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Structure–activity studies of (−)-epigallocatechin gallate derivatives as HCV entry inhibitors

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ABSTRACT

Preventing viral entry into cells is a recognized approach for HIV therapy and has attracted attention for use against the hepatitis C virus (HCV). Recent reports described the activity of (−)-epigallocatechin gallate (EGCG) as an inhibitor of HCV entry with modest potency. EGCG is a polyphenolic natural product with a wide range of biological activity and unfavorable pharmaceutical properties. In an attempt to identify more drug-like EGCG derivatives with improved efficacy as HCV entry inhibitors, we initiated structure–activity investigations using semi-synthetic and synthetic EGCG analogs. The data show that there are multiple regions in the EGCG structure that contribute to activity. The gallate ester portion of the molecule appears to be of particular importance as a 3,4-difluoro analog of EGCG enhanced potency. This derivative and other active compounds were shown not to be cytotoxic in Huh-7 cell culture. These data suggest that more potent, non-cytotoxic EGCG analogs can be prepared in an attempt to identify more drug-like candidates to treat HCV infection by this mechanism.

Hepatitis C virus (HCV) is a threat to human health that has attracted substantial attention to identify shorter, more effective courses of treatment, with fewer adverse events compared to the original oral ribavarin/injectable pegylated interferon regime. Direct acting anti-viral agents such as boceprevir and telaprevir target the NS3-4A protease and are approved for use in patients infected with genotype 1 of the virus.[1] Unfortunately these drugs are significantly less effective in patients who do not respond to ribavarin-interferon treatment. NS5A inhibitors such as daclatasvir are in clinical development as a part of multidrug combinations.[2] Both of these direct acting approaches are associated with resistance (both innate and acquired) and require concomitant use of ribavarin and interferon. A recently approved nucleoside analog, sofosbuvir used in combination with daclatasvir achieved a sustained viral response (SVR) at 24 weeks without added interferon against all HCV genotypes.[3]

Another recently studied approach to HCV treatment involves inhibition of viral entry, as was successfully accomplished for HIV therapy with maraviroc and enfuvirtide that target the CCR5 receptor and gp41 protein, respectively.[4,5] Figure 1 shows representative structures for recently identified small molecule inhibitors of HCV entry. ITX-5061 (**1**) is a re-purposed p38 MAP kinase inhibitor in phase 1 studies that targets the scavenger receptor B1.[6] Fluphenazine (**2**) and topotecan (**3**) were shown to perturb cholesterol packing leading to increased cell membrane fluidity.[7] Triazine **4** was identified by screening using HCV pseudoparticles that incorporated the E1 and E2 envelope proteins from a clinical genotype 1B isolate.[8] The poly phenolic natural product (−)-epigallocatechin gallate (EGCG, **5**) has been shown by two groups to exhibit broad spectrum HCV genotype entry inhibition by preventing viral attachment to the mammalian cell surface.[9,10] EGCG has been widely explored for a range of biological properties and is recognized to have a number of adverse pharmaceutical properties that limit in vivo utility.[11] While **5** is less potent compared to the other entry inhibitors in Figure 1, it demonstrated activity against a wide range of HCV genotypes and is a molecule for which there is very limited structure–activity data for this property. We are investigating SAR studies associated with a range of EGCG activities and now report initial results in a search for more potent, non-cytotoxic HCV entry inhibitors with potentially more drug-like structures.

The semi-synthesis of ester derivatives of EGCG and catechin-derived analogs from epigallocatechin **6** (Fig. 2) was carried out

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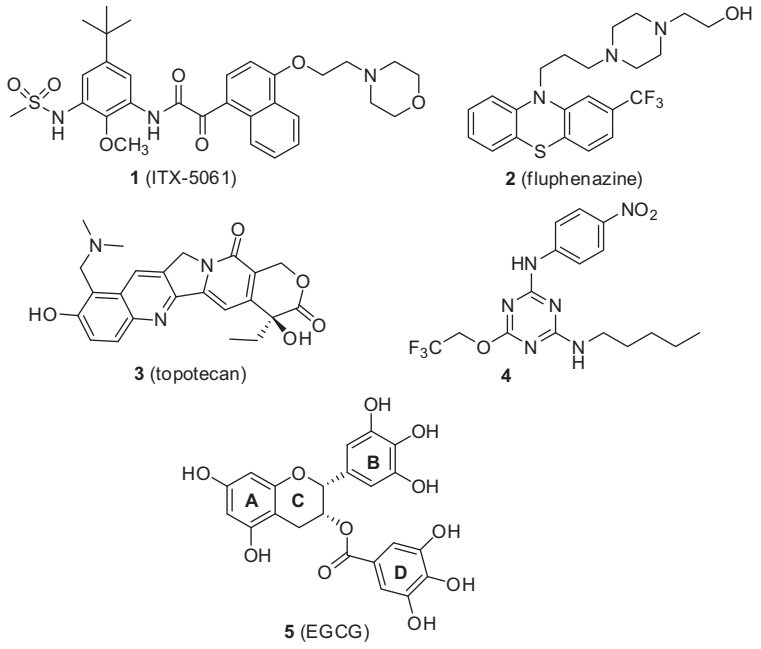


Figure 1.

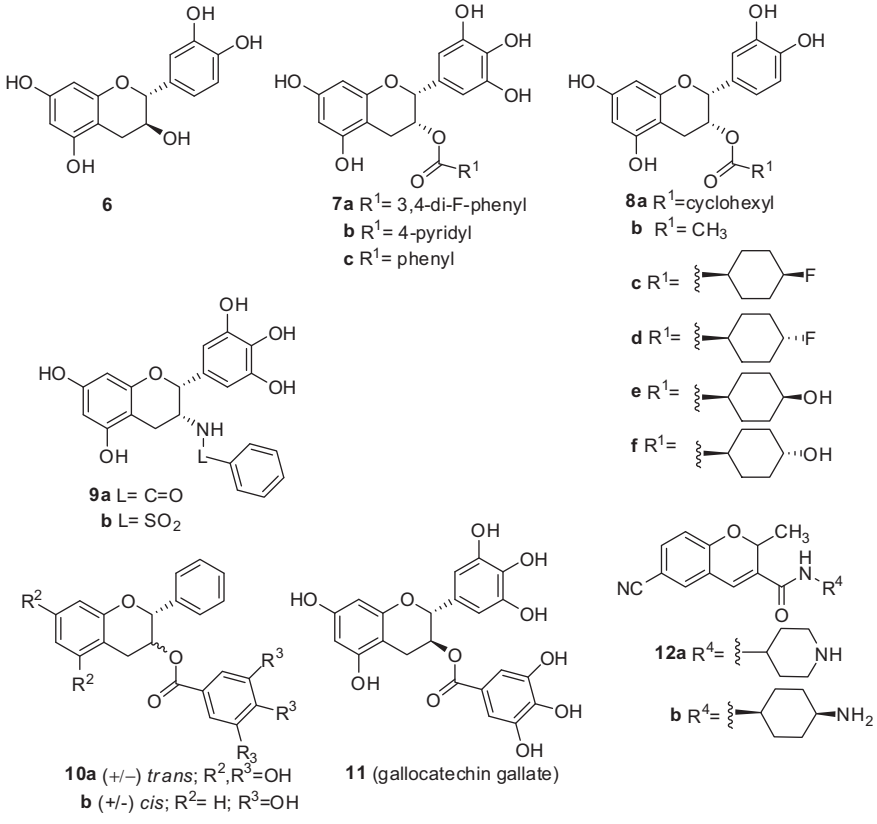
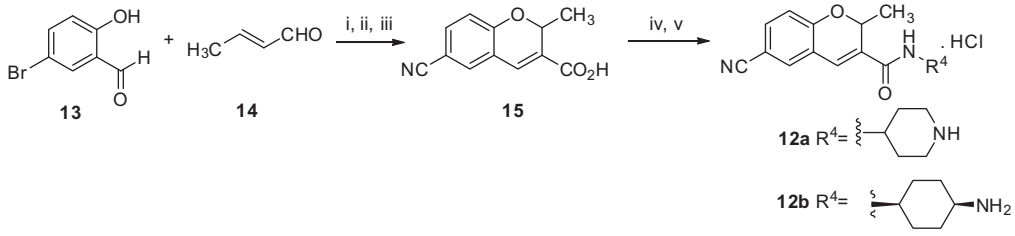


Figure 2.

as reported previously to provide targets **7a–c** and **8a–f**, amide **9a**, sulfonamide **9b** and synthetic, racemic B-ring des-hydroxy compounds **10a** and **b**.[12] A commercially available diastereomer of EGCG, (−)-gallocatechin gallate **11** was included to investigate potential stereochemical effects on inhibition of HCV entry. The preparation of new synthetic analogs **12a** and **b** is outlined in Scheme 1.[13] These compounds were intended to investigate a lower molecular weight, stereochemically less complex core with the ability to rapidly explore amide SAR by parallel synthesis. Cyano-substituted benzopyran acid **15** was obtained in three steps beginning with condensation of bromoacetophenone **13** and crotonaldehyde.[14] Copper-mediated bromine displacement by cyanide was followed by sodium chlorate oxidation. EDC coupling and Boc-deprotection delivered analogs **12a** and **12b**, respectively,



Scheme 1. Reagents and conditions: (i) K_2CO_3, dioxane, 80 °C, 20 h, 41%; (ii) CuCN, NMP, 160 °C, 16 h, 30%; (iii) NaH_2PO_4, $NaClO_2$, 2-methyl-2-butene, *t*-BuOH/THF/H_2O (5:1:2), 80%; (iv) Boc-amine, DMAP, EDCI·HCl, DCM, rt, 5 h; 70%; (v) 4N HCl in dioxane, rt, 4 h, 100%.

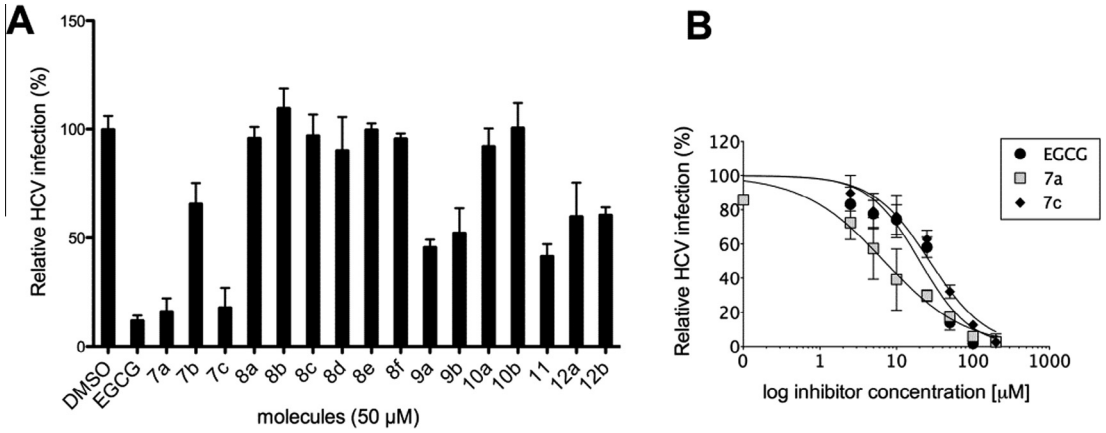


Figure 3. Screening and selected compound IC_{50} results. (A) Anti-HCV entry inhibition of the compounds. Huh-7 cells were inoculated with HCVcc in the presence of each compound at 50 μM for 2 h. DMSO 0.01% was used as a control. Cells were further incubated in compound-free medium for 28 h. Cells were fixed and processed for immunofluorescent detection of infected cells. (B) Dose–response inhibition study of **7a**, **7c** and EGCG. Huh-7 cells were infected with HCVcc for 2 h in the presence of given concentrations of the different compounds and further incubated in compound-free medium for 28 h. Cells were fixed and infectivity quantified by immunofluorescence. Data are means from 3 independent experiments performed in triplicate. Error bars represent standard errors of the mean.

in which the metabolically labile ester and the A-, B- and D-rings of the EGCG core structure were modified.

All compounds were screened initially at a concentration of 50 μM for inhibition of HCV entry in which viral infection was quantified using a previously described immunofluorescence-based assay performed using a high content imaging system.[10] Representative results are illustrated in Figure 3 where the data revealed that the ester can be replaced using an amide or sulfonamide albeit with some decrease in potency compared to EGCG. Stereochemistry in the natural product is important, exemplified by the decreased efficacy of **11** compared to **5**. It is interesting to note that D-ring benzoate **7c** and 3,4-difluorophenyl ester **7a** showed comparable efficacy to EGCG in this screen, suggesting the gallate ester hydroxyl groups are not essential for activity and can be replaced by other polar moieties. Replacement of the gallate ester with a pyridyl heterocycle leads to a weakly active derivative (**7b**) while alicyclic moieties based on the catechin template are not tolerated. Removal of hydroxyl groups from the B-ring of EGCG (**10a**) or in both the B- and D-ring (**10b**) eliminates activity. The new synthetic analogs **12a** and **12b** like the pyridyl ester, amide and sulfonamide demonstrated weak activity in the screen.

Encouraged by the results with **7a** and **7c**, mammalian cell cytotoxicity studies were carried out and the IC_{50} for inhibition of HCV entry was determined. The results of these experiments are illustrated in Figure 4. At concentrations up to 1 mM, neither **7a** nor **7c** reduced cell viability in an MTS assay performed as described.[10] EGCG has an IC_{50} for HCV entry of 20 μM, difluoro **7a** is somewhat more potent with an IC_{50} of 6.9 μM and benzoate **7c** gives a value of 27 μM. This data indicates that fluorination of the D ring leads to

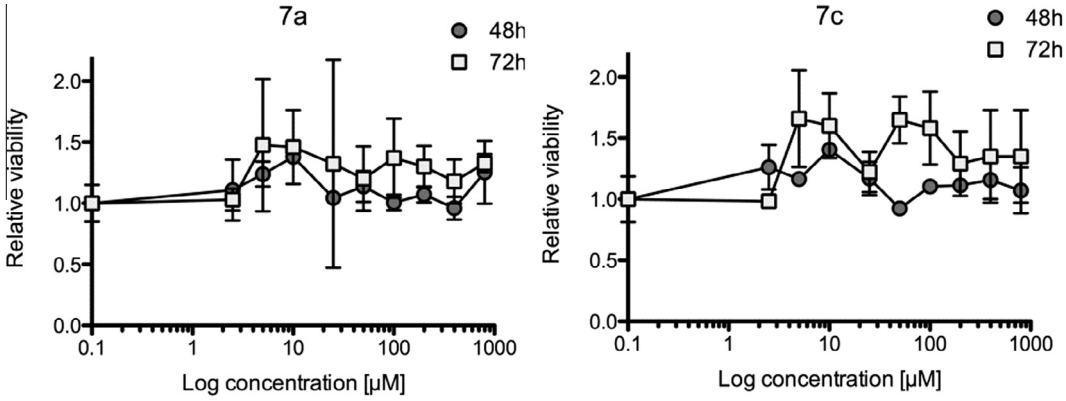


Figure 4. Mammalian cell viability assay.

improved potency compared to the natural product. The structure–activity relationships observed for HCV entry inhibition are distinct from our previously reported study of Hsp90 inhibition of a broader collection of EGCG analogs.[12]

The Huh7 cell viability was monitored using a MTS-based viability assay after 48 h and 72 h of incubation with the compound by determining the OD at 490 nm. Values relative to the condition without molecules expressed as 1 are given. Data are means from 3 independent experiments performed in triplicate. Error bars represent standard errors of the mean.

In conclusion, this work demonstrates important structure–activity relationships for derivatives of the poly-phenolic natural product (−)-epigallocatechin gallate. These initial studies suggest that *cis*-stereochemistry of the natural product is preferred compared to a *trans*-diastereomer. The gallate ester can be replaced by a fluoro-substituted aromatic moiety leading to a moderately more potent inhibitor of HCV entry. This improvement in potency was accomplished without increasing molecular weight and reduces the number of phenolic hydroxyl groups in the natural product. The metabolically labile ester can be replaced by a more stable amide or sulfonamide. It is also possible to eliminate the B-ring with a small alkyl group and replace the poly hydroxy A ring with a cyano-substituted ring. These structure activity observations represent starting points for more detailed exploration of this template and are distinct from previously reported SAR for Hsp90 inhibition. The mechanism and molecular target for HCV entry inhibition by EGCG and these derivatives remain unknown. Identification of more potent chemical probes will prove useful in this regard. Such studies are underway and will be reported in due course.

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