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Anti-orthopoxviral Activity of the 2-Cycloalkylimino-5-(4-Nitrophenyl)-1,3,4-Thiadiazines

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During this study, we synthesized a series of the 2-cycloalkylimino-5-(4-nitrophenyl)-1,3,4-thiadiazines of the general formula (I) wherein the group (N) represents: piperidino-, pyrrolidino-, methylpiperazino-, hexamethyleneimino-group. These derivatives were tested for cytotoxicity and antiviral activity against the orthopoxviruses: vaccinia, cowpox, mousepox, monkeypox in cell cultures. Some of the derivatives show antiviral activity which depended from type of viruses and from the structural features of the compounds. Thus, we find a new class of heterocyclic compounds with antiviral activity against the orthopoxviruses (Fig. 1).

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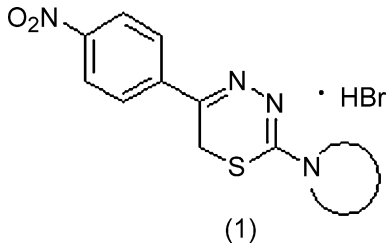

(1)

Fig. 1.

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Development of Novel Cell Lines for the Detection and Quantification of Herpesvirus Replication

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Background: Cell lines that conditionally express reporter molecules in response to infection with human herpesviruses (HHV) would facilitate detection and quantification of HHV, including fastidious or inhibitor resistant clinical isolates. Novel, indicator cell lines were developed that sensitively and specifically express renilla luciferase (rLuc) when infected with HHV.

Methods: Expression cassettes consisting of human herpesvirus 3 (HHV-3) late promoters driving the transcription of rLuc activity, were developed and confirmed by transient transfection experiments. Stable MeWo and U87 cell lines were selected that exhibited minimal basal but high induced levels of rLuc activity upon infection with either human herpesvirus 1 (HHV-1) or HHV-3 and weak activity when infected with human herpesvirus 5 (HHV-5). Detection, quantification, and antiviral susceptibility testing were performed by measuring rLuc levels in HHV infected stable cell lines.

Results: Infection of indicator cell lines with HHV-1 or HHV-3 resulted in high levels of rLuc expression as long as the parental cell line was permissive for infection (e.g. MeWo and U87 cells for HHV-1 and MeWo but not U87 cells for HHV-3). The ability of a MeWo indicator cell line to quantify antiviral activity against HHV-1 was also demonstrated. Titration of the novel compound, PF00558248, an HHV-3 polymerase inhibitor, yielded a 50% effective concentration of 0.4 μM, a value comparable to determinations in other antiviral assays.

Conclusion: A new cell system with conditional expression of reporter activity has been developed providing a new technology with which to detect and quantify HHVs and to study the susceptibility of HHVs to antiviral compounds.

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Discovery of Novel Small Molecule Inhibitors of Dengue Virus Replication

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There is an urgent need for new antivirals for both treatment and control of dengue virus, given that over 50 million people are infected worldwide with dengue every year, including 500,000 cases of the more severe form of the disease, dengue hemorrhagic fever (DHF) and there are no approved vaccines or antiviral drugs available. Vaccine development is promising but faces several significant challenges including the need to balance protection against all four serotypes of the virus equally in order to avoid antibody-dependent enhancement of infection and risk of DHF. An antiviral drug that inhibits viral replication without increasing the risk for ADE would be extremely valuable for both public health by providing a means to control outbreaks, as well as to government stockpiles for biodefense preparedness. The overall goal of the SIGA dengue program is to develop a small molecule therapeutic for the treatment and/or prevention of disease caused by dengue virus.

A sensitive and specific high throughput screening (HTS) assay has been developed to evaluate compounds from the SIGA chemical compound library for inhibitory activity against dengue-2 (DEN-2) virus replication. A CPE-based assay in a 96-well plate format was used to screen compounds at 5 μM for their ability to inhibit DEN-2 CPE. Hits have been identified that are potent (EC50 < 5 μM) and selective (CC50 > 5 μM), with initial structure activity relationship observed in several series. Early hits have structures that are chemically tractable, in that they possess chemically stable functionalities and have potential drug-like qualities. Further evaluation of selected compound series will be conducted to identify potential dengue virus antiviral drug candidates.

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