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New small molecule inhibitors of hepatitis C virus

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ABSTRACT

New small molecule inhibitors of HCV were discovered by screening a small library of indoline alkaloid-type compounds. An automated assay format was employed which allowed identification of dimerization inhibitors of core, the capsid protein of the virus. These compounds were subsequently shown to block production of infectious virus in hepatoma cells.

Hepatitis C, the leading cause of liver disease, affects over 170 million people worldwide.[1] Over three million Americans are infected by the HCV virus,[2] which is particularly lethal for AIDS patients, of whom increasing numbers are co-infected with HIV and HCV.[3] There is no vaccine, and the only available treatment, based on a combination of interferon and ribavirin, cures less than half of the patients.[4] Until recently, the main focus of efforts to develop HCV inhibitors has been on the viral NS3 protease and NS5B polymerase enzymes, but escape mutants have already been reported.[5] The failure to find other new anti-HCV drugs is mainly linked to the dearth of novel targets. In the virus, the core protein is essential for nucleocapsid formation and release of the viral particle from the infected cell. Although HCV is extremely susceptible to mutations, core is the most conserved of all 10 viral proteins, and thus represents an excellent, hitherto-unexplored target for novel anti-HCV drug development.

We recently reported new bioassays based on the inhibition of core dimerization, and hence the inhibition of nucleocapsid formation, with the goal of discovering small molecule inhibitors of this protein–protein interaction.[6] The effectiveness of core fragments and other peptides in inhibiting core dimerization and viral production was also demonstrated, thereby validating the assays as an approach for the discovery of leads that inhibit hepatitis C.[7] We now report the identification of four, small molecule inhibitors of core dimerization revealed through the screening of the CMLD-BU small molecule libraries.

As part of our effort to create diverse, small molecule libraries rich in stereochemistry, a library of indoline alkaloid-type compounds was prepared using inverse electron demand Diels–Alder chemistry. The initial library (**7**, 132 members) originated from the intramolecular cycloaddition of tryptophan derivatives[8] with tethered 1,2,4-triazines following protocols already reported by our group (Scheme 1).[9]

The cycloaddition precursors **3** were prepared by S_NAr displacements following BOC-deprotection of L-tryptophan derivatives **1** on triazine **2**.[10] In situ trifluoroacetylation of the nitrogen linker, accomplished by treatment with trifluoroacetic anhydride, lowers the LUMO of the heteroaromatic azadiene and thereby enables the cycloaddition to proceed immediately after acylation under the reaction conditions without isolation of intermediate **4**. Following deacylation upon chromatography on silica gel, the cycloadducts **6**, which resemble truncated *Aspidosperma*-type alkaloids, were obtained in good to excellent yields. The two newly generated two stereogenic centers at C1 and C9 were formed with complete stereoselectivity controlled by chirality of the tryptophan dienophile, as previously noted.[9] The relative stereochemistry of the cycloadducts was confirmed by NOE studies as previously described.[9] The overall yields of scaffolds **6** ranged from 42% to 64% (three steps) on a 2 g scale (Chart 1). Diversification of scaffolds **6** then proceeded by acylation (17 commercially available acid chlorides) and sulfonylation (10 commercially available sulfonyl

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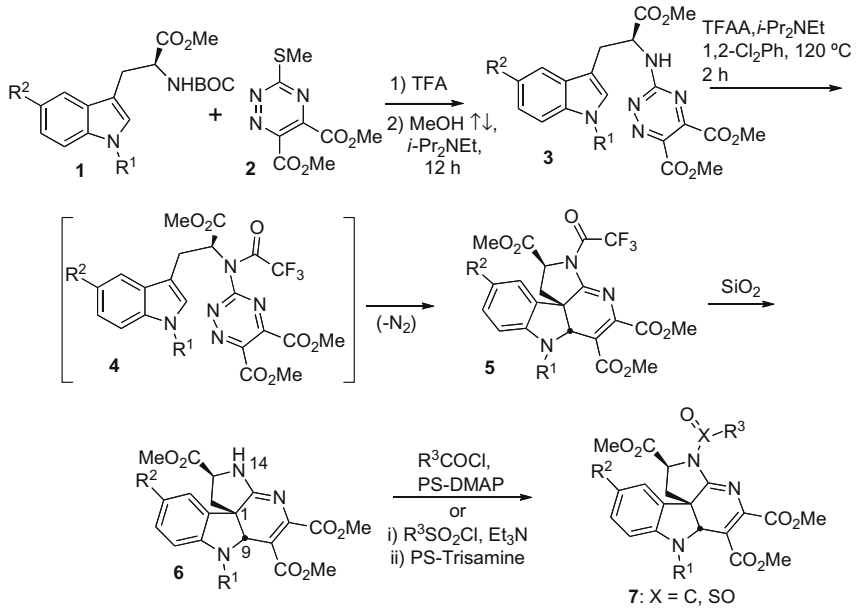


Scheme 1. Preparation of library **7** (homochiral).

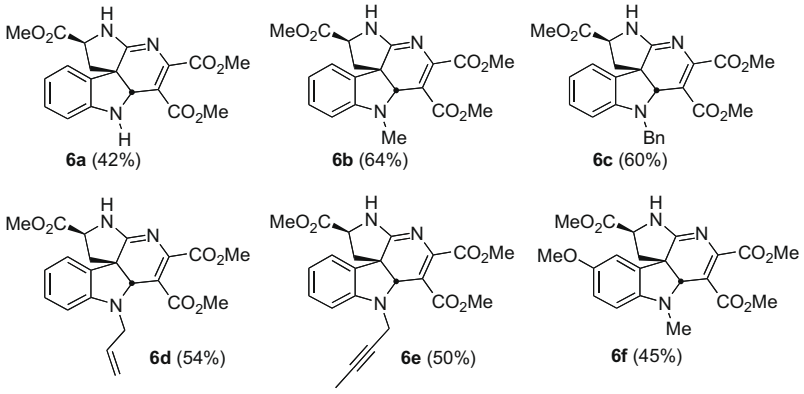


Chart 1. Scaffolds **6** for library preparation (overall yields in parentheses).

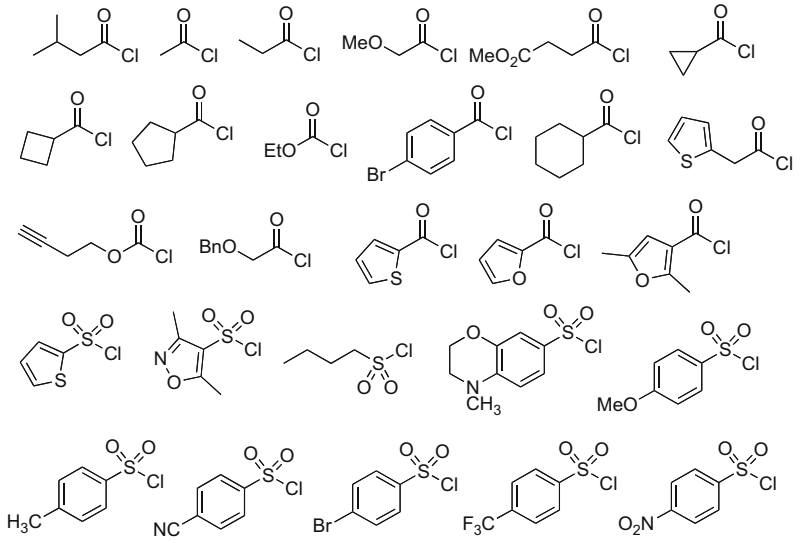


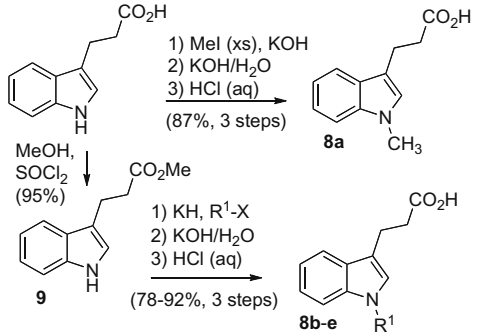
Chart 2. Acylating and sulfonylating reagents for diversification of scaffolds **6**.

chlorides) of N14, producing sublibrary **7** (Chart 2). Reactions were run on a 39 mg/scaffold scale, and the library members were purified by mass-directed HPLC, then plated for storage. Of the 162 reactions attempted, 132 produced sufficient quantities (>40 mg) for inclusion in the library. All library members were stable to storage in the solid state, as well as in DMSO solution over several months.

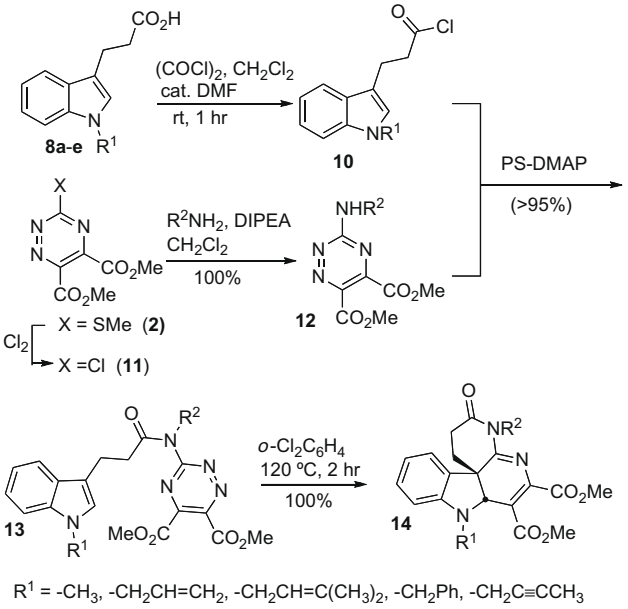
This library was subjected to the core dimerization inhibition assay as a component of a 2240-membered CMLD-BU collection. One member of this library (see below) showed single digit μM IC_{50} activity that was validated by resynthesis and resubmission to the assay, and confirmed in a secondary Alpha screen.[7]

A second generation indoline library with an expanded D-ring was subsequently prepared exploiting the intramolecular inverse electron demand Diels–Alder chemistry beginning with five N-alkylated indolylpropionic acids **8a**–**e** and triazine **2**. The indolylpropionic acids were prepared by straightforward pathways (Scheme 2). For *N*-methylindolylpropionic acid **8a**, permethylation of indolylpropionic acid, followed by basic methyl ester hydrolysis with acid work-up gave **8a** in 87% yield over three steps. For the remaining acids **8b**–**e**, methyl ester **9** formation, then N-alkylation, followed by ester hydrolysis all proceeded uneventfully in good yields.

With the five indolylpropionic acids in hand, the second sublibrary again formed through an intramolecular inverse electron demand Diels–Alder reaction of an indolyl subunit with a tethered triazine, was pursued (Scheme 3). Conversion of the propionic acids to the acid chlorides **10a**–**e**, then amidation of **10** with aminotriazines derived from **2** produced the tethered diene/dieno-



Scheme 2. Preparation of indolylpropionic acids **8**.



Scheme 3. Preparation of δ-lactam sublibrary **14** (racemic).

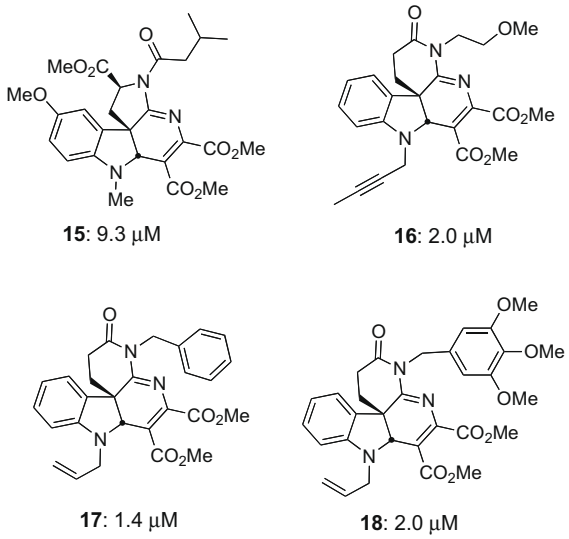


Figure 1. Library members with single digit μM activities (IC_{50}'s) inhibiting core dimerization.

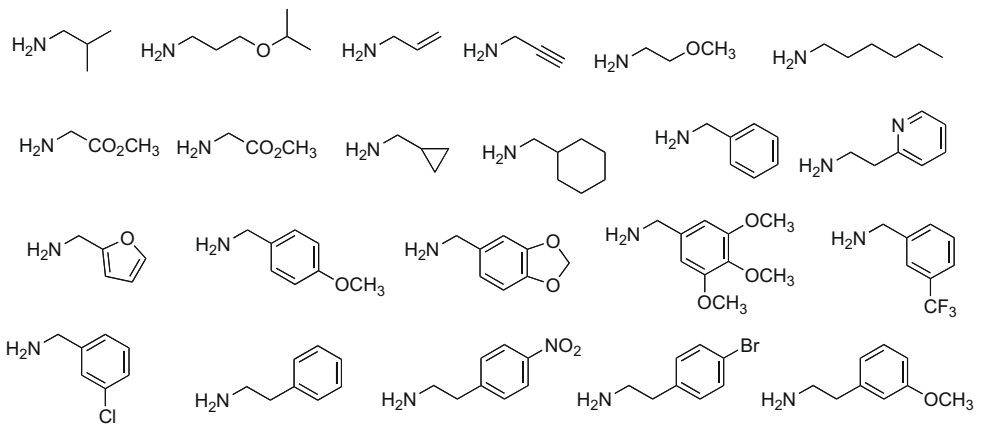


Chart 3. Primary amines used in the preparation of δ-lactam sublibrary **14**.

Table 1
Summary of activities

Compound	CC_{50}[a] (μM)	IC_{50}[b] (μM)	HCV2a T1–EC_{50} (μM)	J6/JFH-1[c] T2–EC_{50} (μM)
BILN2061	5.3	N/A	0.072	0.071
15	>320	9.3	14.8	22.2
16	>320	2.0	4.9	0.7
17	127.2	1.4	2.3	3.2
18	5.3	2.0	3.8	3.0

[a] 50% Cytotoxic conc versus Huh7-5 hepatoma cells average of triplicate runs.
[b] See Figure 2.
[c] EC_{50}'s average of triplicate runs.

phile pairs **13**. Intramolecular cycloadditions by heating in 1,2-dichlorobenzene yielded sublibrary **14** quantitatively. Twenty-two primary amines (Chart 3) were employed in the S_NAr displacements on the chlorotriazine, producing 22 aminotriazines **12**. All amidations and cycloadditions were accomplished smoothly, resulting in a 110-membered, racemic sublibrary **14**; all members were stable to storage in DMSO.

Screening of the sublibrary **14** for inhibition of core dimerization, performed in duplicate, revealed three additional 'hits' with IC_{50} activities in the single digit μM range (Fig. 1 and Fig. 2). The original 'hit' **15** from the initial library is homochiral. The other three 'hits' (**16**–**18**) were from the second generation, δ-lactam sublibrary and are racemic. The activities of all four inhibitors were validated by resynthesis and resubmission to the assay.

Subsequent to the identification of these four hits as core dimerization inhibitors, all four compounds were evaluated for their effect on production of HCV hepatoma cells, as done previously for core-derived peptides which inhibited core dimerization.[6] Cytotoxicity was also examined with hepatoma Huh7-5 cells (Table 1). The compounds were studied in the same cells infected with HCV 2a strain J6/JFH-1.[6] Real-time reverse-transcriptase PCR was done on RNA extracted and purified from the cells treated with increasing concentrations (0.001–100 μM) of each of the compounds **15**–**18**. The EC_{50}'s were calculated at an early (T1) and late (T2) stage, and varied from 2.3 to 20.8 μM at T1, and from 0.7 to 36.3 μM. For comparative purposes, data for BILN2061 a known inhibitor of the NS3/NS4A protease,[11] is included.

From this work, three relatively non-toxic compounds have been identified (**15**–**17**) which could be the basis for the development of new anti-HCV drugs, or which could be used in combination with compounds acting generally, such as interferon, or specifically on other HCV targets. Compound **18**, while of comparable activity against core dimerization, was also cytotoxic. All four of these compounds not only inhibited core dimerization, but they also were shown to be effective in inhibiting HCV 2a strain J6/JFH-1 proliferation in isolated hepatoma cells, with the relatively non-toxic **16** and **17** having EC_{50}'s in the single digit μM range. These two compounds are the two most promising candidates discovered to date in our screening for core dimerization inhibitors. Work continues (Scripps-Florida) to study the binding of these lead compounds with core protein, and to design more potent inhibitors (CMLD-BU) based on these lead structures. Considerable recent progress has been made in identifying small molecules that inhibit protein–protein interactions with the potential of becoming therapeutic targets.[12] Future work will determine whether **15**–**18** or related analogue(s) fall into this emerging class of promising compounds.

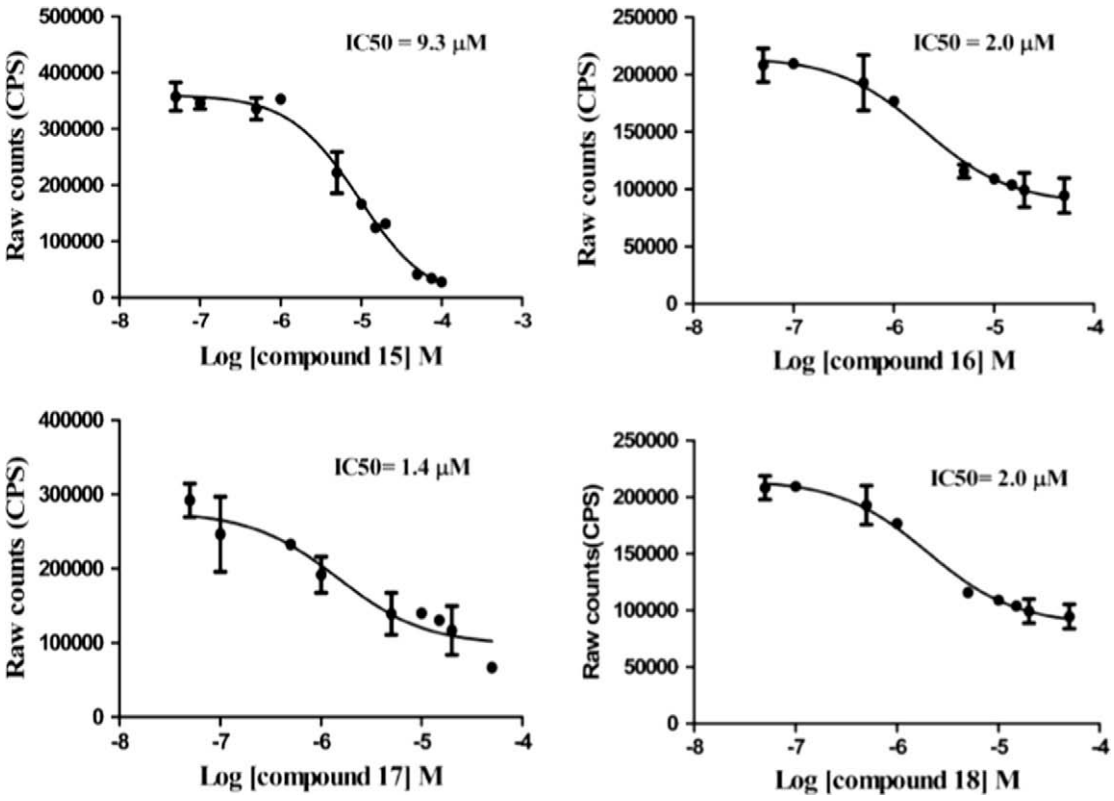


Figure 2. Dose–response analyses of selected compound **15** analogues. Core106 ALPHA screen assay was used for the dose–response analyses. The compounds were dosed from 0 to 100 μM. The IC_{50} were calculated using a non-linear regression 'Log (inhibitor) versus response' with four points for each concentration.

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