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Research paper

Synthesis of novel diflunisal hydrazide–hydrazones as anti-hepatitis C virus agents and hepatocellular carcinoma inhibitors☆

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ABSTRACT

Hepatitis C virus (HCV) infection is a main cause of chronic liver disease, leading to liver cirrhosis and hepatocellular carcinoma (HCC). The objective of our research was to develop effective agents against viral replication. We have previously identified the hydrazide–hydrazone scaffold as a promising hepatitis C virus (HCV) and hepatocelluler inhibitor. Herein we describe the design a number of 2′,4′-difluoro-4-hydroxy-*N*′-(arylmethylidene) biphenyl-3-carbohydrazide **(3a-t)** as anti-HCV and anticancer agents. Results from evaluation of anti-HCV activity indicated that most of the synthesized hydrazone derivatives inhibited viral replication in the Huh7/Rep-Feo1b and Huh 7.5-FGR-JCI-Rluc2A reporter systems. Antiproliferative activities of increasing concentrations of 2′,4′-difluoro-4-hydroxy-*N*′-(2-pyridyl methylidene)biphenyl-3-carbohydrazide **3b** and diflunisal (2.5–40 μM) were assessed in liver cancer cell lines (Huh7, HepG2, Hep3B, Mahlavu, FOCUS and SNU-475) with sulforhodamine B assay for 72 h. Compound **3b** with 2-pyridinyl group in the hydrazone part exhibited promising cytotoxic activity against all cell lines with IC_{50} values of 10, 10.34 16.21 4.74, 9.29 and 8.33 μM for Huh7, HepG2, Hep3B, Mahlavu, FOCUS and SNU-475 cells, respectively, and produced dramatic cell cycle arrest at SubG1/G0 phase as an indicator of apoptotic cell death induction.

1. Introduction

Hepatitis C virus (HCV) is an enveloped virus that is classified in the hepacivirus genus of the *Flaviviridae* family [1]. The virus RNA genome encodes a polyprotein, which is posttranslationally processed by host and virus proteases into 10 mature proteins, of which 4 are structural proteins (C, E1, E2, and p7) and 6 nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2]. HCV is a worldwide infectious pathogen that causes chronic liver diseases, including hepatic fibrosis, hepatic cirrhosis and hepatocellular carcinoma (HCC) [3]. Until recently, HCV-infected patients were treated with a combination of pegylated interferon-α (IFN-α) and the nucleoside analog ribavirin. However, this therapy had limited effectiveness especially in context of patients infected with HCV genotype 1. Furthermore, treatment with interferon is associated with numerous side effects. Recently, new anti-HCV therapies utilizing the direct acting antivirals (DAAs) against the viral proteins HCV NS3-4A protease and NS5B polymerase have been approved. These therapy although more promising have complicated dosing regimens limiting patient compliance [4–7]. Further, the selection of HCV drug resistant variants continues to remain a concern [8,9]. On the other hand, acute and chronic liver diseases that are caused by an infection with hepatitis-C virus (HCV), such as hepatocellular carcinoma and liver cirrhosis have received much attention over the past decade. Recently, HCV is believed to act as carcinogen by virtue of the increased risk of hepatocellular carcinoma among persistently infected patients with chronic active hepatitis. Therefore, it is important to develop new, safer and even more effective agents against HCV infection and resistance emergence.

Diflunisal derivatives [10–14] (Fig. 1) have been reported to possess diverse biological activities such as anticancer, anti-HCV,

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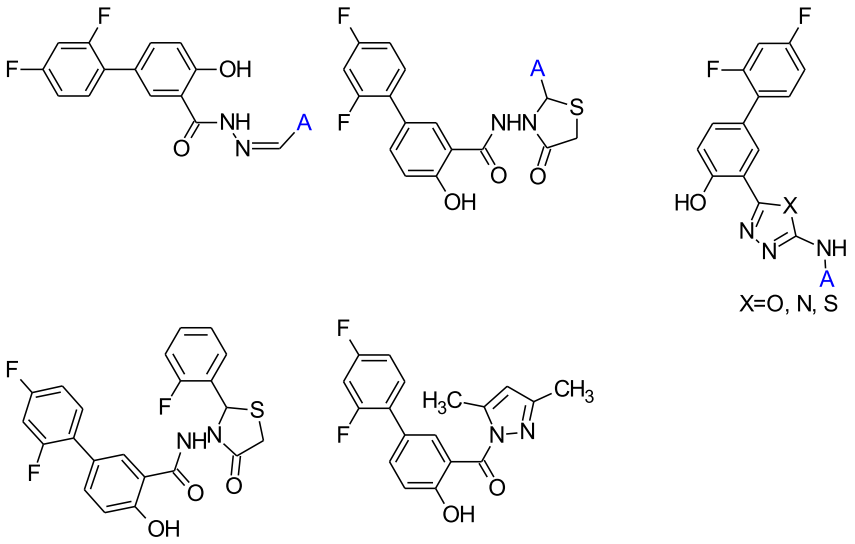


Fig. 1. Diflunisal derivatives.

anticonvulsant, antimicrobial and anti-inflammatory properties [15]. In medicinal chemistry, the presence of a hydrazide–hydrazone group in compounds, has usually led to the development of clinically relevant biological molecules with antimicrobial, anticancer [16,17] and antiviral properties [18].

Recently, our group reported the synthesis of novel hydrazide–hydrazone derivatives and their HCV NS5B inhibition effects [19] (Fig. 2).

Diflunisal is a difluorophenyl derivative of salicylic acid and a non-steroidal drug with analgesic, anti-inflammatory and antipyretic properties. It is a peripherally-acting non-narcotic analgesic drug which functions as a prostaglandin synthase inhibitor. In animals, prostaglandins sensitize afferent nerves and potentiate the action of bradykinin in inducing pain. Since prostaglandins are known to be among the mediators of pain and inflammation, the mode of action of diflunisal may be due to a decrease of prostaglandins in peripheral tissues.

Herein, we report our ongoing efforts towards development of more effective anti-HCV agents. We focused our attention on the hydrazide–hydrazone moiety. Thus, a new series of hydrazide–hydrazone derivatives were synthesized from diflunisal and evaluated for their anti-HCV activity *in vitro* and anticancer activity against hepatocellular cancer cell lines.

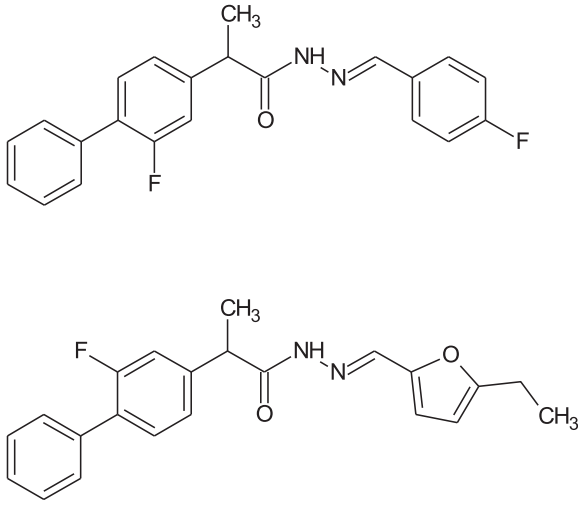


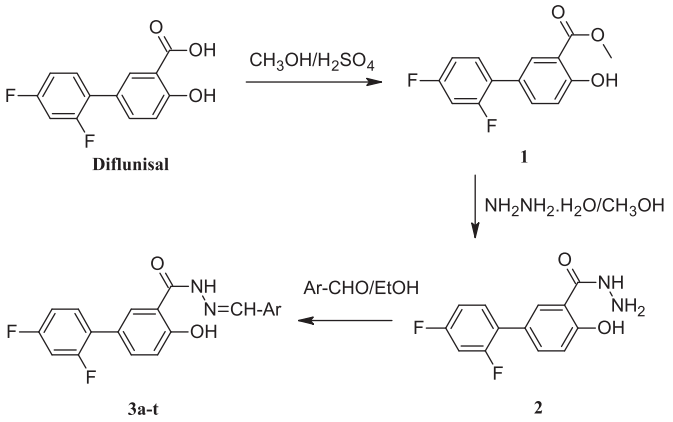
Fig. 2. Hydrazones of anti-HCV activity.

2. Results and discussion

2.1. Chemistry

Methyl 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylate [1] was prepared by the reaction of diflunisal and methanol in the presence of a few drops of concentrated sulfuric acid. 2′,4′-Difluoro-4-hydroxybiphenyl-3-carboxylic acid hydrazide [2] was prepared by heating hydrazine-hydrate and [1] in methanol [10]. After condensing hydrazide with substituted aldehydes in ethanol, novel 2′,4'-difluoro-4-hydroxy-*N*′-(arylmethylidene) biphenyl-3-carbo hydrazide [**3a-t**] were obtained. The synthesis of novel series of hydrazide–hydrazones **3a-t** was performed as outlined in Scheme 1. All synthesized compounds were checked for purity using TLC and HPLC-UV/DAD and were characterized by their melting points, ^{1}H NMR, ^{13}C NMR and HR-MS spectral data.

The FT-IR spectra of hydrazones showed absorption bands at 1583–1614 cm^{-1} due to C=N groups. Moreover, C=O absorption



Scheme 1. The synthetic route for the preparation of the target compounds **(3a-t)**.