 9	631
792	1.1 ± 0.1	>100	>92

In conclusion, a study of anti-HSV suppression by 29 αHTs demonstrates that potency strongly correlates with lipophilicity. While previous lead biaryl molecules **118** and **115** both share a similar biphenyl linkage, substituting this group with other lipophilic side chains led to molecules with similarly high potency. By increasing both lipophilicity and rigidity, as is the case with **360**, further potency enhancements are observed. These studies should help guide the pursuit of therapeutically viable anti-HSV tropolones.

Conflicts of interest

LAM and RPM are co-inventors on a patent application describing the anti-HSV activity of hydroxytropolones.

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Acknowledgements

AJB, AKG, and RPM are grateful for financial support from the National Institutes of Health (SC1GM111158), and LAM appreciates the support from the Pershing Trust.

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