Antiviral Research 82 (2009) 1–11

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

GSK983: A novel compound with broad-spectrum antiviral activity

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ARTICLE INFO

Article history:
Received 21 October 2008
Received in revised form 8 December 2008
Accepted 18 December 2008

Keywords:
Novel antiviral
GSK983
Tetrahydrocarbazole
Interferon-stimulated genes

ABSTRACT

GSK983, a novel tetrahydrocarbazole, inhibits the replication of a variety of unrelated viruses in vitro with EC_{50} values of 5–20 nM. Both replication of the adenovirus Ad-5 and the polyoma virus SV-40, and episomal maintenance of human papillomaviruses (HPV) and Epstein-Barr virus (EBV) are susceptible to GSK983. The compound does not inhibit all viruses; herpes simplex virus (HSV-1), human immunodeficiency virus (HIV), and lytic replication of EBV were not susceptible at concentrations below 1 μM. GSK983 does inhibit the growth of cell lines immortalized by HTLV-1, EBV, HPV, SV40 and Ad-5, with EC_{50} values in the range of 10–40 nM. Depending on the cell line, the compound induces either apoptosis or cytostasis at concentrations over 20 nM. GSK983 also inhibits cell lines immortalized by non-viral mechanisms, but has little effect on primary cells. The CC_{50} values for keratinocytes, fibroblasts, lymphocytes, endothelial, and bone marrow progenitor cells are all above 10 μM. The pattern of inhibition, which includes diverse viruses as well as growth of immortalized cells of varied origins, suggests the target is a host cell protein, rather than a viral protein. Preliminary mechanism studies indicate that GSK983 acts by inducing a subset of interferon-stimulated genes.

1. Introduction

Tremendous progress has been made in treating viral infections over recent decades. Success stories include acyclovir and its prodrug for herpes simplex virus (HSV), two neuraminidase inhibitors for influenza, five antiviral drugs for hepatitis B (HBV), and almost two-dozen human immunodeficiency virus (HIV) drugs. Despite these significant advances, however, many common viral infections still do not have safe and effective treatments. For example, there are no antiviral drugs for either the Dengue virus, which causes 50–100 million cases of Dengue fever a year (Morens and Fauci, 2008), or the oncogenic human papillomaviruses, which cause cervical cancer and have a prevalence of 26.8% in U.S. women (Dunne et al., 2007).

Most of the success stories in the discovery and development of new antiviral drugs have been achieved by focusing on viral targets. The drawback to this approach is that, unless viruses are closely related, separate drug discovery and development efforts are required for each virus. In some cases, multiple inhibitors are needed in combination to combat the rapid development of resistance. This is clearly the case with HIV, and it is expected to be the case with hepatitis C (HCV) (Grunberger et al., 2008). Inhibiting pathogen targets still has great value and will clearly continue to yield beneficial new drugs, but use of host targets in antiviral therapy is growing. For example, one of the newest classes of HIV inhibitors, the CCR5 antagonists, targets the cell surface receptors and blocks entry of the virus (Schlect et al., 2008).

The allure of host targets is the potential to identify and develop broad-spectrum antiviral agents. This is particularly attractive for emerging infections, such as the 2002–2003 SARS coronavirus outbreak. In such cases, a broad-spectrum agent could allow for effective treatment without the lead time necessary to develop a virus-specific therapy. A broad-spectrum inhibitor would also be welcome in the treatment of niche indications, such as progressive multifocal leukoencephalopathy (PML) caused by uncontrolled JC virus infection in the central nervous system of immune-compromised patients. In such cases, medical need is acute, but the patient population is not large enough to provide an economic incentive for development of a virus-specific drug.

Currently, many health care providers use interferons or ribavirin off-label to treat serious viral infections with no approved therapy. These drugs provide benefit in some cases, but the efficacy is typically quite modest, and there are numerous side effects, many of which are serious, associated with use of these drugs (Leyssen et al., 2008; Borden et al., 2007). For details of the adverse events associated with interferon and ribavirin use, see

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doi:10.1016/j.antiviral.2008.12.015

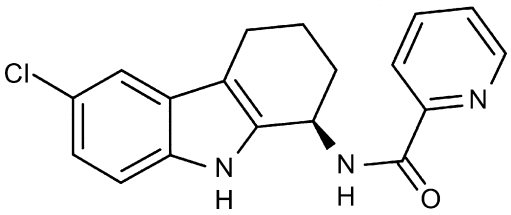

Fig. 1. Structure of GSK983.

http://www.fda.gov/cder/foi/label/2007/103964s5120lbl.pdf and http://www.fda.gov/cder/foi/label/2007/021511s014lbl.pdf.

This manuscript describes a novel antiviral compound, the tetrahydrocarbazole GSK983. It has a unique profile, with initial data that suggest broad-spectrum antiviral activity via a host target. This manuscript describes the antiviral data in a number of in vitro viral replication systems, and it discusses the implications of preliminary mechanism of action studies.

2. Materials and methods

2.1. Compounds

N-[(1*R*)-6-Chloro-2,3,4,9-tetrahydro-1*H*-carbazol-1-yl]-2-pyridinecarboxamide (GSK983, Fig. 1) and its enantiomer GSK984 were synthesized by GlaxoSmithKline, Research Triangle Park, NC (Boggs et al., 2007). The molecular weight of GSK983 is 326. Stock solutions and serial dilutions were prepared in dimethyl sulfoxide (DMSO). In all experiments equal volumes of the serial dilutions were added to growth medium so that all final solutions, including the untreated controls, contained the same concentration of DMSO of ≤1% (v/v).

2.2. Cells and cell lines

The details of the cells and cell lines used in this study and the growth media used for their culture can be found in Supplementary Materials. All are of human origin except for the Vero cell line. Some cell types were prepared in the laboratory as described below.

Peripheral blood mononuclear cells (PBMCs) were prepared from buffy coats as previously described (Ferris et al., 2005).

B cells and CD4 T cells were prepared from human blood obtained with informed consent by negative selection using the StemCell Technologies RosetteSep technique, according to the manufacturer's protocols. Purity of the preparations was 90–95% for B cells and 97% for CD4 cells. B cells were activated by addition of 1 μg/ml soluble CD40 ligand (R&D Systems #617-CL), interleukin-4, 20 ng/ml (R&D Systems #204-IL), and 1 nM phorbol 12-myristate 13-acetate (PMA; Sigma #P-1585). PBMCs and CD4s were activated with phytohemagglutinin and IL2 as previously described (Ferris et al., 2005). In all cases, the cells doubled 3–4 times within 96 h after stimulation.

B-LCL 5/2/1 is a mixed population of B lymphoblastoid cells prepared by infection of PBMCs with the B95.8 strain of EBV as described by Frisan et al. (2001).

W12 medium (Stanley et al., 1989), DMEM, MEM and RPMI 1640 growth media were obtained from Invitrogen (Carlsbad, CA). Eagle MEM was obtained from ATCC. KGM and EGM MV media were obtained from Cambrex (East Rutherford, NJ). Unless otherwise specified, each medium contained 10% (v/v) of fetal bovine serum and either gentamicin (10 μg/ml) or penicillin (100 IU/ml) plus streptomycin (100 μg/ml). All cultures were grown at 37 °C in an atmosphere containing 5% CO_2.

Bone marrow toxicity assays were run by Cambrex Bio Science Walkersville, Inc. (East Rutherford, NJ) using the methods described by Eaves (1995).

2.3. Measurement of cell growth and viability

Most measurements employed the MTS assay (Barltrop et al., 1991; Cory et al., 1991) in which the reduction by cells of 3-(4,5-dimethylthythiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) is measured. CellTiter 96 Aqueous MTS reagent Powder was purchased from Promega and phenazine methosulfate was purchased from Sigma. The suggested protocol from Promega was followed. After a 2 h incubation of cells and reagent at 37 °C and 5% CO_2, the absorbance was read at 490 nm (A_{490}) using a Wallac Victor 1420 Multilabel plate reader. It has been shown that the net A_{490} is proportional to the number (and thus the mass) of viable cells (Cory et al., 1991). We have verified this for W12-20850 cells (data not shown).

In some experiments, sensitivity and dynamic range were increased by carrying out kinetic measurements of A_{490} for 2 h after addition of reagent using a Tecan Spectrafluor Plus plate reader with the plate maintained at 35 °C. A_{490} per hour was then calculated by linear regression of the linear portion of the curve. In other experiments, cell growth and viability were estimated from cellular ATP measurements using the CellTiter-Glo Luminescent Cell Viability Assay (CTG, Promega) according to the manufacturer's protocol. Luminescence was measured using a Wallac 1420 Victor plate reader.

2.4. Adenovirus assay

HFF cells were plated in 96 well plates at 2.5×10^4 cells/well. Sixteen hours after plating, the cells were infected with adenovirus type 5 (ATCC VR-1516) at an MOI of 3 for 2 h. Virus was removed from the cells and 200 μl of fresh medium containing compounds at concentrations ranging from 100 to 0.003 μM were added to each well. After further incubation for 3 days, cell culture fluid was removed and cells rinsed with PBS twice. Intracellular adenovirus DNA from HFFs was quantified by qPCR as described below.

2.5. SV40 assay

Vero cells were plated in 96 well plates at 2×10^4 cells/well. Cells were allowed to adhere to the wells for 4 h and then were infected with SV40 strain Pa-57 (ATCC VR-239) at an MOI of 0.1 for 2 h. Supernatant was removed from the cells and diluted compounds ranging from 100 to 0.003 μM were added. After incubation for 3 days, cells were assayed for growth by MTS and for SV40 DNA by qPCR as described below.

2.6. Hybrid capture assay for HPV16 DNA

W12-20850 cells were seeded at 7.5×10^3 cells/well into a 96 well plate-containing compound at concentrations ranging from 100 to 0.001 μM. Plates were incubated at 37 °C in the presence of 5% CO_2 for 4 days. Cells were lysed in 0.2N NaOH, 1% Igepal, and episomal HPV-16 DNA was quantified using a non-radioactive hybrid capture technique with HPV-16 specific capture and detection probes. Hybridization plates (Nunc Maxisorb 96 well Cat #450320) were coated with a mixture of capture probe and ReactiBind solution (Pierce #17250) for at least 4 h and then washed with 0.2× SSC, 0.05% Tween20 (SSC/T) prior to blocking with 150 μl/well of 0.2N NaOH, 1% Igepal, 10 mg/ml herring sperm DNA for 6–8 h. The hybridization was carried out by mixing 27 μl of lysed cells with 45 μl of denatured detection probe in 6 M guanidine isothiocyanate. To prevent evaporation, 50 μl of mineral oil was added to each well. The plate was then heated to 90 °C for 6.5 min, and the hybridization continued at 42 °C overnight. Assay plates were washed 6 times with SSC/T. Anti-digoxigenin HRP-conjugated antibody (Boehringer Mannheim 1207733, 1:5000) was