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Short communication

Synthesis and biological evaluation of α-aryl-α-tetralone derivatives as hepatitis C virus inhibitors

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ABSTRACT

The synthesis of a novel series of 1-carba-isoflavanones through the α-arylation of α-tetralones is described. Several of these compounds demonstrated potent activity and selectivity *in-vitro* against HCV replicon reporter cells. Compound **10** (LQB-**314**) exhibited the best profile being active and selective in both replicon reporter cells (IC_{50} 1.8 μM, SI > 111 and IC_{50} 4.3 μM, SI > 46 in Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A, respectively). Compound **3** (LQB-307) was the more potent and selective for Huh7.5-FGR-JC1-Rluc2A replicon reporter cells (IC_{50} 1.5 μM, SI > 101.4).

1. Introduction

Hepatitis C Virus (HCV) belongs to the *Flaviviridae* family of viruses and was identified as the causative agent of non-A, non-B hepatitis [1,2], infecting approximately 3% of the world population [3,4]. During chronic HCV infections, the virus induces numerous changes within the host, which increases the risk of developing more serious conditions such as cirrhosis, steatosis, and hepatoma cellular carcinoma [3,5–9]. Until recently, HCV was treated with a combination of pegylated-interferon (PEG-IFNα) and the nucleoside analog ribavirin [10–12]. This therapy had limited effectiveness in maintaining a sustained virological response (SVR), especially when treating patients infected with HCV genotype 1. In addition to lack of SVR, treatment with interferon is associated with numerous side effects. Nowadays, approved anti-HCV therapies utilize direct acting antivirals (DAAs) targeting viral proteins such as HCV NS3-4A protease inhibitors and an NS5B viral polymerase inhibitor [13–16]. The current therapy is much more effective in producing a SVR. However, to treat most HCV genotypes DAAs must be administered in combination with PEG-IFNα and ribavirin [16]. Therefore, IFN-related side effects remain and the complicated dosing regimens of current treatment may further limit patient compliance [13–16]. Further, selection of HCV drug resistant variants remains a major concern associated with current anti-HCV therapies [17,18]. Thus, identification of additional anti-HCV agents remains a priority.

In this letter we report, for the first time, the anti-HCV activity of α-aryl-α-tetralones (1-carba-isoflavanones). These compounds (Fig. 1) are isosters of isoflavanones, a group of naturally occurring isoflavonoids and synthetic derivatives which comprise compounds bearing antineoplasic and antiviral properties [19–22].

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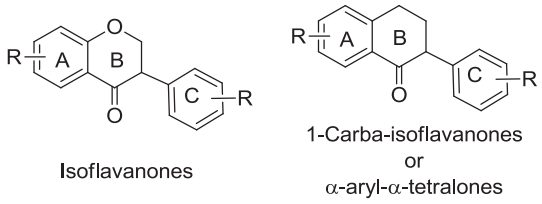


Fig. 1. Isoflavanones and 1-carba-isoflavanones.

2. Results

2.1. Synthesis of α-aryl-α-tetralones

The structures of 1-carba-isoflavanones studied in this work are shown in Fig. 2 and can be divided in three groups. In the first, the A-ring is unsubstituted (LQB-**305**, LQB-**306**); in the second, both A- and C-rings are substituted by methoxy groups (LQB-**307** to LQB-**316**), while in the third, a hydroxy group is present in one of the aromatic rings (LQB-**393** to LQB-**395**).

The concomitant development of metal-catalyzed α-arylation of enolizable compounds with haloarenes, by Buchwald, Myura and Hartwig, made the α-aryl-α-tetralones readily accessible by synthesis [23–25]. Compounds **1** (LQB-**305**) to **12** (LQB-**316**) were synthesized in reasonable to excellent chemical yields as previously described, by palladium catalyzed α-arylation of α-tetralone and methoxylated derivatives with *o*-bromoarenes [26].

The removal of the methoxy groups in these α-aryl-α-tetralones requires the use of boron tribromide (BBr_3) and under these conditions only tetracyclic 5-carba-pterocarpens are obtained [26]. We tried to prepare α-aryltetralone **13** (LQB-**393**), bearing a phenol group at the α-aryl moiety, through the α-arylation of tetralone **1** with *o*-bromo-arenes in which the oxygen at the *o*-position is substituted by more easily removable groups, such as methoxymethyl ether (MOM) or Bn groups (**17a**,**b**, Scheme 1a) [27,28]. The α-arylation of α-tetralones **16** with **17a** and **17b** was accomplished under the same reaction conditions [26], leading to **18a** and **18b** in good yields. We tried to remove the MOM-protecting group in **18a** in acidic medium, but a complex mixture of products was obtained. We also tried the hydrogenolysis of the benzyl group in **18b** with H_2 and Pd/C, but a complex mixture of products was also observed, due to the partial reduction of the carbonyl group and the hydrogenolysis of the resulting alcohol. However, **13** (LQB-**393**) could be prepared from **18b** by hydrogenolysis using Pd/C in the presence of ammonium formate, in acetone–methanol at 50 °C. Interestingly, compounds **14** (LQB-**394**) and **15** (LQB-**395**), bearing a phenol group at the A-ring, were obtained by α-arylation of α-tetralone **19** with bromoarenes **17c** and **17b**, respectively, without protection of the phenol group (Scheme 1).

2.2. Screening of the antiviral activity

The antiviral activity of the target α-aryl-α-tetralones shown in Fig. 1 was investigated in Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon reporter cells, bearing the autonomously replicating HCV RNA of genotype 1b and 2a in the firefly and Renilla luciferase reporters, respectively, as described previously (Table 1) [29–31]. The levels of luciferase activities in these reporter systems, thus serves as a measure of HCV RNA replication. The effect of the compounds on cellular toxicity was determined in non-infected Huh7.5 parental cells (Table 1) [27].

For non-phenolic compounds (**1**–**12**) their potency was found to be strongly dependent on the pattern of substitution at both aromatic rings and the HCV genotype. In context of the Huh7/Rep-Feo1b replicon reporter cells, unsubstituted compounds at the α-tetralone moiety **1** and **2** displayed weak activity and poor selective index (entries 1 and 2). The introduction of a methoxy group at 6-position as in **3** and **4** resulted in higher antiviral activity (entries 3 and 4). The presence of the methoxy group at 7-position in **5** and **6** (entries 5 and 6) led to products with increased potency when

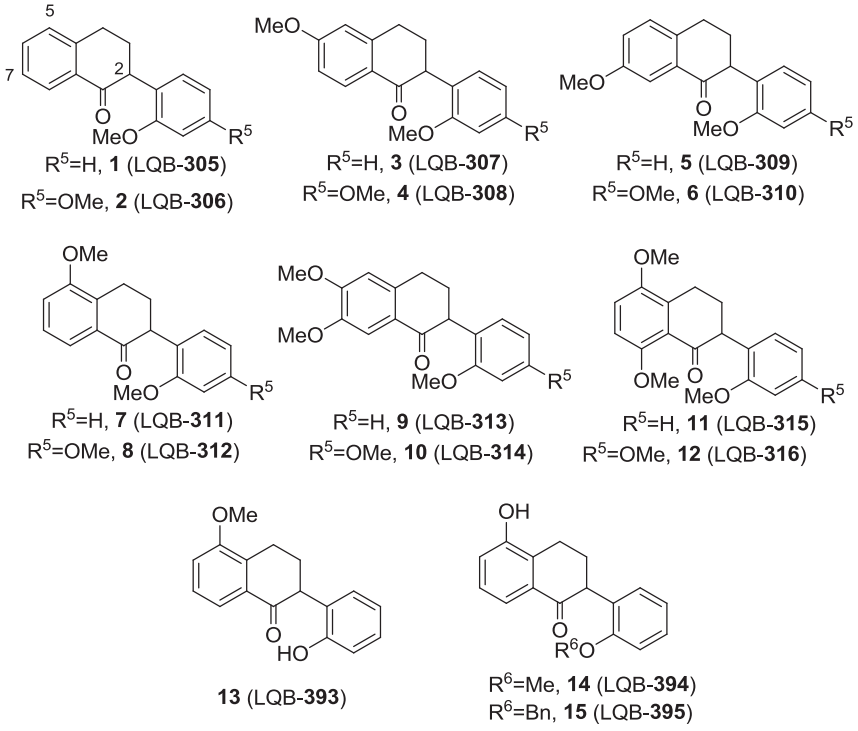
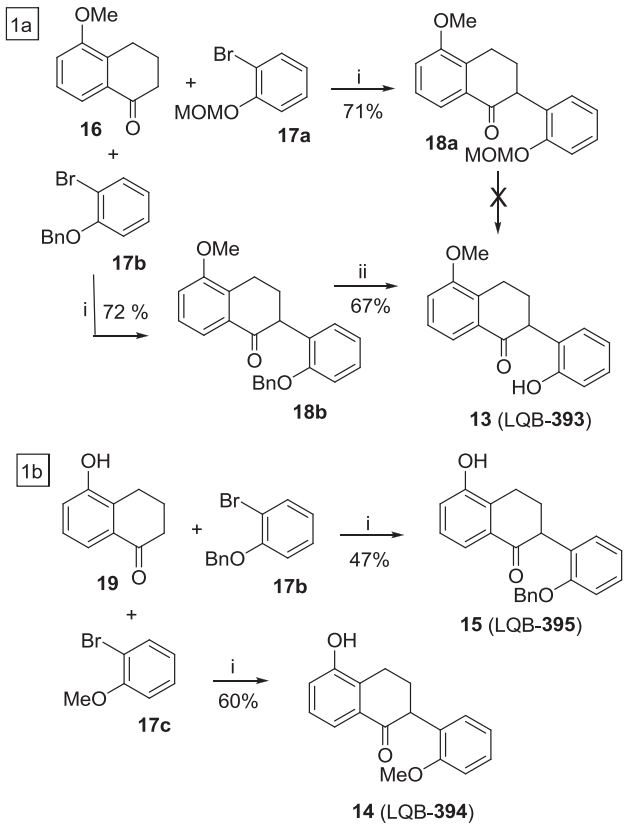


Fig. 2. α-Aryltetralones synthesized and studied in this work.



i, Dioxane-water (4/1),$Pd_2(dba)_3$ (2.5 mol%), KOH (2.5 eq.), MW 80W, tBu_3PHF_4 (10 mol%), 100^0C, 40 min.
ii, HCO_2NH_4 (10 equiv.), Pd/C (10 %), acetone/methanol (10/1) 50^0C

Scheme 1. Synthesis of phenolic α-aryltetralones.

compared to unsubstituted derivatives at the α-aryl-tetralone moiety (entries 1 and 2), but less potent than 5-methoxy derivatives **3** and **4** (entries 3 and 4). The presence of methoxy group at 5-position in compounds **7** and **8** (entries 7 and 8) also resulted in products with diminished activity compared to 3 and 4. However, in all these case low to moderated SI were observed. The presence of two methoxy groups at the α-tetralone moiety in **9** decreased the potency (entry 9), but **10**, bearing two methoxy groups at both aromatic rings, was the most potent compound in this series, showing an excellent SI > 111 (entry 10).

Non-phenolic compounds (**1**–**12**) were, in general, more potent on Huh7.5-FGR-JC1-Rluc2A replicon reporter cells. For example, **5** and **6** were 4–5-fold more potent when compared to their activity on Huh7/Rep-Feo1b replicon reporter cells (entries 1 and 2). Except for **6** (entry 6) and **12** (entry 12), the activity increased by the addition of methoxy groups at the A-ring, while the introduction of a second methoxy group at the C ring led to a decrease in their activity, except for **8** (entry 8). The more interesting compound was **3** (EC_{50} 1.5 μM, SI 101.4).

Furthermore, the presence of hydroxy groups at the aromatic rings, as in **13**, **14** and **15** showed to be detrimental for compound potency on Huh7/Rep-Feo1b replicon reporter cells (entries 13–15). However, these compounds present a higher potency on Huh7.5-FGR-JC1-Rluc2A replicon reporter cells, but low SI.

3. Conclusions

In conclusion, this letter describes the synthesis and anti-HCV activity evaluations of α-aryl-α-tetralones (1-carba-isoflavanones), which are amenable for further structural optimization. Overall, 4 compounds displayed EC_{50} values ≤8 μM against HCV genotype 1b while 7 compounds exhibited EC_{50} values below 5 μM against HCV genotype 2a. Considering the selective index, **3** (LQB-**307**) emerged as the most promising lead against HCV genotype 2a, displaying EC_{50} value of 1.5 μM and SI > 101.4. Notably, **10** (LQB-**314**), the most potent and selective against Huh7/Rep-Feo1b replicon reporter cells (IC_{50} 1.8 μM, SI > 111), was also potent and selective against Huh7.5-FGR-JC1-Rluc2A replicon reporter cells (IC_{50} 4.3 μM, SI > 46). Work is now in progress to evaluate the anti-HCV activity of other analogs and to study its mechanism of action.

Table 1
Anti-HCV activity of α-aryl-α-tetralones.

Ent	Comp.	CC_{50} (μM)[a]	Huh7/Rep-Feo1b			Huh7.5-FGR-JC1-Rluc2A		
			Inhibition (50 μM, %)[b]	EC_{50}[c]	SI[d]	Inhibition (50 μM, %)[b]	EC_{50}[c]	SI[d]
1	**1**	117.4 ± 20.1	48.4 ± 9.6	50.1 ± 3.2	2.3	84.4 ± 6.6	14.3 ± 1.8	8.2
2	**2**	63.6 ± 7.1	55.6 ± 2.4	44.3 ± 5.2	1.4	83.9 ± 6.2	11.0 ± 1.9	5.7
3	**3**	152.2 ± 18.6	86.4 ± 9.0	10.6 ± 0.7	14.3	88.8 ± 1.7	1.5 ± 0.8	101.4
4	**4**	<50	89.5 ± 0.4	7.5 ± 0.5	<6.6	93.4 ± 3.3	3.3 ± 0.8	<15.3
5	**5**	111.2 ± 5.7	85.4 ± 3.1	29.5 ± 1.0	3.7	91.8 ± 3.6	3.6 ± 0.9	30.8
6	**6**	<50	95.2 ± 1.4	14.3 ± 0.9	<3.5	97.6 ± 0.4	15.0 ± 1.6	<3.3
7	**7**	>200	51.7 ± 4.2	48.5 ± 3.9	>4.1	86.6 ± 5.7	10.8 ± 1.1	>18.5
8	**8**	104.5 ± 12.1	96.5 ± 1.5	12.8 ± 1.6	8.2	99.5 ± 0.3	3.2 ± 1.8	32.6
9	**9**	126.2 ± 18.9	60.2 ± 12.7	42.5 ± 0.9	2.9	79.9 ± 6.1	4.8 ± 0.8	26.2
10	**10**	>200	89.6 ± 4.0	1.8 ± 0.5	>111.1	86.8 ± 1.6	4.3 ± 1.6	>46.1
11	**11**	>200	80.6 ± 5.2	8.0 ± 0.9	>24.9	88.8 ± 1.5	6.8 ± 1.1	>29.5
12	**12**	<50	73.8 ± 7.6	8.2 ± 1.2	<6.0	86.2 ± 4.4	21.5 ± 2.8	<2.3
13	**13**	111.1 ± 9.2	26.1 ± 9.1	ND	ND	86 ± 6	10.8 ± 0.9	10.2
14	**14**	>200	41.2 ± 10.0	ND	ND	44 ± 10	ND	ND
15	**15**	<50	99.1 ± 2.3	6.21 ± 0.9	<8.0	98 ± 1	4.4 ± 0.9	<11.3

[a] CC_{50} values were evaluated in Huh7.5 parental cells by the MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation kit, Promega). Cells treated with equal amounts of DMSO served as viability control.
[b] Compounds were screened at 50 μM concentrations against Huh7.5/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2Ab replicon reporter cells. Cells treated with equal amounts of DMSO served as control.
[c] EC_{50} values were computed from dose–response curves using half-log dilutions of the compounds employing CalcuSyn V2 software.
[d] SI: selectivity index represents the ratio of CC_{50} to EC_{50}. The data represents an average of three independent experiments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.01.057.

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