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Nucleotide Analogs that Induce Cellular Production of Antimicrobial Proteins

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Innate immunity plays a significant role in the antimicrobial defense against viruses, as well as, prokaryotic and eukaryotic pathogens. Cell-mediated immune response to viral infections occurs through cellular pathogen recognition receptors (PRRs) that bind to pathogen-associated molecular patterns (PAMPs) and activation of intracellular signaling pathways. Recently, peptides homologous to beta chain of hemoglobin (Hb-beta) have been reported to play an important role in the cellular antimicrobial defense. The Hb-beta, also called extra-erythrocytically expressed hemoglobin [EEEH], is produced by macrophages, alveolar epithelium, and vaginal tissues. Interestingly, AMPs homologous to EEEH are known to be upregulated in the gills and skin epithelium of fish and have potent antiparasitic and antibacterial activity. We have recently discovered that the orally bioavailable dinucleotide compound SB 44 (a prodrug of the anti-HBV compound SB 40) induces increase in plasma EEEH in HBV transgenic mice which correlated with its anti-HBV activity. Thus, for example, orally administered SB 44 at 1, 5, 10, and 100 mg/kg showed a dose-dependent increase in plasma EEEH that correlated with dose-dependent reduction of liver HBV DNA. The sublingual administration of a polyether formulation of the dinucleotide – SB 40 – also caused sustained increase in plasma EEEH in woodchucks. The presence of EEEH with a MW of 15 kDa was confirmed by SDS gel electrophoresis followed by proteomic analysis. We therefore hypothesize that a component of the antiviral action of the dinucleotides SB 40 and SB 44 involves the upregulation of the EEEH that is mediated through the host innate immune system. Furthermore, we believe that EEEH is a potential biomarker that indicates the presence of an active innate immune defense against viral infections.

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Synthesis and Antiviral Evaluation of 3-(2,3-Dihydroxypropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-ones

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Bicyclic nucleoside analogues (BCNAs) are potent and selective inhibitors of varicella-zoster virus (VZV) replication. The antiviral compound Cf 1743 is one of the most potent and selective antiviral agents and its orally bioavailable prodrug FV100 (5′-valine ester of Cf 1743) is currently in Phase II clinical development for the treatment of herpes zoster (shingles). SAR studies of the BCNAs showed that sugar modifications are not well tolerated for maintaining potent anti-VZV activity. Thus, while 2′-deoxyribosides are potent and selective anti-VZV agents, their ribo- and arabino-analogues are significantly less active. Furthermore, 2′,3′-dideoxy analogues and N-3 alkyl derivatives showed poor activity against VZV but they surprisingly exhibited activity against human cytomegalovirus (HCMV) infection. Replacement of the sugar at the N-3 position of the BCNAs by the (2-hydroxyethoxy)methyl group (as in the antiherpes drug acyclovir) afforded compounds with weak activity against both VZV and HCMV. Although phosphorylation of the BCNAs by the VZV-encoded thymidine kinase (TK) is a prerequisite for their anti-VZV activity, acyclic nucleotide analogues (2-phosphonomethoxyethyl derivatives) of the furo[2,3-*d*]pyrimidin-2(3*H*)-ones were not active at sub-toxic concentrations. To help further elucidate the mechanism of antiviral action of the BCNAs, novel 3-(2,3-dihydroxypropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-ones were synthesized. The target compounds were prepared by the Sonogashira coupling of various 1-alkynes with 1-(2,3-dihydroxypropyl)-5-iodouracil, followed by in situ Cu(I)-promoted intramolecular cyclization. The activity of these compounds against VZV and HCMV will be reported.

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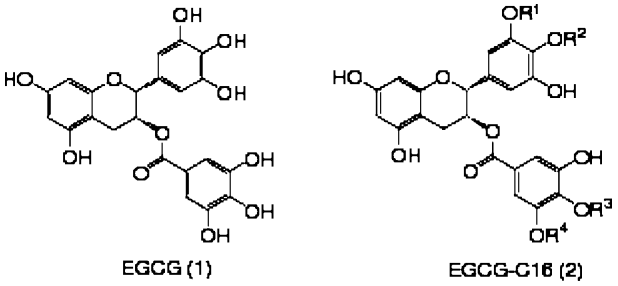

Fig. 1. Chemical structure of EGCG (**1**) and EGCG-C16 (**2**). **2** is a mixture of four regio-isomers (**2a–d**). **2a**: R^2=R^3=R^4=H, R^1=$CO(CH)_{14}CH_3$; **2b**: R^1=R^3=R^4=H, R^2=$CO(CH)_{14}CH_3$; **2c**: R^1=R^2=R^4=H, R^3=$CO(CH)_{14}CH_3$; **2d**: R^1=R^2=R^3=H, R^4=$CO(CH)_{14}CH_3$. The ratio of each regio-isomer **2a**:**2b**:**2c**:**2d** is 38:35:7:20, respectively.

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Combating Drug-resistant Influenza Viruses with Novel Green Tea Catechin Derivatives

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Seasonal influenza epidemic and pandemic outbreaks cause significant disease burdens and mortality in humans. The 1918 Spanish flu pandemic was caused by an influenza A (H1N1) virus and resulted in the death of millions of people worldwide. With the ever-present threat of an influenza pandemic, antiviral compounds are in great demand, yet such drugs for the treatment and prophylaxis of influenza are limited. A neuraminidase (NA) inhibitor, oseltamivir phosphate, is the most commonly used antiviral drug. However, reports found that some new seasonal influenza viruses possess resistance to oseltamivir phosphate.

(−)-Epigallocatechin-3-*O*-gallate (EGCG; **1**), a major green tea component (*Camellia sinensis*), has been recognized to possess antiviral activities. Recently, we have reported a method to synthesize EGCG-fatty acid monoesters using lipase-catalyzed transesterification and confirmed that EGCG-fatty acids showed improved influenza virus inhibitory effect in an alkyl length dependent manner (Mori et al., 2008).

Here we studied the anti-influenza virus activity of EGCG (**1**, Fig. 1) and EGCG-C16 (**2**, Fig. 1) on drug-resistant influenza A (H1N1)