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Synthesis and biological evaluation of helioxanthin analogues

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ABSTRACT

Helioxanthin and analogues have been demonstrated to suppress gene expression of human hepatitis B virus. In the continuous attempt to optimize antiviral activity, various structural motifs were grafted on the helioxanthin scaffold. Many such analogues were synthesized and evaluated for their anti-hepatitis B virus activity. Structure–activity relationships of these helioxanthin derivatives are also discussed. Among these new compounds, **15** exhibits the highest activity against HBV (EC_{50} = 0.06 μM). This compound can suppress viral surface antigen and DNA expression. Furthermore, viral RNA is also diminished while the core promoter is deactivated upon treatment by **15**. A plausible working mechanism is postulated. Our results establish helioxanthin lignans as potent anti-HBV agents with unique mode of action. Since their antiviral mechanism is distinct from current nucleoside/nucleotide drugs, helioxanthin lignans constitute a potentially new class of anti-HBV agents for combination therapy.

1. Introduction

The hepatitis B virus (HBV) is a major world health problem. According to the world health report released by WHO in 1997, at least 300 million of are chronically infected with HBV despite the availability of an effective vaccine.[1] The majority of cases occur in developing country in which nearly 10% of the population are infected. Long term HBV infection can result in acute and fulminant liver failure. Many chronic patients latter develop liver cirrhosis and eventually hepatocellular carcinomas. It has been estimated that HBV-related complications cause 600,000–1.2 million deaths every year. No therapeutic strategy that can completely eradicate HBV from the host is hitherto available. Yet, to diminish virus replication is still crucial for the patients, since it would not only prevent further infection but also attenuated inflammation response to viral expression.

Knowledge of the viral life cycle and pathogenesis can be an important guide in the development of effective therapies. The infection of HBV begins with the internalization viral DNA which is a circular 3.2 kb genome composed of a partially double-stranded DNA.[2] Its genome encodes for four main genes by a series of overlapping reading frames. The core gene encodes for core antigen (HBcAg) and the precore gene encodes for the e antigen (HBeAg). The three envelope genes PreS1, PreS2 and S encode for the large, middle, and small envelope proteins, respectively. Finally, the polymerase gene encodes for the multifunctional polymerase, and the X gene encodes for the X protein. After translocated into the hepatocyte nucleus, the genome is converted into covalently closed circle DNA [cccDNA] which serves as the template for the transcription of all viral mRNA. During the subsequent replication, the mRNA not only serves as the template for reverse transcription but also encodes the viral core protein and the HBV polymerase. In the cytoplasm, the mRNA is translated to make viral proteins. After encapsulation and maturation, the viral particles are released from the infected cells.

Interferon (INF) treatment is the first approved therapy for HBV related liver disease.[3] This class of cytokine can bind to receptors on the hepatocyte membrane and trigger a cascade of intracellular immune response to combat HBV proliferation.[4] However, the incidence of adverse effect in INF treatment, ranging from fatigue to decrease in platelets, makes it unsuitable for some patients. In addition, INFs need to be administrated by weekly injection which diminishes its practicality in area with limited medical resources. Recently, several nucleoside and nucleotide analogues were launched to treat chronic HBV infection. This class of compound suppresses HBV reproduction by inhibiting the viral polymerase.[5] Lamivudine[6] [(−)-L-2′,3′-dideoxy-3′-thiacytidine] is the first nucleotide analogue licensed for chronic hepatitis B infection. Since then, adefovir[7,8] [PMEA, 9-(2-phosphonylmethoxyethyl)adenine], entecavir[9] (BMS-200475, carbocyclic 2′-deoxyguanosine), telbivudine[10] (synthetic L-thymidine nucleoside analogue), and levudine[11] [L-FMAU, 1-[(2*S*,3*R*,4*S*,5*S*)-3-fluoro-4-hydroxy-5-(hydroxymethyl) oxolan-2-yl]-5-methyl-pyri-midine-2,4-dione] were also approved for clinical use. Unfortunately, drug resistant mutant often emerge

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when the target of the drug is polymerase. Indeed, resistant and cross-resistant strand against lamivudine (3TC) and adefovir appeared after only one to 2 years of treatment.[12,13] To overcome the drug-resistance problem, it is essential to develop non-nucleotide drugs that can block viral replication at a different stage during its life cycle. One successful example of this strategy is the heteroarylpyrimidine Bay 41-4109 reported by Deres and Schroder.[14] The authors demonstrated that this drug impedes HBV replication by inhibiting the assembly of nucleocapsids. Such approach not only opens unique opportunities in anti-HBV research, but may also provide new insights to the development of antiviral agent in general (Fig. 1).

Natural products constitute an inexhaustible source of inspiration and challenge for many disciplines in science. For medicinal chemistry, natural products provide instrumental lead structures that can be developed into useful pharmacological targets against pathogenic agents. Arylnaphthalene lignans are a subclass of botanical lignans. Their basic structural feature is a naphthalene core with an aryl group at 1 position. A diverse range of medicinal potentials, including antitumor[15–17] and antiviral activities,[18] were discovered for this class of compounds. Helioxanthin is an arylnaphthalene lactone lignan isolated from *Taiwania cryptomerioides* Hayata[19] and *Heliopsis scabra* Dunal.[20] It was found helioxanthin and its derivatives can inhibit HBV production in various strand of HBV infected cellular models with sub-micromolar concentration.[21,22] We have also reported helioxanthin can effectively inhibit HBV gene expression and replication in 3TC-resistance strand of human hepatoacyte.[23] Many helioxanthin congeners were synthesized and two derivative, lactam **5-4-2** and phthalazin dione **8-1**, are even more potent than the parent compound against HBV.[24] Furthermore, we have verified that such compounds most likely target the viral mRNA production machinery instead of the polymerase. With this antiviral mechanism, relapse associated with drug resistance is much less likely. In our previous study, we have synthesized a wide range of helioxanthin analogues and tested their anti-HBV activities. A preliminary structure–activity relation (SAR) model was also proposed.[23] In our ongoing venture to discover new anti-HBV agents and investigate their mechanism, a new series of nearly 40 helioxanthin derivatives were synthesized and their anti-HBV activities assessed. Here, we report the synthetic strategy and the results of anti-HBV testing. A new compound with potency exceeding that of the parent helioxanthin was revealed. We also put forward a refined SAR model to guide future investigation.

2. Results

2.1. Synthesis of helioxanthin analogues

In our effort to explore various anti-HBV agents, we constantly go back helioxanthin analogues for inspiration. Our strategy is to identify essential structural motifs that enable a helioxanthin analogue to block HBV production. These key structural elements will then be combined on the arylnaphthalene scaffold to produce more potent helioxanthin derivatives. In our earlier studies, we have discovered a few compounds with comparable antiviral activity to the parent compound.[23] The present study is an extension of our previous effort with similar approach.

There are several different synthetic strategies to arylnaphthalene lignans in the literature, including the dimerization of dehydro-cinnamate,[25,26] Pd-catalyzed benzannulation,[27] and Diels–Alder approach.[28] Although each methodology has its merits, we found the Diels–Alder approach developed by Charlton has the widest substrate scope and therefore enables us to generate a library of derivatives in a few routine steps. The synthesis (Scheme 1) starts with piperonal. The aldehyde was first protected as acetal before *ortho*-metallation was carried out with *n*-butyl lithium. The aryl lithium thus generated can react with various aldehydes to furnish the hydroxyacetal **2**. When heated in acetic acid solution, **2** was converted into bezo[*c*]furan in situ to react with maleic anhydride in the key Diels–Alder cycloaddition. The adduct underwent spontaneous aromatization under the reaction condition to give **3** which was then reduced to give the lactone helioxanthin **4** (separated from the regioisomer retro-**4**). When we substituted fumaronitrile for maleic anhydride as the dienophile, dinitrile **5** is produced instead (Scheme 2).

By employing various aldehydes after the lithiation step, we synthesized helioxanthin analogues of which the [1,3]dioxole rings on the aryl moiety were opened. Lactones (**12**, **17**, **22**, **26** and **29**) and their regioisomers (**13**, **18**, and **23**) were prepared as described previously.[23] Phenolic hydroxyl groups were integrated as benzyl protected ether and deprotected after the anhydrides were reduced to lactones. Compounds **14** and **19** thus produced are acetylated to give **15** and **20**, respectively. **16**, **21**, and **25** were

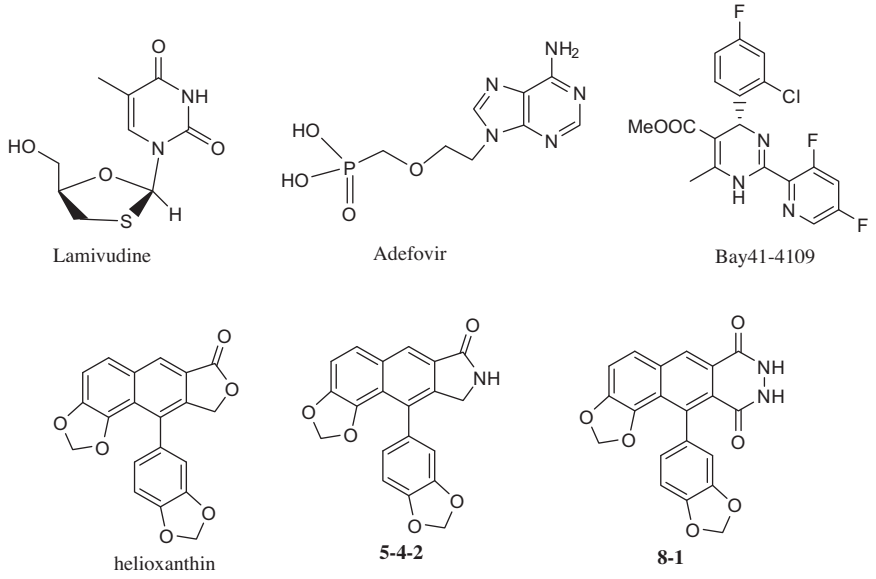

Figure 1. Examples of anti-HBV compounds.