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Potent inhibitors of hepatitis C core dimerization as new leads for anti-hepatitis C agents

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ABSTRACT

New indoline alkaloid-type compounds which inhibit HCV production by infected hepatoma cells have been identified. These compounds, dimeric-type compounds of previously known inhibitors, display double digit nanomolar IC_{50} and EC_{50} values, with cytotoxicity CC_{50} indexes higher than 36 μM, thus providing ample therapeutic windows for further development of HCV drugs.

Hepatitis C virus (HCV),[1] which infects 130–170 million people worldwide,[2] is the main cause of liver disease in humans. This single-strand, positive RNA virus encodes ten proteins[3] all of which are essential for viral infection and propagation. Currently, the only treatment of HCV infection is a 48-week regimen, a combination of interferon and ribavirin, that cures less than half the people infected, depending on viral genotype and strain.[4] Several of the viral proteins have been targeted for drug development, with the viral NS3 protease[5] and NS5B polymerase[6] key among them, and several candidates have advanced significantly in the drug pipeline.[7]

Core, the HCV capsid protein, is the most conserved of the ten viral proteins across all HCV genotypes.[8] Core is responsible for nucleocapsid formation, recruitment of HCV replicase proteins to lipid droplets, and assembly of the viral particles in infected cells. As such, core is an interesting target for HCV drug development distinct from the HCV protease and polymerase enzymes, from which resistant mutants have already emerged.[9] With combination therapies likely to evolve for HCV treatment,[10] inhibitors of core dimerization, and hence nucleocapsid formation, could play a key role.

We have been interested in the discovery of new, small molecule inhibitors of core dimerization as lead structures for anti-HCV agents. To this end, we have reported new assays to screen for inhibitors of core dimerization[11] and of interactions of core with other HCV proteins, including NS3 helicase.[12] Screening of a small molecule library led to the identification of **1** as an initial hit for further elaboration (Fig. 1)[13] An additional focused library was prepared, which revealed three new core dimerization inhibitors as racemates (**2**–**4**) with IC_{50}'s in the single digit μM range. All four inhibitors were also found to inhibit HCV production in infected Huh-7.5 hepatoma cells; only **4** showed levels of cytotoxicity comparable to activity against core dimerization. We now report a successful search for more potent inhibitors of core

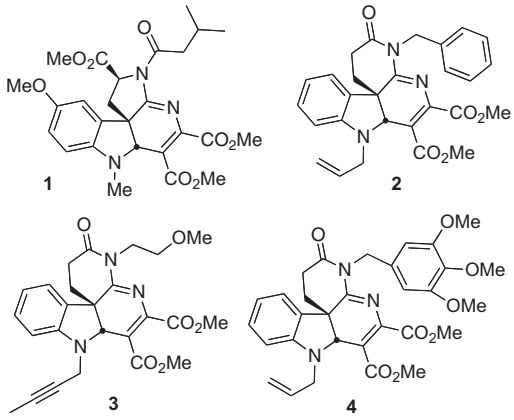

Figure 1. Previously reported inhibitors of core dimerization.[11]

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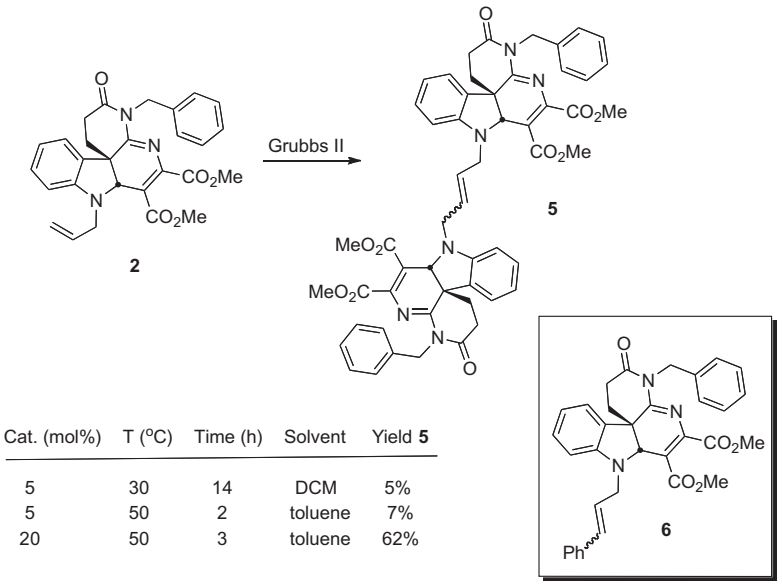
protein dimerization based on the structures of **2**–**4**, which are also effective in blocking HCV proliferation in infected cells.

Since inhibition of core dimerization requires blockage of a protein–protein interaction (PPI)[14] our initial approach to improve on the activity of **1**–**4** was to prepare a dimer of the most active inhibitor **2** since dimerization of inhibitors of PPI's have been shown to be an effective strategy to improve activity.[15–17] The hope was that a second binding site or 'hot spot' might be found on the surface of core for the second heterocycle to occupy that would magnify the dimerization inhibition. It was also understood that since **2** was racemic, dimerization of **2** would produce a mixture of enantiomers and as well as the meso diastereomer which would be likely difficult to separate. Nonetheless, if such a mixture would show improved activity over the original monomer, this would suggest that attachment of the second heterocyclic subunit could be a viable approach to preparing compounds with improved activity.

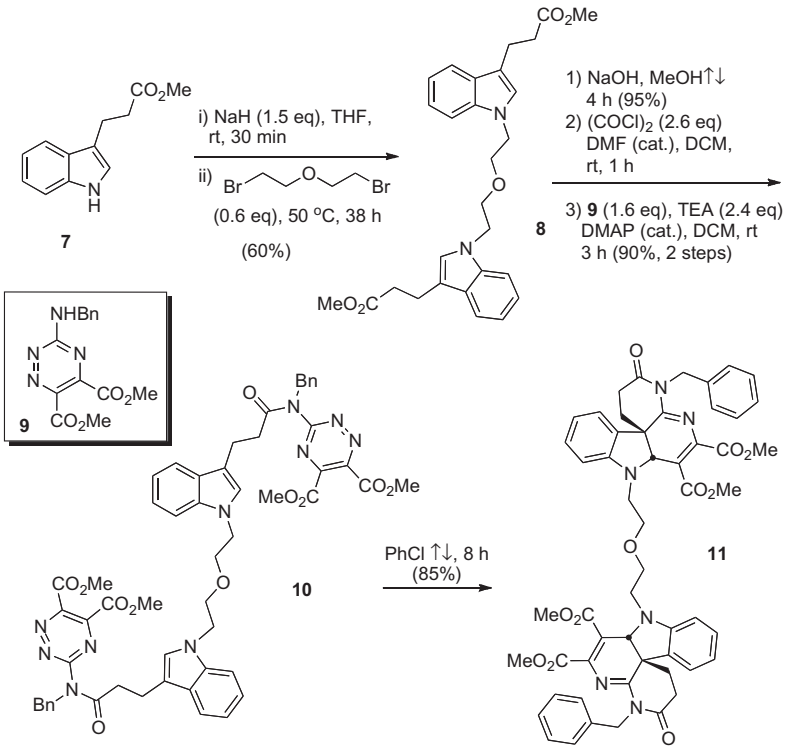
Attempts to dimerize **2** through metathesis gave disappointingly low yields unless 20 mol % of Grubbs II catalyst was employed (Scheme 1). Under optimal conditions, a 62% yield of dimer **5** was obtained as a mixture of *E*- and *Z*-isomers of the racemate and the meso diastereomer, along with **6** (15%), resulting from metathesis with the benzylidene ligand of the catalyst.

Due to the difficulty in purification, the poor yields in the subsequent non-chemoselective hydrogenation of the olefinic double

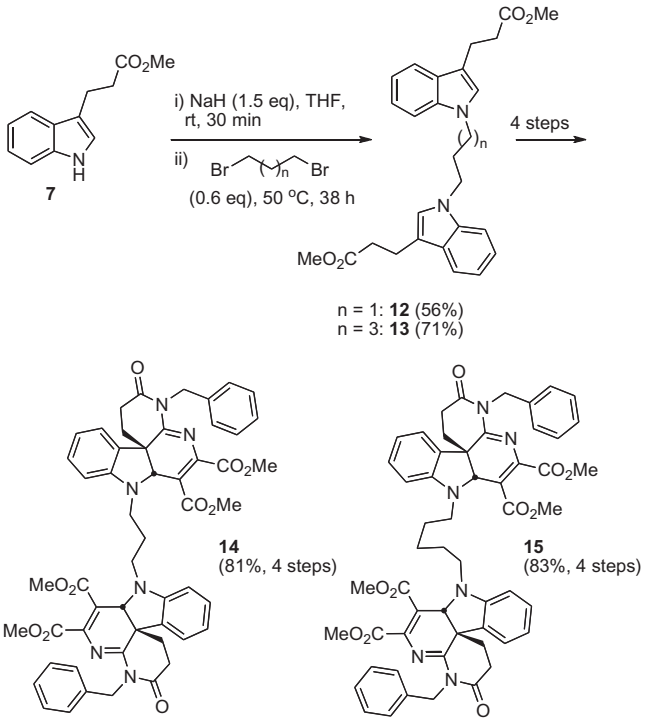


Cat. (mol%)	T (°C)	Time (h)	Solvent	Yield **5**
5	30	14	DCM	5%
5	50	2	toluene	7%
20	50	3	toluene	62%

Scheme 1. Dimerization of **2** through metathesis.



Scheme 2. Preparation of dimer **11** with an ether linkage between the monomeric subunits.



Scheme 3. Preparation of dimers **14** and **15** with all-carbon tethers linking the monomeric subunits.

bond of **5**, as well as the relatively poor yield of **5** without the use of a large amount of catalyst, an alternative approach was explored.

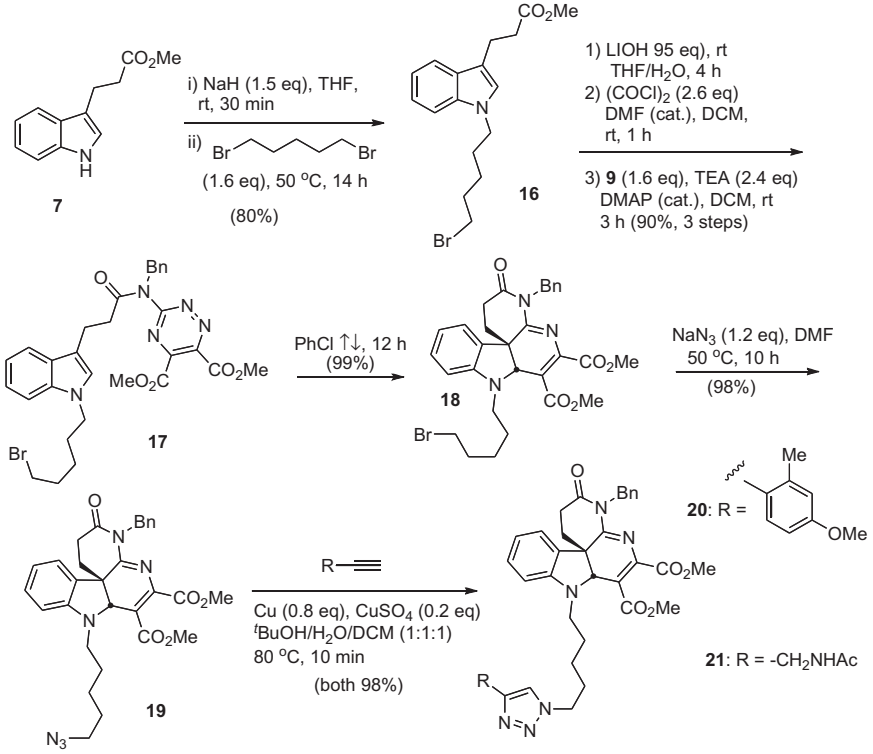
Dimer **11** was ultimately prepared by first linking two indolylpropionate esters **7** with dibromoethyl ether, producing the symmetric dimer **8** (60%, Scheme 2). Basic hydrolysis of the esters,

Table 1
Summary of bioactivities

Compd	IC_{50}[a] (μM)	T1-EC_{50}[b] (μM)	T2-EC_{50}[c] (μM)	CC_{50}[d] (μM)	MW	*c* log *P*
1[e]	9.3	14.8	22.2	>320	513	4.7
2[e]	1.4	2.3	3.2	127.2	485	6
3[e]	2.0	4.9	0.7	>320	465	4.6
4[e]	2.0	3.8	3.0	5.3	575	5.3
11	0.098	0.48	2.5	>100	960	10.2
14	2.9	0.088	0.735	>36	930	10.5
15	0.72	0.09	29.9	>36	958	10.1
20	0.092	1.4	1.1	>100	702	7.9
21	0.341	26.2	56.2	150	653	4.4
BILN2061[f]	N/A	0.072	0.071	5.3	––	––

[a] Values are means of three experiments.
[b] Values are means of three experiments. T1 corresponds to initial 72 h culture of cells in presence of inhibitors. See Refs. 11, 13.
[c] Values are means of three experiments. T2 corresponds to second 72 h culture of fresh cells, infection resulting from virus secreted in T1. See Refs. 11, 13.
[d] Values are means of three experiments. 50% Cytotoxic concn versus Huh-7.5 hepatoma cells.
[e] Data from Ref. 13.
[f] Known inhibitor of the HCV NS3/NS4A protease[20].

conversion to the bis-acid chloride, then acylation of triazine **9** as previously described in the preparation of monomer **2**[13] all proceeded uneventfully, yielding dimer **10** (86% over three steps). Double inverse electron demand Diels–Alder cycloadditions in refluxing chlorobenzene produced the target dimer **11**. Beginning with the 3- and 5-carbon tethers, 1,3-dibromopropane and 1,5-dibromopentane, dimers **14** and **15** were similarly prepared in 45% and 59% overall yields, respectively (five steps each beginning with **7**, Scheme 3). In each of the tethering reactions with the respective dibromides, it was imperative to keep the number of equivalents of dibromide relatively low (0.6 equiv) to avoid monoalkylations (vide supra). Dimers **11**, **14**, and **15** were also mixtures of enantiomers and the meso diastereomer.



Scheme 4. Preparation of tethered bis-heterocycles **20** and **21** via click dipolar addition chemistry.

In the core dimerization assay, all three dimers were active, with dimeric mixture **11** having an IC_{50} value of 98 nM, more than an order of magnitude greater than that observed for the monomer **2** (Table 1). None of the dimers showed significant cytotoxicity (CC_{50}'s >36 μM). Whole cell assays with Huh-7.5 hepatoma cells infected with HCV 2a strain J6/JFH-1 were treated with increasing concentrations (0.001–100 μM) of the dimers to assess their effect on HCV propagation as previously described.[11,13] The known NS3/NS4A protease inhibitor BILN2061 was included as a positive control. The EC_{50}'s were calculated at an early (T1) and late (T2) stage. Sensitivity to the nature of the tether linking the dimers is readily apparent, with **14** emerging as the best inhibitor (EC_{50} T1: 88 nM, T2: 735 nM). Dimeric mixture **15** was also quite active in early stage inhibition of HCV infectivity (T1 EC_{50} 90 nM), but with a dramatic loss of activity at late stage (T2 EC_{50} 29.9 μM). The main drawbacks with these dimers are their high MW and *c* log *P* values (Table 1).

With the validation of tethering a second heterocyclic unit to the basic active scaffold as an approach to enhanced activity against core dimerization as well as inhibition of HCV production in infected cells, two dimer 'mimics' were then prepared in an effort to discover inhibitors equally potent to **11** and **14**, but without the meso diastereomer contamination (Scheme 4). To this end, alkylation of **7** with 1,5-dibromopentane (1.6 equiv) produced the monobromide **16** in 80% yield, with only a small amount of tethered dimer **13** (9%). Conversion to the corresponding acid chloride and attachment of triazine **9** proceeded smoothly under standard conditions, as did the cycloaddition to give **18**. No unwanted chemistry of this primary alkyl bromide disrupted the strategy. Azide displacement to **19**, then Cu-catalyzed dipolar cycloaddition[18] with appropriate alkynes produced racemic triazoles **20** and **21**, with the cycloadditions both occurring in 98% yield.

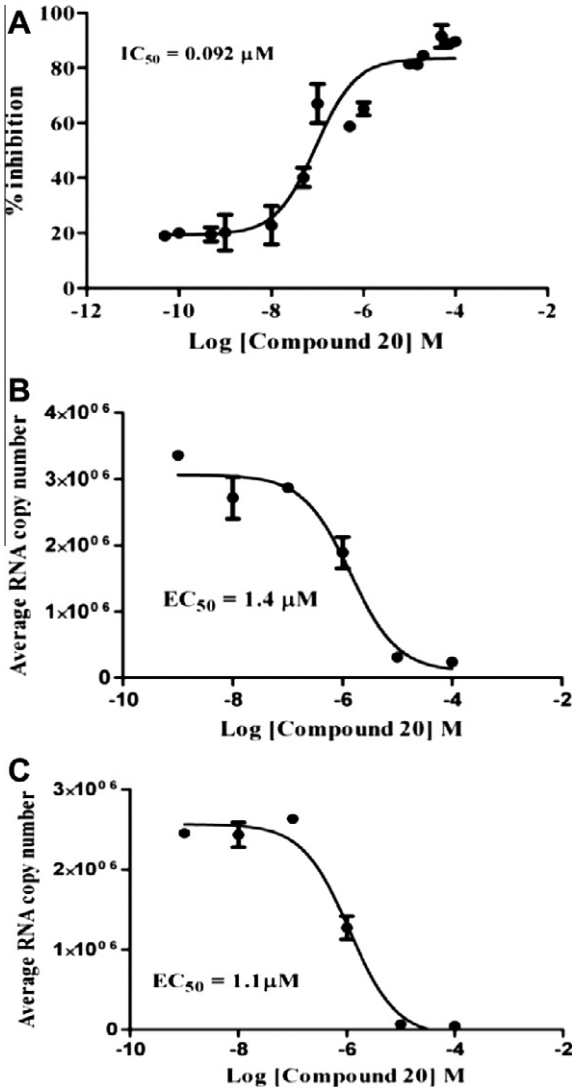


Figure 2. Dose–response analyzes of **20** using Core106 ALPHA screen assay.[11] The compound was dosed from 0 to 100 μM; IC_{50} and EC_{50}'s were calculated using a non-linear regression 'log[inhibitor] versus response' with four points per concentration. (A) IC_{50}; (B) T1-EC_{50}; (C) T2-EC_{50}.

Both **20** and **21** proved to be excellent inhibitors of core dimerization, with sub-micromolar IC_{50}'s (92 and 341 nM, respectively); the activity of **20** was comparable to that of dimer **11**. Equally important, the cytotoxicities of **20** and **21** were in the high μM range, and the whole cell activity of **20** remained in the single digit μM region. The main drawback to be addressed is that the most effective of these new tethered bis-heterocycles (**20**, Fig. 2) also has the highest MW (702) and the highest *c* log *P* value (7.9).

In conclusion, several dimers of the previously reported core dimerization inhibitor **2** have been prepared, and been shown to be more effective core dimerization and HCV inhibitors than the original lead compound, though with cellular activity that declines with increasing incubation time, perhaps due to compound instability in the cellular assays. All compounds were stable upon storage. Using click chemistry,[19] two racemic tethered bis-heterocycles (**20** and **21**) were also prepared and shown to be even more effective inhibitors of core dimerization as well as inhibitors of HCV production in infected hepatoma cells. Further studies to (i) probe the nature of the interaction of these inhibitors with core, (iii) resolve and screen the enantiomers of **20** and **21**; and (iii) prepare an additional focused library of analogues of **20** and **21** are underway. Completion of these studies should allow for a better SAR understanding.

Acknowledgments

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