Medicinal Chemistry Research
https://doi.org/10.1007/s00044-019-02365-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH



# In vitro activity of novel derivatives of 1,3-oxazole-4-carboxylate and 1,3-oxazole-4-carbonitrile against human cytomegalovirus

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Received: 9 January 2019 / Accepted: 21 May 2019


## Abstract

Ten 5-functionalized derivatives of 1,3-oxazole-4-carboxylate and 1,3-oxazole-4-carbonitrile were synthesized and their antiviral activities against the human cytomegalovirus (HCMV) were evaluated in vitro. Bioassays showed that seven compounds exhibited considerably higher antiviral activity ($EC_{50}$: < 0.05 μM) against a normal laboratory HCMV strain (AD-169) in human foreskin fibroblast cells than Ganciclovir ($EC_{50}$ = 0.32 μM), an anti-HCMV agent in clinical use. Additionally, the HCMV-resistant isolate (GDGr $K_{17}$) was tested for sensitivity to 1,3-oxazole derivatives with most antiviral potency against the strain AD169. A one of them (5-((2-hydroxyethyl)(methyl)amino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile) showed very high potency ($EC_{50}$: < 0.05; $CC_{50}$: >150 μM, and $SI_{50}$ = 3125) towards the resistant isolate compared to standard drugs Cidofovir ($EC_{50}$ = 0.10 μM, $CC_{50}$: >30 μM and $SI_{50}$: <4). But, in contrast to the primary assays, the antiviral activity of these compounds against both the normal strain and the resistant isolate of HCMV were considerably less than one of Cidofovir in secondary assay. These results provided evidence that derivatives of 1,3-oxazole could be useful for developing new anti-HCMV drugs.

**Keywords** Antiviral discovery · 1 · 3-oxazole derivatives · Human cytomegalovirus · HFF cells

## Introduction

Human cytomegalovirus (HCMV) is a ubiquitous pathogen that severely affects individuals with impaired or immature immune-defence functions (Collins-McMillen et al. 2018; Li et al. 2018; Yong et al. 2018). Since HCMV infects ~60% of people in the developed world and over 99% in developing countries, the risk of morbidity due to HCMV disease is a significant problem for public health (Griffiths et al. 2015). So far, there is no widely available vaccine against HCMV. Therefore, anti-HCMV drugs and immunotherapy have been approved for the treatment and prophylaxis of HCMV diseases (Ahmed 2011; Tan 2014; Hanson and Swaminathan 2015). HCMV is a double-stranded DNA virus that belongs to the β-subfamily in large family *Herpesviridae*. It is among the largest of the known human herpes virus. The genome of HCMV has been completely sequenced and encodes more than 200 proteins (Chee et al. 1990). Not all of the proteins have been identified and the functions of all identified proteins have not been characterized. However, three of the proteins are critical to the activity of antiviral agents. The first protein is HCMV DNA polymerase. This protein is the common, ultimate target for antiviral drugs currently approved for the treatment of HCMV infections. At present, anti-HCMV drugs that inhibit HCMV DNA polymerase are the nucleoside analogs Ganciclovir, and its oral prodrug Valganciclovir, Cidofovir, and the pyrophosphate analog foscarnet (Britt and Prichard 2018). All these substances inhibit HCMV replication. However, these drugs have poor oral bioavailability and many shortcomings, including associated toxicities, and the emergence of drug-resistant viruses (Ahmed 2011). The second, the UL97 protein

**Supplementary information** The online version of this article (https://doi.org/10.1007/s00044-019-02365-x) contains supplementary material, which is available to authorized users.

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Published online: 31 May 2019

kinase of HCMV (HCMV phosphotransferase) is responsible for phosphorylating antiviral drugs to their active forms (Prichard 2009). The HCMV UL97 kinase inhibitor maribavir is new perspective drug because of oral bioavailability and lack of cross-resistance with currently used drugs (Frange and Leruez-Ville 2018). The third, the subunit UL56 of the HCMV terminase enzyme complex is responsible for the terminal phase of the virus life cycle. Letermovir inhibits the terminal phase of the virus life cycle by targeting the subunit UL56 (Chou 2015). It is currently the most active molecule against HCMV and a preserved activity against viruses resistant to currently available molecules (Lischka et al. 2010; Frange and Leruez-Ville 2018). Combination of anti-HCMV agents that involve drugs with different mechanisms of action, which could reduce the incidence of drug resistance, is not yet available because of limited number of anti-HCMV agents and the lack of clinical trials (Cai et al. 2014).

Specificity against virus replication is a key issue in chemotherapy. Because of the close interaction between viral reproduction and normal cellular metabolism, it was originally thought difficult to interrupt the viral life cycle without adversely affecting the host cell metabolism. However, it is now clear that several events associated with viral replicative amplification either do not occur in normal uninfected cells or are controlled by virus-specified enzymes that differ structurally and functionally from the corresponding host cell enzymes. For example, Cidofovir is an acyclic nucleoside phosphonate, which nonspecifically blocks viral replication because of more efficient conversion of the prodrug to the active precursor in virus-infected cells or by selective inhibition of a viral DNA polymerase (Magee et al. 2005). It also inhibits human polymerases but this action is 60–80 times weaker than its actions on viral DNA polymerases (Upadhyayula and Michaels 2013).

Until recent years, there were rather few chemotherapies for HCMV infections. The five antiviral drugs approved by the Food and Drug Administration have shown adverse reactions and the antiviral drug resistance were reported (Michel and Mertens 2006; Chou 2008). Hence, this warrants the need for urgent development of novel antiviral drugs. 1,3-Oxazole compounds containing nitrogen and oxygen atoms in the five-membered aromatic ring are readily able to bind with a variety of biological structures via different non-covalent interactions, and thus display various biological activities (Joshi et al. 2017). 1,3-Oxazole derivatives are among the most useful heterocyclic compounds from both synthetic and medicinal chemistry aspects (Zhang et al. 2018). A series of novel 1,3-oxazole derivatives were synthesized and their antiviral activities against the hepatitis C virus (HCV), the coxsackie and HIV-1 viral strains were evaluated in vitro (see rev. Swellmeen 2016). Some 1,3-oxazole derivatives showed strong activity against the HCV and HIV-1 at low concentrations ($EC_{50} < 2.0$ μM and 14 nM, respectively) and had low cytotoxicity. Recently, we have synthesized novel multi-substituted 1,3-oxazole derivatives with different moieties, such as piperazine, piperidine, sulfonamide, and carbonitrile for evaluation of their antiviral activities (Kachaeva et al. 2017). Bioassays showed that the synthesized compounds 1-((2,5-diphenyl-1,3-oxazole-4-yl)sulfonyl)-3-methylpiperidine, 2,5-diphenyl-1,3-oxazole-4-sulfonamide, 1-((2,5-diphenyl-1,3-oxazole-4-yl)sulfonyl)piperidine, and 4-cyano-2-phenyl-1,3-oxazole-5-sulfonamide exhibited potent antiviral activity against low-risk HPV-11 ($IC_{50}$: 1.7–9.6 μM) in a transient DNA replication assay and exhibited low cytotoxicity in HEK293 cells compared to Cidofovir, an antiviral agent in clinical use. These data indicate that 1,3-oxazole derivatives are promising compounds in the search for design of new anti-HCMV drugs. The number of existing drug as antiviral is not enough and development of new antiviral drugs is still in requirement because of the viral resistance. There are no reports of anti-HCMV activity of 1,3-oxazole derivatives in available literature.

The present study is an exploratory investigation of anti-HMCV activity by novel 1,3-oxazole derivatives designed and synthesized in Kyiv, and evaluated with in vitro antiviral assays against HCMV.

## Material and methods

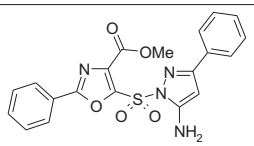
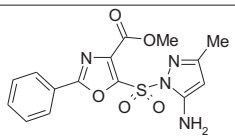
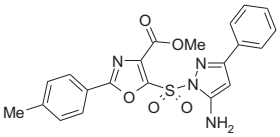
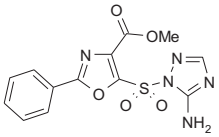
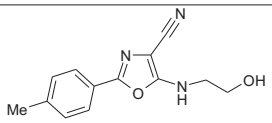
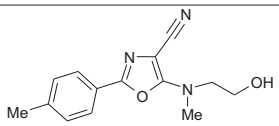
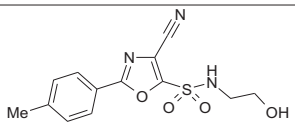
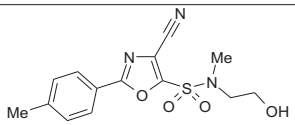
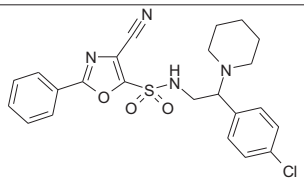
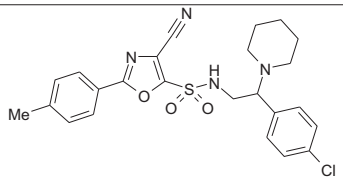
### Chemistry

1,3-Oxazole-5-sulfonyl amides **1**–**4** and **7**–**10** (Table 1) have been synthesized from sulfonyl chlorides (Kornienko et al. 2012) by refluxing with appropriate amine and excess of triethylamine (Kachaeva et al. 2018)

By the reaction of 2-acylamino-3,3-dichloroacrylonitrile (Drach et al. 1974) with appropriate amine using excess of triethylamine (Drach et al. 1974) 5-amino-1,3-oxazoles **5** and **6** (Table 1) have been prepared.

The structure of synthesized 1,3-oxazole derivatives are confirmed by NMR ($^1$H and $^{13}$C NMR), IR spectroscopy, chromato-mass, and elemental analysis. All CH-proton signals of compound **10** are visible in the $^1$H NMR spectrum. Signal of the OH group of compound **7** is seen at 3.94, of compound **6**—at 4.92 ppm. $NH_2$ group (**4**) is observed in the $^1$H NMR spectrum at 7.58 ppm, in IR—at 3424 $cm^{-1}$. The intensive absorption bands of $SO_2$-group of compounds **4**, **7**, and **10** are observed in the IR spectra at 1154–1164 and 1339–1395 $cm^{-1}$. Also the broad intensive bands at 1741 $cm^{-1}$ corresponded to ester C=O bond of compound **4** and intensive bands at 2198–2253 $cm^{-1}$ corresponded to CN group of **6**, **7**, and **10** were observed.

**Table 1** Chemical structures of compounds

| Compound | Structure | Name |
|---|---|---|
| 1 |  | methyl 5-((5-amino-3-phenyl-1*H*-pyrazol-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate |
| 2 |  | methyl 5-((5-amino-3-methyl-1*H*-pyrazole-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate |
| 3 |  | methyl 5-((5-amino-3-phenyl-1*H*-pyrazole-1-yl)sulfonyl)-2-(4-methylphenyl)-1,3-oxazole-4-carboxylate |
| 4 |  | methyl 5-((5-amino-1*H*-1,2,4-triazole-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate |
| 5 |  | 5-((2-hydroxyethyl)amino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile |
| 6 |  | 5-((2-hydroxyethyl)(methyl)amino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile |
| 7 |  | 4-cyano-*N*-(2-hydroxyethyl)-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide |
| 8 |  | 4-cyano-*N*-(2-hydroxyethyl)-*N*-methyl-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide |
| 9 |  | *N*-(2-(4-chlorophenyl)-2-(1-piperidinyl)ethyl)-4-cyano-2-phenyl-1,3-oxazole-5-sulfonamide |
| 10 |  | *N*-(2-(4-chlorophenyl)-2-(1-piperidinyl)ethyl)-4-cyano-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide |

## General chemistry methods

Melting points were determined on a Fisher–Johns apparatus. IR spectra were recorded on a Vertex-70 spectrometer from KBr pellets. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Varian Mercury 400 (400 MHz) and Bruker Avance DRX 500 (500 and 125 MHz, respectively) spectrometers in $(CD_3)_2SO$ or $CDCl_3$ taking its residual protons signal as a standard. LCMS analysis was performed on an Agilent 1200 Series system equipped with a diode array and a G6130A mass-spectrometer (atmospheric pressure electrospray ionization). Combustion elemental analysis was performed in the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine analytical laboratory.

Chemicals and reagents were purchased from commercially available sourses. 2-Methylaminoethanol, 2-aminoethanol and 1*H*-1,2,4-triazole-5-amine were purchased from Aldrich. 2-(4-Chlorophenyl)-2-piperidin-1-ylethanamine was synthesized by the previously described method (Briner et al. 2006).

## General procedure for the synthesis of compounds 1–4 and 7–10

To a solution of appropriate 1,3-oxazole-5-sulfonyl chloride (0.01 mol) and amine (0.011 mol) in THF (30 ml), $Et_3N$ (0.011 mol) was added. The mixture was refluxed for 2 h and kept at 20–25 °C for 12 h. The precipitate was filtered off the solvent was removed in a vacuum. The residue was treated with water, filtered off, dried at 70–80 °C and recrystallized from ethanol or acetonitrile.

## General procedure for the synthesis of compounds 5 and 6

To a solution of *N*-(2,2-dichloro-1-cyanovinyl)-4-methylbenzamide (0.01 mol) in 30 ml of THF, triethylamine (0.022 mol) and an appropriate amine (2-methylaminoethanol or 2-aminoethanol (0.011 mol) were added. The mixture was stirred at room temperature during 12 h. The residue was triturated with water to give a crude product which was separated, dried at 70–80 °C, and recrystallized from ethanol.

**Methyl 5-((5-amino-3-phenyl-1*H*-pyrazol-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (1)** have been described in Kachaeva et al. (2018).

**Methyl 5-((5-amino-3-methyl-1*H*-pyrazol-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (2)** have been described in Kachaeva et al. (2018).

**Methyl 5-((5-amino-3-phenyl-1*H*-pyrazol-1-yl)sulfonyl)-2-(4-methylphenyl)-1,3-oxazole-4-carboxylate (3)** have been described in Kachaeva et al. (2018).

**Methyl 5-((5-amino-1*H*-1,2,4-triazol-1-yl)sulfonyl)-2-phenyl-1,3-oxazole-4-carboxylate (4)**

White solid (75%); mp (acetonitrile) 179–181 °C; IR (KBr) $\nu_{max}/cm^{-1}$ 1049, 1162 ($SO_2$), 1339 ($SO_2$), 1418, 1741 (C=O), 3424 ($NH_2$). $^{1}H$ NMR (500 MHz, $(CD_3)_2SO$) δ 3.89 (3H, s, $CH_3O$), 7.58 (s, 2H, $NH_2$), 7.61–7.64 (2H, m, ArH), 7.68–7.74 (2H, m, ArH), 8.00–8.03 (2H, m, ArH). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 53.8, 124.6, 127.7, 130.1, 133.7, 136.1, 145.8, 154.0, 158.2, 159.3, 162.9. LCMS, *m/z* 350 $[M+1]^+$. Anal. calcd. for $C_{13}H_{11}N_5O_5S$: C, 44.70%; H, 3.17%; N, 20.05%; S, 9.18%. Found: C, 44.71%; H, 3.15%; N, 20.12%; S, 9.26%.

**5-(2-Hydroxyethylamino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile (5)** have been described in Kozachenko et al. (2012).

**5-(2-Hydroxyethylmethylamino)-2-(4-methylphenyl)-1,3-oxazole-4-carbonitrile (6)** White solid (70%); mp (ethanol) 104–106 °C; IR (KBr) $\nu_{max}/cm^{-1}$ 1048, 1634, 2198 (CN), 3446 (OH). $^{1}H$ NMR (400 MHz, $(CD_3)_2SO$) δ 2.34 (3H, s, $CH_3$), 3.21 (3H, s, $NCH_3$), 3.54–3.57 (2H, m, $CH_2$), 3.62–3.65 (2H, m, $CH_2$), 4.92 (1H, t, $J = 5.2$ Hz, OH), 7.30 (2H, d, $J = 8.0$ Hz, ArH), 7.72 (2H, d, $J = 8.0$ Hz, ArH). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 21.5, 38.0, 54.0, 59.9, 84.8, 116.8, 123.1, 125.4, 129.5, 140.5, 150.9, 160.5. LCMS, *m/z* 258 $[M+1]^+$. Anal. calcd. for $C_{14}H_{15}N_3O_2$: C, 65.36; H, 5.88; N, 16.33. Found: C, 65.28; H, 5.86; N, 16.35.

**4-Cyano-*N*-(2-hydroxyethyl)-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide (7)** White solid (74%); mp (ethanol) 132–134 °C; IR (KBr) $\nu_{max}/cm^{-1}$ 1054, 1154 ($SO_2$), 1351 ($SO_2$), 1499, 1554, 2253 (CN), 3513 (OH). $^{1}H$ NMR (400 MHz, $(CD_3)_2SO$) δ 2.41 (1H, s, $CH_3$), 3.17 (2H, t, $J =$ 4.8 Hz, $CH_2$), 3.43–3.44 (2H, m, $CH_2$), 3.94 (1H, br s, OH), 7.44 (2H, d, $J = 7.6$ Hz, ArH), 7.92 (2H, d, $J = 7.6$ Hz, ArH), 8.92 (1H, s, NH). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 21.7, 46.0, 60.1, 111.5, 116.1, 122.1, 127.6, 130.5, 143.9, 154.1, 163.1. LCMS, *m/z* 308 $[M+1]^+$. Anal. calcd for $C_{13}H_{13}N_3O_4S$: C, 50.81%; H, 4.26%; N, 13.67%; S, 10.43%. Found: C, 50.78%; H, 4.24%; N, 13.60%; S, 10.55%.

**4-Cyano-*N*-(2-hydroxyethyl)-*N*-methyl-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide (8)** have been described in Kachaeva et al. (2018).

***N*-(2-(4-Chlorophenyl)-2-piperidin-1-ylethyl)-4-cyano-2-phenyl-1,3-oxazole-5-sulfonamide (9)** have been described in Kachaeva et al. (2018).

***N*-(2-(4-Chlorophenyl)-2-(1-piperidinyl)ethyl)-4-cyano-2-(4-methylphenyl)-1,3-oxazole-5-sulfonamide (10)** Yellow solid (69%); mp (ethanol) 148–150 °C; IR (KBr) $\nu_{max}/cm^{-1}$ 1092, 1161 ($SO_2$), 1395 ($SO_2$), 1496, 1588, 1616, 2253 (CN), 3321 (NH). $^{1}H$ NMR (500 MHz, $(CD_3)_2SO$) δ 1.18 (2H, br s, $CH_2$), 1.35 (4H, br s, $2CH_2$), 2.12 (2H, br s, $CH_2$), 2.26 (2H, br s, $CH_2$), 2.41 (3H, s,

$CH_3$), 3.41–3.48 (2H, m, $CH_2$, CH), 3.59–3.66 (2H, m, $CH_2$), 7.18 (2H, d, $J = 8.5$ Hz, ArH), 7.27 (2H, d, $J = 8.5$ Hz, ArH), 7.43 (2H, d, $J = 8.0$ Hz, ArH), 7.87 (2H, d, $J = 8.0$ Hz, ArH). $^{13}$C NMR (125 MHz, $(CD_3)_2SO$) δ 21.71, 24.4, 26.0, 44.5, 50.6, 63.1, 67.9, 111.5, 115.8, 121.9, 127.6, 128.2, 130.6, 132.5, 135.4, 143.9, 154.2. LCMS, *m/z* 485 $[M + 1]^+$. Anal. calcd for $C_{24}H_{25}ClN_4O_3S$: C, 59.44; H, 5.20; N, 11.55; S, 6.61. Found: C, 59.41; H, 5.22; N, 11.65; S, 6.72.

## Antiviral and cytotoxicity assays

### CPE assay

For the primary assay, antiviral activity and cytotoxicity were evaluated using CellTiter-Glo™ Assay (Promega Corporation, USA) by methods reported recently (Hartline et al. 2018). This is a simple, quantifiable method of determining viral-induced cytopathic effects (CPE) and compound toxicity in host cells. Briefly, CPE reduction assays were performed in medium consisting of MEM with Earle's salts, 2% FBS and standard concentrations of L-glutamine, penicillin, and gentamycin. Cells were seeded into 384-well microtiter plates and subsequently incubated at 37 °C in a humidified 5% $CO_2$ incubator for 24 h to allow the formation of confluent monolayers. Dilutions of test compounds were then prepared directly in the plates in a series of five-fold dilutions in duplicate wells to yield final concentrations that range from 300 to 0.1 μM or from 10 to 0.003 μM. Monolayers were then infected at a multiplicity of infection of 0.005 PFU/cell with HCMV and incubated further until 100% CPE was observed in the virus control wells. Cytopathology was determined by the addition of CellTiter-Glo reagent according to the manufacturer's suggested protocol. Concentrations of test compounds sufficient to reduce CPE by 50% ($EC_{50}$) were interpolated from the experimental data. Cytotoxicity was also determined with CellTiter-Glo and concentrations of the compounds that decreased cell viability by 50% ($CC_{50}$) were also calculated from the data and selective index (SI) values were calculated as the $CC_{50}/EC_{50}$ as a measure of antiviral activity.

### Yield reduction assays

Selected compounds with good antiviral activity were evaluated further with virus yield reduction (VYR) assays. Briefly, monolayers of HFF cells in 96-well plates were infected with HCMV a multiplicity of infection of 0.5 PFU/cell in the presence of dilutions of the test compounds. At 4 days following infection, the plates containing monolayers of infected cells were frozen to lyse the cells, then the contents were mixed and the titer of the progeny virus was determined in tissue culture infectious dose assays performed in HFF cells in 384-well plates.

**Table 2** Antiviral activity against the normal strain as well as the resistant isolate (GDGr K17) of HCMV, and cytotoxicity of the 1,3-oxazole derivatives in HFF cell line. Compound concentrations are in μM

| Compound | Strain AD169 | | | GDGr K17 (resistant isolate) | | |
|---|---|---|---|---|---|---|
| | $EC_{50}$ | $CC_{50}$ | $SI_{50}$ | $EC_{50}$ | $CC_{50}$ | $SI_{50}$ |
| **1** | <0.05 | 17.0 | >356 | >1.20 | 4.5 | <4 |
| **2** | <0.05 | 87.2 | >1818 | >30 | 74.0 | <2 |
| **3** | <0.05 | 16.0 | >334 | <0.05 | 4.0 | >83 |
| **4** | >6.00 | 29.0 | <5 | | | |
| **5** | <0.05 | >150 | >3125 | >30 | 106.2 | <4 |
| **6** | <0.05 | >150 | >3125 | <0.05 | >150 | >3125 |
| **7** | <0.05 | 88 | >1833 | >30 | 53.7 | <2 |
| **8** | <0.05 | 16.4 | >342 | 0.05 | 2.46 | 46 |
| **9** | >6.00 | 20.7 | <3 | | | |
| **10** | >6.00 | 12.6 | <2 | | | |
| Ganciclovir | 0.32 | >150 | >463 | 17 | >150.00 | >8 |
| Cidofovir | | | | 0.10 | >30.00 | <4 |

Control and drug concentrations ranges are 0.048–150 μM. Vehicle is DMSO. $EC_{50}$ and $CC_{50}$ are compound concentrations that reduce viral replication and cell viability, respectively, by 50% in CellTiter-Glo (cytopathic effect/toxicity) assay. Selectivity index ($SI_{50}$) is calculated as the $CC_{50}$ value divided by the $EC_{50}$ value

## Results and discussion

The effects of the 1,3-oxazole derivatives on antiviral activity against a normal laboratory HCMV strain, AD-169, and their cytotoxicity were first evaluated on HFF cells using CellTiter-Glo (cytopathic effect/toxicity) assay (Table 2).

All compounds exhibited antiviral activity against the normal HCMV strain AD169 and, with the exception of **4**, **9**, and **10**, had $EC_{50}$ values of <0.05 μM, and were considerably more active than Ganciclovir ($EC_{50} = 0.32$ μM) in this assay. The most selective compounds were **5** = **6** >> **7** ≈ **2** >> Ganciclovir > **1** ≈ **8** and **3**.

The HCMV-resistant isolate (GDGr K17) was tested for sensitivity to the compounds with high antiviral activity against the normal HCMV strain AD169 (Table 2). The 1,3-oxazole derivatives **3** and **6** showed very high potency ($EC_{50}$: < 0.05 μM) towards the resistant isolate compared to standard drug Cidofovir ($EC_{50} = 0.10$ μM). Compound **8** showed moderate activity and was still two times more potent ($EC_{50} = 0.05$ μM) than Cidofovir. The most selective compounds were **6** >> Cidofovir >> **3** > **8** > **1** = **5** > **2** = **7** > Ganciclovir.

**Table 3** Anti-HCMV activity and cytotoxicity of 1,3-oxazole derivatives in yield reduction assays in HFF cells. Compound concentrations are in μM

| Compound | $EC_{50}$ | $EC_{90}$ | $CC_{50}$ | $SI_{50}$ | $SI_{90}$ |
|---|---|---|---|---|---|
| *AD169* | | | | | |
| **5** | >20.00 | >20.00 | 46.70 | <2 | <2 |
| **6** | >100.00 | >100.00 | >100.00 | 1 | 1 |
| **7** | >20.00 | >20.00 | 73.30 | <4 | <4 |
| Ganciclovir | 4.65 | 15.8 | >100.00 | >21 | >6 |
| Cidofovir | 0.63 | 2.9 | >20.00 | >31 | >6 |
| *GDGr K17 (resistant isolate)* | | | | | |
| **5** | >20.00 | >20.00 | 46.70 | <2 | <2 |
| **6** | >100.00 | >100.00 | >100.00 | 1 | 1 |
| **7** | >20.00 | >20.00 | 73.30 | <4 | <4 |
| Ganciclovir | 30.8 | 98.7 | >100.00 | >3 | >1 |
| Cidofovir | 0.97 | 3.4 | >20.00 | >20 | >5 |

Drug concentration ranges are 0.032–100 μM. Control concentration ranges are 0.8–100 (Ganciclovir) and 0.16–20 μM (Cidofovir). Vehicle is DMSO. $EC_{50}$ and $CC_{50}$—compound concentrations that reduce viral replication and cell viability by 50% in yield reduction assay. Selectivity index ($SI_{50}$) is calculated as the $CC_{50}$ value divided by the $EC_{50}$ value

Compounds **5**, **6**, and **7**, which showed high activity against the normal strain of HCMV in the primary in vitro antiviral assays, were chosen for the secondary assays. The effects of compounds **5**, **6**, and **7** on the viral replication and the cell cytotoxicity in a HFF culture using yield assay were investigated.

In contrast to the primary assay, the antiviral activity of these compounds against both the susceptible and the resistant isolate GDGr K17 of HCMV was not detectable (Table 3). Differences in the antiviral activity measured by CPE assay and the yield reduction assay may be related to compound exposure is 14 days and 4 days, respectively.

In this assay, compounds **5** and **7** have shown the identical antiviral activity against the normal strain AD169 and the resistant isolate GDGr K17, which is more than for compound **6** but less than for control drugs.

Thus, only 5-amino- and 5-sulfonamide derivatives with hydroxyethyl group (compounds **5** and **7**) displayed the mild biological activity ($EC_{50}$: >20.0 μM) among the three analogs of 1,3-oxazole-4-carbonitrile tested in the secondary assay. Introducing into the 5-position of the 1,3-oxazole ring another substituents led to a negative impact on HCMV inhibition.

All synthesized compounds belong to 2-phenyl-1,3-oxazoles. It was shown that compounds of this type are potent inhibitors of histone demethylases in vitro (Dulla et al. 2013). As mentioned above, HCMV infects a broad range of the population and establishes life-long latency in the infected individuals. Periodically the latently infected virus can reactivate and provoke morbidity in immunocompromised individuals. Upon reactivation, viral lytic gene transcription is initiated by the expression of viral immediate early (IE) genes, and histone demethylases play important roles in the activation of viral IE gene expression. Pharmacological inhibition of the HDM leads to severely impaired viral IE gene expression and may block HCMV lytic infection and reactivation (Gan et al. 2017). This may suggest hypothetical mechanism of anti-HCMV action of 1,3-oxazoles derivatives here.

Large numbers of compounds for their ability to inhibit have been synthesized and tested in HFF cells in vitro but only few have been developed (Fader et al. 2016; Britt and Prichard 2018). Therefore, a problem has occurred with the adequate of model cell line and/or experimental methods that is used for antiviral screening of new compounds (Griffiths 2002). HCMVs are highly restricted in their tropism, so the human virus is challenging to study outside of cultured cells. In fibroblast cell cultures HCMV replicates produce CPE very slowly. This slow appearance of CPE is misleading about the true nature of CMV replication. In vivo, in its natural human host, HCMV replicates rapidly (Emery et al. 1999). This contrast between findings in vivo and in vitro is only one of the differences between replication of the virus in cell cultures and the human host. Thus, we cannot exclude the possibility that these molecules may demonstrate potent antiviral activity in animal models.

## Conclusion

Ten five-functionalized derivatives of 1,3-oxazole-4-carboxylate and 1,3-oxazole-4-carbonitrile have been synthesized and evaluated for in vitro antiviral activity and cytotoxicity. Bioassays showed that some of the synthesized compounds exhibited considerably higher antiviral activity ($EC_{50}$: < 0.05 μM) against a normal laboratory HCMV strain (AD-169), and the HCMV-resistant isolate (GDGr K17) in HFF cells than Ganciclovir. In contrast to the primary assays, the antiviral activity of these compounds against both the normal strain and the resistant isolate of HCMV were less than one of cidofovir in secondary assay. Additional research will be required to understand fully the activities of these compounds against HCMV.

## Disclaimer

This material should not be interpreted as representing the viewpoint of the National Institute of Allergy and Infectious Diseases (USA) and its Collaborative Antiviral Testing Group.

**Acknowledgements** We would like to thank Enamine Ltd. for the material and technical support. These studies were funded in whole or in part with Federal funds from the National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract HHSN272201100016I (MNP).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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