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Short communication

Bicyclic octahydrocyclohepta[*b*]pyrrol-4(1*H*)one derivatives as novel selective anti-hepatitis C virus agents

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ABSTRACT

We report the discovery of the bicyclic octahydrocyclohepta[*b*]pyrrol-4(1*H*)-one scaffold as a new chemotype with anti-HCV activity on genotype 1b and 2a subgenomic replicons. The most potent compound **34** displayed EC_{50} values of 1.8 μM and 4.5 μM in genotype 1b and 2a, respectively, coupled with the absence of any antimetabolic effect (gt 1b SI = 112.4; gt 2a SI = 44.2) in a cell-based assay. Compound **34** did not target HCV NS5B, IRES, NS3 helicase, or selected host factors, and thus future work will involve the unique mechanism of action of these new antiviral compounds.

1. Introduction

The hepatitis C virus (HCV) was discovered in 1989 as the etiological agent causing non-A non-B hepatitis [1,2]. HCV is now understood to be a diverse genus of positive sense single-stranded RNA viruses, which is part of the *Flaviviridae* family. There are presently eleven known HCV genotypes (gt1-gt11) whose RNA sequences differ by up to 30–50% [3]. Within the HCV genotypes, there are also several subtypes (designated as 1a, 1b, 1c, *etc.*) [4]. HCV infection typically causes few symptoms, but about 85% of patients develop chronic infection, which leads to progressive liver damage, fibrosis, cirrhosis, hepatocellular carcinoma, and ultimately liver failure. Presently, 130–170 million people are chronically infected with HCV [5], about 35,000 of whom die each year [6]. Fortunately, unlike other chronic viral diseases like AIDS, antiviral therapy can eliminate detectable HCV in patients. Such a sustained virologic response (SVR) also prevents cirrhosis, hepatocellular carcinoma and related HCV-induced mortality.

Early HCV therapies relied on agents that modulate the host antiviral response like interferon-alpha (IFN-α) and ribavirin (RBV) [7]. INF-α/RBV therapy results in an overall 50%–75% SVR (depending on HCV genotype and numerous other factors), but the therapy is poorly tolerated. INF-α-based therapy has therefore been

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replaced with combinations of antiviral drugs that target viral proteins, called direct-acting antivirals (DAAs). DAA drug targets include the proteins encoded by the 9600 nucleotide HCV viral genome: core, E1, E2 p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [8]. Core, E1 and E2 constitute the viral particle, p7 and NS2 are involved in virion assembly and release, and the NS3 through NS5B proteins are involved in RNA replication [9,10]. In early stage discovery efforts, DAA candidates are typically tested using recombinant HCV proteins, or RNA replicons that are capable of autonomous replication in cells because they contain portions of the HCV genome [9].

The U.S. Food and Drug Administration (FDA) approved the first two HCV DDAs (telaprevir and boceprevir) in 2011. Both were NS3/4A protease inhibitors, but they were only effective in triple therapies when combined INF-α and RBV [11]. In 2014, the FDA approved the second generation NS3/4A protease inhibitor simeprevir, the NS5B nucleotide inhibitor sofosbuvir, and the NS5A inhibitor daclatasvir. More recently approved HCV DAAs include ledipasvir, ombitasvir, dasabuvir, grazoprevir, and elbasvir [12–15]. Notably, these DAAs can be used in all-oral, INF-free therapies, which cure most HCV patients [12]. Despite this enormous progress toward ultimate HCV eradication, HCV DAA development still needs to address high manufacturing costs, the evolution of drug resistant HCV alleles, and side effects [16]. In this context, we herein report the discovery of new anti-HCV compounds that have an octahydrocyclohepta[*b*]pyrrol-4(1*H*)-one core.

2. Results and discussion

Last year, we reported a new compound class possessing a 2-phenyl-4,5,6,7-tetrahydro-1H-indole core that inhibits HCV genotype 1b and 2a replication in cells [17,18]. Inspired by this success, we tested an additional set of representative heterobicyclic compounds (**1**–**21** [19–22], Fig. S1 Supporting Information) that was available from the EDASA Scientific public repertory of compound stocks (http://www.edasascientific.com/page/catalogue). The compounds were first tested at 50 μM using HCV replicons derived from HCV gt1b and gt2a, which are two widely studied HCV genotypes. Each compound was tested using Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A cells, which carry the autonomously replicating RNA from HCV gt1b and gt2a, and the firefly and *Renilla* luciferase reporters, respectively. The compounds that lowered cellular HCV replicon content more than 50% at 50 μM were further evaluated in concentration-response assays (Table S1 Supporting Information). A selectivity index (SI) was calculated by comparing the concentrations needed to reduce cell viability by 50% (CC_{50}) with EC_{50} values. Five compounds (**6**, **16**, **18**, **19**, and **20**) showed an ability to selectively reduce levels of both gt1b and gt2a replicons in cells with little apparent toxicity (SI > 10). In addition, derivatives **1**, **2**, and **3** reduced cellular levels of gt2a replicons. Compound **6** [22] was the most attractive among all the compounds tested, displaying low cytotoxicity (CC_{50} > 200) and an ability to reduce both genotype 1b (EC_{50} = 7.1 μM) and 2a (EC_{50} = 6.1 μM) HCV cellular replicon levels (Table 1).

Based on the promising data from HCV replicon assays, and the consolidated synthetic procedure to obtain the bicyclic octahydrocyclohepta[*b*]pyrrol-4(1*H*)one scaffold [22], we designed and synthesized eighteen new close derivatives of **6 (22**–**39)**, that have been clustered in five groups accordingly to their chemical modification: a) replacement of the ethyl ester with a carboxylic acid or amides, b) "trans" variation of the ring junction, c) tosyl (Ts) modification and replacement, d) keto group functionalization or reduction, and e) methyl insertion at the bicyclic junction (Fig. 1, Schemes 2 and 3, and Fig. S2 in Supporting Information).

The starting octahydrocyclohepta[*b*]pyrrol-4(1*H*)-ones **40**–**43** [22] were obtained *via* an aza-Cope-Mannich reaction from corresponding amino vinylic alcohols and either glyoxylic acid or ester following previously disclosed conditions (Scheme 1).

The relatively mild, high-yielding and operationally simple conditions of this tandem process, accompanied by an accurate and predictable stereochemical outcome, are the main virtues which provided the permanent access to the requisite bicyclic proline derivatives in desirable quantities for further chemical modifications.

Since compound **6** is an ethyl ester, we also tested the biological effect of its corresponding acid **22** and of a similar acid **23,** and its corresponding ethyl ester **24**, where a methyl group was inserted at 3a position of the octahydrocyclohepta[*b*]pyrrol-4(1*H*)-one scaffold (Scheme 2). The two acid derivatives **22** and **23** were prepared by the tosylation of the appropriate amino acids **40** and **41** [22]. Compound **24** was prepared in a quantitative yield by direct esterification, with acetyl chloride and ethanol, of the corresponding acid **23**.

We also investigated whether changing stereochemical relationship of the ring fusion on "*cis*-" to "*trans*-" might affect the biological behavior of the octahydrocyclohepta[*b*]pyrrol-4(1*H*)-one scaffold. The "*trans*" variation of **6**, compound **25**, was obtained by applying the general procedure for tosylation starting from the amino ester **42** (Scheme 2) [22].

Taking into account that amides are much more stable metabolically than esters, another modification of **6** was achieved by replacing its ester moiety with an amide group. Thus, amides **26**–**29** were synthesized by a HBTU coupling reaction of the corresponding acid **22** and appropriate amine (Scheme 2).

Variations of the tosyl moiety were also investigated by synthesizing compounds **30**–**33,** which were prepared by treating the amino ester **43** with the corresponding acyl or sulfonyl chloride (Scheme 3).

Finally, compounds derived from functionalization (**34**–**37**) or reduction (**38**) of the keto group of compound **6** were also synthesized. Hydrazide **34**, semicarbazide **35** and two oximes (**36**, **37**) were prepared by refluxing compound **6** in ethanol with appropriate nucleophile (Scheme 3). By reducing the keto group of **6** with $NaBH_4$, it was possible to obtain compound **38**, though in low yields. Its further treatment with acetic anhydride furnished compound **39** (Scheme 3).

It should be noted that compounds **22**–**39** contain three stereo centers (all racemic mixtures), two of which are placed near electron withdrawing groups. However, reaction conditions were very mild and no epimerization (based on ^{1}H and ^{13}C NMR data) was observed during the synthesis of compounds **22**–**39.** The relative configuration was assigned based on the parent compounds **40**–**43**.

Reduction of **6** led exclusively to one stereoisomer (**38**). The configuration of the newly formed stereocenter was elucidated using X-Ray analysis of derivative **39** (Fig. 2, CCDC 1454446 and Supporting Information).

All the synthesized compounds were then tested in HCV replicon assays using the same protocol described above for the initial set of 21 compounds (Table 2). The new derivatives **22**–**39** that reduced HCV replicon levels in cells more than 50% at 50 μM, were then evaluated for their toxicity (CC_{50}), potency (EC_{50}) and selectivity (SI) (Table 2).

These data highlighted that all the chemical modifications involving the replacement of the ethyl ester with a carboxylic acid (**22** and **23**), methyl insertion (**24**), fused ring stereochemistry variation (**25**), ester-to-amide replacement (**26**–**29**), tosylate residue modification (**30**–**33**) and keto group reduction (**38** and **39**) were generally detrimental, as inactive or less active/selective derivatives than the parent compound **6** were obtained.

Conversely, out of the series of derivatives obtained through the