**RESEARCH ARTICLE**

RO0504985 is an Inhibitor of CMGC Kinase Proteins and has

Anti-Human Cytomegalovirus Activity

Blair L Strang1, 2

Institute for Infection & Immunity, St George’s, University of London, London, UK1; Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA, USA2

Corresponding Author: Blair L Strang, Institute for Infection & Immunity, St George’s, University of London, London, UK. Email: [bstrang@sgul.ac.uk](mailto:bstrang@sgul.ac.uk)

Word Count (Abstract): 140

Word Count (Main Text): 3,054

**ABSTRACT**

Public-private partnerships allow many previously unavailable compounds to be screened for antiviral activity. Here a screening method was used to identify an oxindole compound, RO0504985, from a Roche kinase inhibitor library that inhibited human cytomegalovirus (HCMV) protein production. RO0504985 was previously described as an inhibitor of cyclin-dependent kinase 2 (CDK2). However, using kinase selectivity assays it was found that RO0504985 was an inhibitor of several CMGC group kinase proteins, including CDK2. Using virus yield reduction assays it was observed that RO0504985 inhibited replication of different HCMV strains at low micromolar concentrations. Western blotting was used to investigate how RO0504985 inhibited HCMV replication. Treatment of HCMV infected cells with RO0504985 inhibited production of the immediate early viral IE2 proteins and the late viral protein pp28. Thus, RO0504985 inhibited HCMV replication by preventing production of specific HCMV proteins necessary for virus replication.

**KEYWORDS**

Human, cytomegalovirus, screen, kinase, CDK, CMGC

**ABBREVIATIONS**

# CDK: cyclin-dependent kinase, CK: casein kinase, CLK: cdc2-like kinase, CMGC: kinase group named using the initials of several members of that group, DYRK: dual specificity tyrosine-phosphorylation-regulated kinase, GSK: glycogen synthase kinase, HIPK: homeodomain-interacting protein kinase, JNK: c-Jun N-terminalkinase, MAPK: mitogen-activated protein kinase, MOK: MAPK/MAK/MRK overlapping kinase, MSSK: muscle-specific serine kinase, NLK: Nemo-like kinase,SAPK: stress-activated protein kinase.

**HIGHLIGHTS**

* Screening of kinase inhibitors in HCMV infected cells was conducted via a public-private partnership.
* An oxindole compound, RO0504985, was found to have anti-HCMV activity.
* RO0504985 was discovered to be an inhibitor of a range of CMGC protein kinases.
* RO0504985 inhibited HCMV replication by preventing production of viral immediate-early and late proteins.

**1. INTRODUCTION**

Several factors, including bioavailability, drug resistance and toxicity, limit the use of currently available anti-human cytomegalovirus (HCMV) drugs ([Biron, 2006](#_ENREF_3); [Coen and Schaffer, 2003](#_ENREF_5); [Mercorelli et al., 2008](#_ENREF_16); [Schleiss and McVoy, 2004](#_ENREF_23); [Villarreal, 2003](#_ENREF_26)). While several novel anti-HCMV drugs are now under development ([Coen and Schaffer, 2003](#_ENREF_5); [Mercorelli et al., 2008](#_ENREF_16)), it remains necessary to expand our understanding of what compounds have anti-HCMV activity.

Screening of compound libraries is a useful methodology to survey compounds for potential anti-viral activity. However, the number of libraries currently available for screening is limited. Plus, there is often little diversity of chemical structures within those collections and the compounds within each library are often poorly characterised ([Drewry et al., 2014](#_ENREF_7); [Knapp et al., 2013](#_ENREF_13)).

Public-private partnerships are one route to expand the number of compounds available for screening ([Drewry et al., 2014](#_ENREF_7); [Knapp et al., 2013](#_ENREF_13)). Here, a screening methodology was used to identify potential anti-HCMV compounds within a Roche kinase inhibitor library. This collection is comprised of a diverse range of previously reported compounds that inhibit kinase proteins from several proteins kinase groups.

**2. MATERIALS AND METHODS**

**2.1 Compounds.** A Roche Kinase Inhibitor library (Table S1) was supplied to the Institute of Chemistry and Chemical Biology-Longwood (Harvard Medical School) by Hoffmann-La Roche Inc. All compounds were resuspended in dimethyl sulfoxide (DMSO).

**2.2 Cells and viruses.** Human foreskin fibroblast (HFF) cells (clone Hs29) were used. HCMV strains AD169 and Merlin RCMV1111 ([Stanton et al., 2010](#_ENREF_25)) were gifts from Don Coen (Harvard Medical School) and Richard Stanton (Cardiff University), respectively.

**2.3 Screening of compounds.** See Supplementary Material.

**2.4 Viral yield reduction.** HFF cells were plated at a density of 5 × 104 cells per well. After 24 hours incubation, cells were infected with HCMV at a multiplicity of infection (MOI) of 1. After virus adsorption for 1 hour at 37°C, cells were washed and incubated with 0.5 ml of media containing different dilution series of DMSO or RO0504985 that covered a range of concentrations from above 10M to below 0.01M. Plates were incubated for 96 hours at 37°C. Titers were determined by serial dilution of viral supernatant onto HFF monolayers which were covered in DMEM media containing 10% FBS and 0.6% methylcellulose. Cultures were incubated for 14 days, cells were stained with crystal violet and plaques were counted. The final concentration of DMSO in all samples was maintained at <1% (v/v).

**2.5 MTT assays.** HFF cells were seeded at a density of 1 × 104 cells per well. After overnight incubation to allow cell attachment, cells were treated for 96 hours with a dilution series of DMSO or RO0504985 that covered a range of concentrations from 10M to below 0.1M. Cytotoxicity or inhibition of cell division was then determined with an MTT assay (GE Healthcare) according to the manufacturer's protocol, in which the ability of the ability of NAD(P)H-dependent cellular oxidoreductase enzymes to reduce the tetrazolium dye MTT (3-(4,5-[di](https://en.wikipedia.org/wiki/Di-)[methyl](https://en.wikipedia.org/wiki/Methyl)[thiazol](https://en.wikipedia.org/wiki/Thiazole)-2-yl)-2,5-di[phenyl](https://en.wikipedia.org/wiki/Phenyl)tetrazolium bromide) to [formazan](https://en.wikipedia.org/wiki/Formazan). The final concentration of DMSO in all samples was maintained at <1% (v/v).

**2.6 Kinase selectivity analysis.** RO0504985 was submitted to the Eurofins Pharma Discovery (Dundee) **KinaseProfiler™ service to assay the ability of** 1M of compound or an equivalent volume of DMSO to inhibit the panel of 43 CMGC kinase proteins shown in Figure 2. Full details of each kinase protein inhibition assay can be obtained by contacting Eurofins Pharma Discovery. Briefly, recombinant protein kinases were tested for their ability to inhibit removal of a radiolabeled phosphate from γ-33P-ATP. Each in vitro kinase reaction was initiated by adding of 10 M MgATP. After 40 minutes incubation at room temperature, reactions were stopped by adding 3% phosphoric acid. Ten μL of each reaction was spotted onto Filtermat and washed (three times for 5 minutes in 75 mM phosphoric acid and once in methanol) prior to drying and scintillation counting.

**2.7 Western blotting.** At time points indicated in the Figure cells were washed with PBS and resuspended in Laemmli buffer containing 5% -mercaptoethanol. Proteins were separated on 8% or 10% polyacrylamide gels. Membranes were probed with antibodies recognizing IE1/2, pp28, pp65, UL44, UL84, (Virusys, 1:1000 dilution), IE2 proteins (clone 5A8.2, Millipore, 1:1000 dilution), UL85, UL86 (both kind gifts from Wade Gibson, Johns Hopkins University School of Medicine, 1:1000 dilution) and -actin (SIGMA, 1:5000 dilution). All primary antibodies were detected using anti-mouse- or anti-rabbit-horseradish peroxidase (HRP) conjugated antibodies (Millipore and Cell Signaling Technology, respectively. Used at 1:10,000 and 1:2000 dilution, respectively.). Chemiluminescence solution (GE Healthcare) was used to detect secondary antibodies on film. Where shown, the presence of -actin was used as a control to demonstrate similar amounts of cell lysate were assayed. Where indicated, relative band intensity (band intensity relative to -actin signal in the same lane) was analysed using ImageJ software, obtained from the National Institutes of Health (USA).

**3. RESULTS AND DISCUSSION**

**3.1 Kinase inhibitor screening.** A Roche Kinase Inhibitor library of 235 compounds (Table S1) was screened at a concentration of 9.4 M to identify compounds that inhibited production of the HCMV protein pp28 in cells infected with high passage strain AD169 ([Wilkinson et al., 2015](#_ENREF_28)). Eighteen compounds (Table S2) produced a notable decrease in the number of cells in the assay (see Supplementary Material for criteria) and were judged to be cytotoxic to HCMV infected cells. Remaining data from the screen (217 compounds) was then converted to a z-score (the number of standard deviations from the mean of the data ([Birmingham et al., 2009](#_ENREF_2); [Zhang et al., 1999](#_ENREF_29))) to show an increase or decrease (positive or negative z-score, respectively) in the number of pp28 positive cells (Fig. 1A and Table S3).

There was no obvious overall relationship between compound structure or putative compound target and the positive or negative z-score assigned to each compound, except for 5 structurally related quinolinyl-methylene-thiazolinone compounds (RO4600445, RO4569139, RO4509200, RO4915610 and RO4554339) that had negative z-scores of -2.0 to -2.3 (Figs. 1A, 1B and Table S3). Using MTT assays it was found that these quinolinyl-methylene-thiazolinone compounds had 50% cellular cytotoxicity (CC50) values of greater than or equal to 8M in uninfected cells and 50% effective dose (ED50) values of 1-2M against HCMV strain AD169 (Table 1). Therefore, cytotoxicity and/or inhibition of cell division may have made a contributed to the low z-scores for these compounds observed in the screen (Fig.1A and Table S3). However, low ED50 values of the quinolinyl-methylene-thiazolinone compounds compared to their CC50 values (selectivity indexes (SI), Table 1) demonstrated that these compounds had anti-HCMV activity that was not related to cytotoxicity or inhibition of cell division.

The aforementioned quinolinyl-methylene-thiazolinone compounds have been reported to be potent and selective inhibitors of CDK1 (Table S3), a kinase from the CMGC protein kinase group. Multiple mechanisms are employed by HCMV to ensure CDK1 is present in the infected cell ([Sanchez et al., 2003](#_ENREF_21)), and treatment of infected cells with compounds that inhibit CDK proteins, including CDK1, inhibit HCMV replication ([Hertel et al., 2007](#_ENREF_11); [Sanchez and Spector, 2006](#_ENREF_22)). It is possible that CDK1 has multiple roles in facilitating HCMV replication. Analysis of these roles is complicated as inhibitors of CDK1 may also inhibit other CDK proteins. Using western blotting it was found that treatment of infected cells with quinolinyl-methylene-thiazolinone compounds resulted in a decrease in detection of HCMV Early protein UL44 and HCMV Late protein pp28, that correlated with a decrease in z-score for the compounds, but no obvious decrease in detection of either HCMV Immediate-Early proteins IE1 or IE2 (Fig. S1). This suggested that quinolinyl-methylene-thiazolinone compounds acted on virus replication after the production of immediate early proteins. A previous report indicated that treatment of cells with roscovitine (an inhibitor of CDK proteins (including CDK1)) lead to a reduction in IE1 and IE2 protein production, but no reduction in UL44 and pp28 production ([Sanchez and Spector, 2006](#_ENREF_22)). Thus, it was speculated that the quinolinyl-methylene-thiazolinone compounds examined here had as yet unrecognised targets in HCMV infected cells and did not inhibit CDK1 in the context of HCMV replication.

The compound with the greatest negative z-score in the screen was RO0504985 (Figs. 1A and 1C), a oxindole compound reported to inhibit the kinase CDK2 ([Dermatakis et al., 2003](#_ENREF_6)). CDK2, like other CDK proteins, is from the CMGC protein kinase group. RO0504985 compound was chosen to be the focus of further study.

**3.2 Inhibition of low and high passage HCMV strains by RO0504985.** Viral yield reduction assays were used to assess replication of either AD169 or low passage HCMV strain (Merlin(RCMV1111)), whose genomic content is similar to that of a clinical virus isolate ([Wilkinson et al., 2015](#_ENREF_28)), in the presence of RO0504985. The ED50 values of RO0504985 against AD169 and Merlin(RCMV1111) were 0.01M and 1M, respectively (Table 2). Thus, RO0504985 could inhibit replication of different HCMV strains. It was unclear, however, why RO0504985 had less anti-HCMV activity against Merlin, compared to AD169. It can be speculated that any of the open reading frames present in low passage strain Merlin, but not in high passage strain AD169 ([Wilkinson et al., 2015](#_ENREF_28)), were responsible for the different effects of RO0504985 on different HCMV strains. Anti-HCMV drugs such as ganciclovir have been reported to have ED50 values of approximately 1M under similar conditions ([Loregian and Coen, 2006](#_ENREF_14); [Markham and Faulds, 1994](#_ENREF_15)) and a selectivity index of approximately 200 ([Loregian and Coen, 2006](#_ENREF_14)). Therefore, RO0504985 has similar anti-HCMV to the frontline HCMV drug ganciclovir. However, the selectivity index of RO0504985 against different HCMV strains, suggests that ganciclovir may have a preferable safety profile compared to RO0504985 depending on the HCMV strain that is being targeted.

To ensure that the anti-HCMV activity of RO0504985 was not due to cellular cytotoxicity or inhibition of cell division, an MTT assay was used to assess the number of uninfected cells in the presence of RO0504985 compared to treatment of cells with DMSO. It was found that the CC50 value of RO0504985 was 10M (Table 2). Therefore, when comparing CC50 and ED50 values for RO0504985 (selectivity indexes, Table 2) it was likely that cellular cytotoxicity or inhibition of cell division did not obviously contribute to the ED50 value of RO0504985. However, the CC50 value of RO0504985 did imply that cellular cytotoxicity or inhibition of cell division could have contributed to the very low z-score assigned to RO0504985 in the screen (Fig. 1).

**3.3 RO0504985 kinase selectivity analysis.** Oxindole compounds can inhibit a range of kinase proteins from several kinase groups ([Elkins et al., 2016](#_ENREF_8)). Previous screening experiments suggest that to have anti-HCMV activity oxindole compounds must be able to potently inhibit several CDK proteins, plus several other CMGC kinase group proteins ([Khan et al., 2017](#_ENREF_12)). It remains unknown which combinations of CMGC group kinase proteins must be inhibited for an oxindole compound to have anti-HCMV activity. RO0504985 has been reported to inhibit CDK2 ([Dermatakis et al., 2003](#_ENREF_6)), but it was unknown if other CMGC proteins could be inhibited by this compound. Therefore, *in vitro* kinase inhibition assays were used to assess the ability of RO0504985 to inhibit a panel of CMGC kinase group proteins, including CDK proteins CDK1-7 and CDK9 (Fig. 2). RO0504985 was a potent inhibitor (less than 10% kinase activity remaining) of all CDK proteins tested and several CLK, DYRK, GSK and SRPK proteins. Plus, RO0504985 was also an inhibitor of various other CMGC kinase proteins (less than 50% kinase activity remaining). Thus, RO0504985 was an inhibitor of a range of CMGC protein kinase proteins *in vitro*.

Several CMCG kinase proteins are thought to be involved in HCMV replication. Treatment of HCMV infected cells with roscovitine, which inhibits a range of CDK proteins, inhibits several facets of productive HCMV replication ([Sanchez et al., 2007](#_ENREF_20); [Sanchez and Spector, 2006](#_ENREF_22)) and proteins such as CK2 and MAPK1 are involved in phosphorylation of at least one HCMV protein ([Barrasa et al., 2005](#_ENREF_1); [Harel and Alwine, 1998](#_ENREF_9); [Heider et al., 2002](#_ENREF_10); [Rodems and Spector, 1998](#_ENREF_19)). It is unclear or unknown if CLK, DYRK, GSK or SRPK proteins potently inhibited by RO0504985 are required for efficient HCMV replication. It is possible that inhibition of the aforementioned CMGC kinase proteins by RO0504985, alone or in combination, results in anti-HCMV activity in infected cells. As the function of many kinase proteins in infected cells is unknown or unclear, it is possible that inhibition of kinase proteins by RO0504985 their inhibition has both direct and indirect on HCMV replication. It is possible that RO0504985 also inhibits proteins in other kinase groups, but, as mentioned above, our previous analysis of HCMV replication inhibition by oxindole compounds suggests that inhibition of CMGC kinase proteins, not inhibition of proteins from other kinase groups, is responsible for anti-HCMV activity ([Khan et al., 2017](#_ENREF_12)).

Furthermore, the potential for RO0504985 to inhibit the HCMV encoded kinase UL97 was also examined. Treatment of infected cells with the UL97 inhibitor maribavir inhibits UL97 autophosphorylation and results in loss of high molecular weight UL97 that can be visualised by western blotting ([Silva et al., 2011](#_ENREF_24)).. Treatment of infected cells with RO0504985 did not affect production of high molecular weight UL97 found by western blotting (data not shown). Therefore, the anti-HCMV activity of RO0504985 compounds was unlikely to have been due to inhibition of UL97 kinase activity.

These data will assist future studies of RO0504985 and CMGC kinase proteins. Plus, these data furthers our understanding of kinase proteins required for HCMV replication and stimulates questions about the relationship between the role of CDK proteins and other CMGC proteins in the infected cell. Combining the data presented here with other reports ([Khan et al., 2017](#_ENREF_12)) and analysis of further oxindole compounds will create structure-activity relationships and illuminate how inhibition of CMGC proteins relates to anti-HCMV activity.

**3.4 Inhibition of HCMV protein production.** Finally, how RO0504985 inhibited HCMV replication was investigated. Western blotting was used to assay production of HCMV proteins from each kinetic class of viral transcription (immediate-early to early to late) shown in Figure 3A. Compared to treatment of HCMV infected cells with DMSO (Fig. 3A, lanes 2-4), densitometry analysis revealed treatment of HCMV infected cells with RO0504985 (Fig. 3A, lanes 5-7) resulted in an approximately 2-fold decrease in production of immediate-early proteins IE2-86 and IE2-60 and an approximately 4-fold decrease in late protein pp28. To confirm this analysis using western blotting, HFF cells were again infected with AD169 and treated with either DMSO or RO0504985. At 72 h.p.i. the defect in IE2 and pp28 protein production was assayed by comparing a 2-fold serial dilation series of lysate from DMSO treated cells to undiluted lysate of RO0504985 treated cells (Fig. 3B and 3C, respectively). Consistent with the aforementioned densitometry analysis, approximately 2-fold and 4-fold decreases in IE2 and pp28 protein production, respectively.

Treatment of infected cells with RO0504985 had no obvious effect on the accumulation of any other immediate-early (IE1 and IE2-40), early (UL44, UL84, pp65) or late (UL85, UL86) protein examined (Fig. 3A).

Both IE2-86 and pp28 are essential for virus replication ([Britt et al., 2004](#_ENREF_4); [Pizzorno et al., 1988](#_ENREF_18)), therefore, a lack of either or both of these proteins could reflect the anti-HCMV activity of RO0504985. IE2-60 is a protein produced from an internal start codon in the open reading frame encoding IE2-86 and is required for efficient HCMV replication ([White et al., 2007](#_ENREF_27)). Thus, inhibition of IE2-60 production could also have contributed to the anti-HCMV effects of RO0504985.

Inhibition of a broad range of CDK proteins by roscovitine results in loss of both IE1 and IE2 proteins in HCMV infected cells ([Sanchez and Spector, 2006](#_ENREF_22)). Thus, it is likely that inhibition of HCMV replication by RO0504985 did not involve inhibition of CDK proteins. However, several factors could link inhibition of CMGC kinases with inhibition of IE2 and pp28 protein production. RO0504985 is an inhibitor of CMGC kinase proteins CK2 and MAPK1 (Fig. 2), both of which have been reported to phosphorylate IE2-86 ([Barrasa et al., 2005](#_ENREF_1); [Harel and Alwine, 1998](#_ENREF_9); [Heider et al., 2002](#_ENREF_10); [Rodems and Spector, 1998](#_ENREF_19)). It is unknown if CMGC kinase proteins can phosphorylate IE2-60. It has been reported that mutation of certain CK2 phosphorylation sites in IE2 resulted in inhibition of IE2-86 production, plus defects in the production of several of other viral proteins including UL44, pp65 and pp28 ([Barrasa et al., 2005](#_ENREF_1)). This suggests the possibility that there is a link between inhibition of IE2 phosphorylation by CK2 in the presence of RO0504985 and inhibition of IE2 and pp28 production. However, it is unknown how the presence of RO0504985 allowed efficient production of pp65 and UL44 when CK2 function should be inhibited. It has been reported that mutation of MAPK1 phosphorylation sites in IE2-86, or treatment of infected cells with an inhibitor of MEK signaling (an upstream regulator of MAPK1), had no obvious effect on immediate-early transcription or protein production ([Heider et al., 2002](#_ENREF_10); [Rodems and Spector, 1998](#_ENREF_19)). However, it is unknown what effect, if any, inhibition of MAPK has on pp28 production.

It is possible that there were also direct effects of RO0504985 on pp28 production that did not involve inhibition of IE2 protein production. Inhibition of pp28 phosphorylation leads to pp28 instability ([Munger et al., 2006](#_ENREF_17)). However, it is unknown if pp28 can be phosphorylated by any CMGC kinase protein that is inhibited by RO0504985. An alternative possibility is that RO0504985 is an inhibitor of HCMV UL26, whose function is unknown, as mutation of UL26 leads to hypophosphorylation and instability of pp28 ([Munger et al., 2006](#_ENREF_17)).

**ACKNOWLEDGMENTS**

Thanks to Paul Gillespie and colleagues at Roche for their assistance with this project and to Don Coen for his support of this study, providing reagents and his comments on the manuscript. Reagents were generously provided by Wade Gibson and Richard Stanton. We acknowledge Robin Leach and Anna Woodward of Eurofins Pharma Discovery for providing information on kinase assays. Special thanks go to all members of Institute of Chemistry and Chemical Biology-Longwood for their assistance in all aspects of the screening process. Finally, thanks to the anonymous Reviewers whose comments and suggestions have greatly improved this manuscript.

**FUNDING**

This work was supported by New Investigator funds from St George’s, University of London, a St George’s Impact & Innovation Award and a PARK/WestFocus Award (all to B.L.S.). B.L.S. was also supported by grants awarded to Don Coen (Harvard Medical School) from the National Institutes of Health (R01 AI019838 and R01 AI026077). The funders had no role in experimental design, data collection, data interpretation or the decision to submit the work for publication.

**REFERENCES**

Barrasa, M.I., et al., 2005. The phosphorylation status of the serine-rich region of the human cytomegalovirus 86-kilodalton major immediate-early protein IE2/IEP86 affects temporal viral gene expression. J Virol 79, 1428-1437.

Birmingham, A., et al., 2009. Statistical methods for analysis of high-throughput RNA interference screens. Nature methods 6, 569-575.

Biron, K.K., 2006. Antiviral drugs for cytomegalovirus diseases. Antiviral research 71, 154-163.

Britt, W.J., et al., 2004. Rapid genetic engineering of human cytomegalovirus by using a lambda phage linear recombination system: demonstration that pp28 (UL99) is essential for production of infectious virus. J Virol 78, 539-543.

Coen, D.M., Schaffer, P.A., 2003. Antiherpesvirus drugs: a promising spectrum of new drugs and drug targets. Nat Rev Drug Discov 2, 278-288.

Dermatakis, A., et al., 2003. Synthesis of potent oxindole CDK2 inhibitors. Bioorganic & medicinal chemistry 11, 1873-1881.

Drewry, D.H., et al., 2014. Seeding collaborations to advance kinase science with the GSK Published Kinase Inhibitor Set (PKIS). Current topics in medicinal chemistry 14, 340-342.

Elkins, J.M., et al., 2016. Comprehensive characterization of the Published Kinase Inhibitor Set. Nat Biotechnol 34, 95-103.

Harel, N.Y., Alwine, J.C., 1998. Phosphorylation of the human cytomegalovirus 86-kilodalton immediate-early protein IE2. J Virol 72, 5481-5492.

Heider, J.A., et al., 2002. Characterization of a human cytomegalovirus with phosphorylation site mutations in the immediate-early 2 protein. J Virol 76, 928-932.

Hertel, L., et al., 2007. Viral and cell cycle-regulated kinases in cytomegalovirus-induced pseudomitosis and replication. PLoS pathogens 3, e6.

Khan, A.S., et al., 2017. High Throughput Screening of a GlaxoSmithKline Protein Kinase Inhibitor Set Identifies an Inhibitor of Human Cytomegalovirus Replication that Prevents CREB and Histone H3 Post-Translational Modification. J Gen Virol.

Knapp, S., et al., 2013. A public-private partnership to unlock the untargeted kinome. Nature chemical biology 9, 3-6.

Loregian, A., Coen, D.M., 2006. Selective anti-cytomegalovirus compounds discovered by screening for inhibitors of subunit interactions of the viral polymerase. Chem Biol 13, 191-200.

Markham, A., Faulds, D., 1994. Ganciclovir. An update of its therapeutic use in cytomegalovirus infection. Drugs 48, 455-484.

Mercorelli, B., et al., 2008. Human cytomegalovirus DNA replication: antiviral targets and drugs. Reviews in medical virology 18, 177-210.

Munger, J., et al., 2006. UL26-deficient human cytomegalovirus produces virions with hypophosphorylated pp28 tegument protein that is unstable within newly infected cells. J Virol 80, 3541-3548.

Pizzorno, M.C., et al., 1988. trans-activation and autoregulation of gene expression by the immediate-early region 2 gene products of human cytomegalovirus. J Virol 62, 1167-1179.

Rodems, S.M., Spector, D.H., 1998. Extracellular signal-regulated kinase activity is sustained early during human cytomegalovirus infection. J Virol 72, 9173-9180.

Sanchez, V., et al., 2007. Nuclear export of the human cytomegalovirus tegument protein pp65 requires cyclin-dependent kinase activity and the Crm1 exporter. J Virol 81, 11730-11736.

Sanchez, V., et al., 2003. Mechanisms governing maintenance of Cdk1/cyclin B1 kinase activity in cells infected with human cytomegalovirus. J Virol 77, 13214-13224.

Sanchez, V., Spector, D.H., 2006. Cyclin-dependent kinase activity is required for efficient expression and posttranslational modification of human cytomegalovirus proteins and for production of extracellular particles. J Virol 80, 5886-5896.

Schleiss, M.R., McVoy, M.A., 2004. Overview of congenitally and perinatally acquired cytomegalovirus infections: recent advances in antiviral therapy. Expert review of anti-infective therapy 2, 389-403.

Silva, L.A., et al., 2011. Sites and roles of phosphorylation of the human cytomegalovirus DNA polymerase subunit UL44. Virology 417, 268-280.

Stanton, R.J., et al., 2010. Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. The Journal of clinical investigation 120, 3191-3208.

Villarreal, E.C., 2003. Current and potential therapies for the treatment of herpes-virus infections. Prog Drug Res 60, 263-307.

White, E.A., et al., 2007. The IE2 60-kilodalton and 40-kilodalton proteins are dispensable for human cytomegalovirus replication but are required for efficient delayed early and late gene expression and production of infectious virus. J Virol 81, 2573-2583.

Wilkinson, G.W., et al., 2015. Human cytomegalovirus: taking the strain. Medical microbiology and immunology 204, 273-284.

Zhang, J.H., et al., 1999. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Journal of biomolecular screening 4, 67-73.

**FIGURE LEGENDS**

**Fig. 1 Compounds assigned z-scores.** (A) The ability of compounds within the Roche Kinase collection to inhibit HCMV strain AD169 protein production in HFF cells was assessed using the screen described in Materials & Methods. After exclusion of compounds judged to by cytotoxic, each compound was assigned a z-score (the number of standard deviations from the mean value of the screen) to describe the number of cells expressing viral antigen pp28 Thus, negative and positive z-scores represent fewer or greater numbers of cells expressing pp28, respectively. A plot of all z-scores is shown,where each data point represents a single compound. A list of each compound with its assigned z-scores is shown in Supplementary Table S3. The z-scores of several compounds discussed in the text are highlighted. (B and C) The structures and z-scores (stated in parentheses) of quinolinyl-methylene-thiazolinone compounds and RO0504985, respectively.

**Fig. 2 CMGC Kinase inhibition by RO0504985.** RO0504985 (1M) was tested for its ability to inhibit removal of a radiolabelled phosphate from an ATP substrate by CMGC group kinase proteins, compared to kinase proteins incubated with DMSO.. Each data point represents the percentage kinase activity compared to DMSO. The data from two independent experiments (black and grey bars, respectively) is shown. Inhibition of kinase activity and potent inhibition of kinase activity was set at 50% and 10% remaining kinase activity, respectively.

**Fig. 3 Western blotting of HCMV infected cells treated with RO0504985.** HFF cells were infected with AD169 at an MOI of 1, then treated with either 0.01M RO0504985 or the equivalent volume of DMSO at the time of infection. Cell lysates were prepared for western blotting at (A) 24-72 (hours post infection (h.p.i.)) or (B and C) 72 h.p.i.. Uninfected cells harvested at the time of infection are shown as 0 h.p.i..Proteins recognised by the antibodies used are indicated to the right of each figure. The positions of molecular mass markers (kDa) are indicated to the left of each figure. In Figure (A) the proteins that belong to the kinetic classes of immediate-early (IE), early (E) and late (L) protein production proteins are indicated. In Figures (B) and (C) a 2-fold dilution series of lysate from infected cells treated with DMSO is indicated above each Figure.

**TABLES**

**Table 1.** Anti-HCMV activity and cytotoxicity of quinolinyl-methylene-thiazolinone compounds.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | HCMV strain | EC501 | CC502 | SI3 |
| RO4600445 | AD169 | 2.6 +/- 1.1 | 8 | 3 |
| RO4569139 | AD169 | 2.6 +/- 0.5 | 10 | 4 |
| RO4509200 | AD169 | 1.3 +/- 0.5 | 9 | 7 |
| RO4915610 | AD169 | 1.0 +/- 0 | 8 | 8 |
| RO4554339 | AD169 | 1.0 +/- 0 | 8 | 8 |

1 50% Effective Dose (ED50). Data shown is the mean +/- standard deviation values (M) from three independent experiments.

2 50% Cytotoxic concentration (CC50). Data shown is the mean value from two independent experiments (M).

3Selectivity Index (SI): CC50/EC50

**Table 2.** Anti-HCMV activity and cytotoxicity of RO0504985 against different HCMV strains.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | HCMV strain | EC501 | CC502 | SI3 |
| RO0504985 | AD169 | 0.01+/- 0.005 | 10 | 1000 |
| RO0504985 | Merlin(RCMV1111) | 1.3 +/- 0.5 | - | 8 |

1 50% Effective Dose (ED50). Data shown is the mean +/- standard deviation values (M) from three independent experiments.

2 50% Cytotoxic concentration (CC50) (M). Data shown is the mean value from two independent experiments (M).

3Selectivity Index (SI): CC50/EC50

**SUPPLIMENTARY MATERIAL CAPTIONS**

**Supplementary Material.** Kinase inhibitor screening and screening data analysis.

**Supplementary Figure 1 Western blotting of HCMV infected cells treated with** quinolinyl-methylene-thiazolinone compounds**.** HFF cells were infected with AD169 at an MOI of 1, then treated with the compounds noted above the figure (1M) or the equivalent volume of DMSO at the time of infection. Cell lysates were prepared for western blotting at 72 hours post infection. Uninfected cell lysate prepared at the time of infection were also assayed. Proteins recognised by the antibodies used are indicated to the right of each figure. The positions of molecular mass markers (kDa) are indicated to the left of each figure.

**Supplementary Table S1.** List of compounds within the Roche Kinase Inhibitor library.

**Supplementary Table S2.** List of compounds judged to be cytotoxic in the screening process.

**Supplementary Table S3.** List of compounds assigned z-scores from analysis of screening data.