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Original article

Synthesis and SAR optimization of diketo acid pharmacophore for HCV NS5B polymerase inhibition

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ABSTRACT

Hepatitis C virus (HCV) NS5B polymerase is a key target for anti-HCV therapeutics development. Here we report the synthesis and biological evaluation of a new series of α,γ-diketo acids (DKAs) as NS5B polymerase inhibitors. We initiated structure–activity relationship (SAR) optimization around the furan moiety of compound **1a** [IC_{50} = 21.8 μM] to achieve more active NS5B inhibitors. This yielded compound **3a** [IC_{50} = 8.2 μM] bearing the 5-bromobenzofuran-2-yl moiety, the first promising lead compound of the series. Varying the furan moiety with thiophene, thiazole and indazole moieties resulted in compound **11a** [IC_{50} = 7.5 μM] bearing 3-methylthiophen-2-yl moiety. Finally replacement of the thiophene ring with a bioisosteric phenyl ring further improved the inhibitory activity as seen in compounds **21a** [IC_{50} = 5.2 μM] and **24a** [IC_{50} = 2.4 μM]. Binding mode of compound **24a** using glide docking within the active site of NS5B polymerase will form the basis for future SAR optimization.

1. Introduction

Hepatitis C virus (HCV) infection has emerged as a significant global public health problem. HCV is responsible for the development of malignant chronic liver disease, including liver cirrhosis and hepatocellular carcinoma, which frequently ends in liver failure [1–4]. An estimated 200 million cases of HCV infections exist worldwide, of which over 4.1 million are in the United States [5]. Despite its large medical and economic impact, neither a vaccine nor a therapy with effective broad spectrum mode of action against all genotypes of HCV is available [6–8]. Current HCV therapy comprising of pegylated interferon α (PEG–IFN-α) in combination with ribavirin has found limited patient compliance due to severe adverse effects [9,10]. Therefore, there is an urgent need to develop novel and efficacious antiviral therapeutics targeting HCV.

The HCV non-structural protein 5B (NS5B), a 66 kDa RNA-dependent RNA polymerase (RdRp), is an attractive therapeutic target, since it plays an important role in replicating the HCV RNA genome and the host lacks its functional equivalent [11–13]. The crystal structure of HCV NS5B polymerase reveals a classical "right hand" shape with characteristic fingers, palm and thumb subdomains. The combination of crystallographic, biochemical and mutagenesis studies has facilitated the identification of five distinct non-nucleoside inhibitor (NNI) binding sites on NS5B [14–17]. Allosteric pockets, AP-1 and AP-2 are located in the thumb subdomain, whereas AP-3 and AP-4 are partially overlapped and located in the palm subdomain, in close proximity to the active site, and AP-5 is located in the fingers subdomain [17–20]. The NS5B polymerase active site is located in the palm subdomain, which contains two Mg^{2+} ions that are coordinated by conserved aspartic acid residues Asp220 and Asp318 [18]. The Mg^{2+} ions serve the dual role of positioning/stabilizing the pyrophosphate leaving group on the incoming nucleoside triphosphate (NTP) and activating the 3′-OH of the growing RNA for nucleophilic attack on the α-phosphate group of NTP [14,15].

Derivatives of 4,5-dihydroxypyrimidine carboxylic acid [19] and α,γ-diketo acids (DKAs) have been reported previously as pyrophosphate (PPi) mimetic inhibitors of HIV-1 IN [20] and HCV NS5B polymerase [19,21]. These compounds function as product-like analogs and chelate the divalent metal ions at the active site of NS5B [22,23]. In continuation of our efforts toward identification and development of NS5B PPi mimetic inhibitors [24], we have explored the effect of various substituted aryl/heteroaryl specificity domain of DKAs on NS5B inhibition. Herein we

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describe the synthesis, SAR, and molecular modeling of optimized DKA analogs.

2. Results and discussion

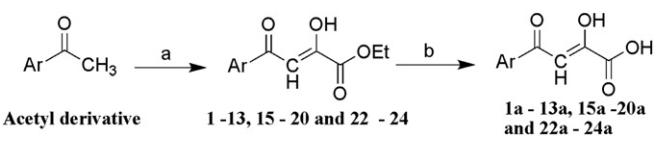
2.1. Chemistry

The α,γ-diketo esters (**1**–**13**, **15**–**20** and **22**–**24**) were synthesized by Claisen condensation of appropriate acetyl derivative with diethyl oxalate in the presence of freshly prepared sodium ethoxide. The resulting α,γ-diketo esters were readily hydrolyzed by treating with 1N sodium hydroxide to corresponding DKAs (**1a**–**13a**, **15a**–**20a** and **22a**–**24a**) (Scheme 1) [25]. Target compound **14a** was prepared in good yields from the 1*H*-indazole-3-carboxylic acid as shown in Scheme 2. The 3-acetyl-1*H*-indazole intermediate was synthesized from 1*H*-indazole-3-carboxylic acid by initial conversion into Winreb amide using the one-pot reaction: mixed anhydride method followed by nucleophilic attack of *N*,*O*-dimethylhydroxylamine hydrochloride. The Winreb amide thus formed was further treated with methyl magnesium bromide to yield 3-acetyl-1*H*-indazole [26]. The 3-acetyl-1*H*-indazole was converted to α,γ-diketo ester **14** and further converted to compound **14a** as described above. Target compound **21a** was prepared from **20a** by reacting it with sodium azide in the presence of the catalytic amount of ammonium chloride (Scheme 3) [27].

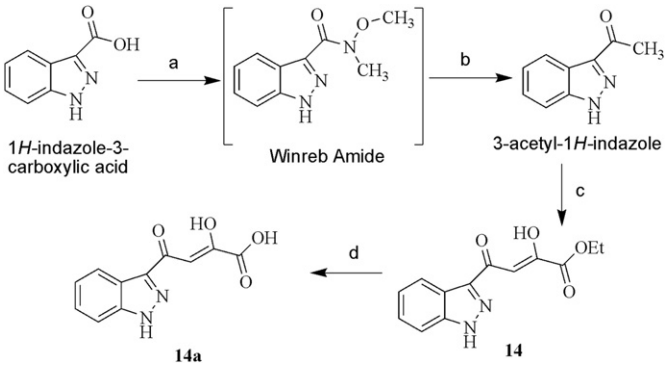
2.2. Structure–activity relationship

The HCV NS5B RdRp inhibitory activity of DKAs is reported in Table 1. All the DKAs exhibited appreciable inhibition of HCV NS5B polymerase, with IC_{50} values ranging from 2.4 μM to 63.9 μM. Having identified compound **1a**, a modest NS5B inhibitor as the initial hit, we sought to explore SAR around the aryl/heteroaryl specificity domain while keeping the DKA metal chelating pharmacophore constant. Replacing furan moiety with benzofuran led to compound **2a** which was marginally less active. Since the benzofuran ring is in conjugation with the γ-keto group, we expected that the electron withdrawing bromo group would increase the acidity of the diketo moiety and its ionized enolic form would be more suitable for strong metal chelation, thereby resulting in a relatively more active inhibitor. Consistent with this hypothesis, compound **3a** bearing 5-bromo substitution on the benzofuran ring exhibited ~3.5-fold improvement in inhibitory activity over compound **2a**. Bioisosteric replacement of the benzofuran moiety by benzothiophene resulted in compound **4a**, with marginal loss of activity with respect to **2a**. By contrast, replacement of benzothiophene (compound **4a**) with thiophene (compound **5a**), increased inhibitory activity by ~2.2-fold. We further explored the influence of varying substituents on the thiophene ring of compound **5a**. Substitution of the methyl group at 5-position of the thiophene ring resulted in compound **6a** with approximately 3-fold decrease in activity than the parent compound. Bioisosteric replacement of the methyl group with chloro (compound **7a**) or bromo (compound **8a**) groups resulted in ~2-fold enhancement of inhibitory activity, whereas 5-iodo derivative, compound **9a**, was found to be relatively less active than the methyl analog. We also explored the effect of variation in the position of the substitution around the thiophene ring. Moving the methyl group from the 5th to the 4th-position (compound **10a**), improved the activity of the compound by ~2-fold; whereas moving it to the 3rd-position yielded compound **11a** with ~7-fold higher inhibitory activity. Replacement of the thiophene ring with thiazole (compound **12a**) led to ~ 1.8-fold decrease in activity. Introduction of the pyrrole ring in place of the furan ring yielded compound **13a** with similar activity. Introduction of the indazole moiety in place of the benzofuran (compound **14a**) conferred slightly improved inhibitory activity. Among several corresponding α,γ-diketo esters tested (data not shown), only compounds **14** and **17** conferred inhibitory activity comparable to their acid counterparts, thus underscoring the observation that carboxylate anion moiety may be critical for chelating the active site divalent metal ions.

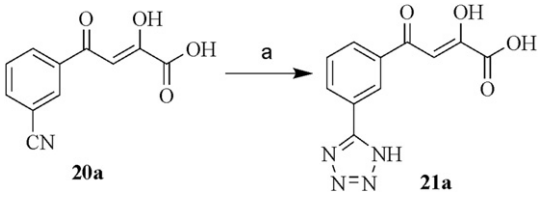
We next explored the influence of substituted phenyl ring versus the heterocyclic ring on NS5B inhibition. *Para*-biphenyl derivative **15a**, the first analog explored, was ~4-fold less active than the previously reported unsubstituted phenyl analog (IC_{50} = 5.7 μM) by Summa and colleagues [28], thus suggesting possible steric hindrance by the second phenyl ring within the active site of NS5B polymerase. The 2-nitrophenyl and 2-fluorophenyl analogs (compounds **16a** and **17a**, respectively) were ~2-fold more active than **15a**, while the 2-methylphenyl derivative **18a** was comparable in activity to the unsubstituted phenyl analog [28]. Further, the 3-fluorophenyl (compound **19a**) and 3-cyanophenyl (compound **20a**) analogs were 7–8 fold less active, while the 3-(1*H*-tetrazol-2-yl)phenyl analog (compound **21a**) was marginally more active than the unsubstituted phenyl analog [28]. The 2,6-difluorophenyl derivative **22a** exhibited comparable activity to 2-fluorophenyl analog, thus suggesting that the second *ortho*-fluoro substituent may not be important for NS5B inhibition. The 2,4-dichlorophenyl analog (compound **23a**) was 8-



Scheme 1. (a) Diethyl oxalate, sodium ethoxide, anhydrous THF, rt, 65–86%; (b) 1 N NaOH, THF/methanol 1:1, rt, 70–86%.



Scheme 2. (a) (i) Isobutyl chloroformate, *N*-methylmorpholine, −20 °C, 4 h, (ii) *N*,*O*-dimethylhydroxylamine hydrochloride in triethylamine, rt, 6 h; (b) anhydrous THF, methyl magnesium bromide (12% in THF), −78 °C, 2 h, rt, 5 h, 55% over two steps; (c) diethyl oxalate, sodium ethoxide, anhydrous THF, rt, 78%; (d) 1 N NaOH, THF/methanol 1:1, rt, 72%.



Scheme 3. (a) Ammonium chloride, sodium azide, DMF, 110 °C, 6 h, 75%.