Recent advances in the development of HBV capsid assembly modulators
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Hepatitis B virus (HBV) infections represent a significant burden on global public health. Current HBV treatments using nucleos(t)ide analogs (NAs) and PEG interferons cannot fully alleviate this burden as they do not affect the transcriptional activity of the tenacious covalently closed circular DNA (cccDNA) responsible for viral persistence. Capsid assembly modulators (CAMs) disrupt the encapsidation of pre-genomic RNA and can cause nucleocapsid disassembly, thereby affecting multiple steps of HBV replication and reduction of cccDNA pools. This review provides a concise overview of the development of CAMs and the progress achieved in understanding their interactions with HBV core proteins.

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Introduction
Hepatitis B virus (HBV) infection and its associated liver diseases, such as cirrhosis and hepatocellular carcinoma, result in nearly one million deaths worldwide, annually according to World Health Organization estimates [1]. The disease disproportionately affects China, accounting for one-third of all HBV chronic carriers in the world with more than 90 million people affected. Current therapeutics for HBV rely on either nucleos(t)ide analogs (NAs) [2] or immunotherapy [3]. NAs such as lamivudine and entecavir effectively target the viral reverse transcriptase to suppress virus production, but do not decrease the formation of covalently closed circular DNA (cccDNA), which is responsible for viral persistence [4,5]. On the other hand, interferon therapy has only been effective in a small minority of patients and exhibits severe side effects [6]. There is clearly an on-going need for alternative therapeutic targets to increase cure rates and decrease the global threat posed by HBV, despite the availability of vaccines to manage this disease. This review provides a summary of recent advances in the development of small molecule capsid assembly modulators (CAMs) and the progress achieved in understanding their interactions with HBV core proteins, with a particular emphasis on articles published in the past three years.

Nucleocapsid assembly as a drug target
Nucleocapsids play a role during genome packaging, reverse transcription, intracellular trafficking, and maintenance of the HBV viral replication cycle [7**]. During its assembly process, core protein homodimers encapsulate the HBV pre-genomic RNA (pgRNA) and polymerase to form biologically competent HBV nucleocapsids. Elucidation of this poorly understood assembly process has been the focus of significant research efforts. Contrary to a prior assumption that the assembly process results in perfect icosahedral nucleocapsids, a recent study using charge detection mass spectrometry (CDMS) showed that many of the capsid particles generated in the assembly process are defective and overgrown, but self-correct over time, suggesting that completion is a distinct phase in the assembly reaction [8**]. It is important to note that this study was conducted in vitro, in a truncated capsid without packaging materials and will need further experiments to explore this finding. Nonetheless, nucleocapsid assembly and its correction process provide an attractive target for treating HBV infections. Small molecule therapeutics can destabilize the HBV core protein, increase the capsid assembly rate, and form either aberrant or empty, nonfunctional capsid particles [9].

Capsid assembly modulators
In recent years, significant attention has been paid to the discovery and development of CAMs, which generally fall within one of five main classes: phenylpropenamides (PPAs), heteroaryldihydropyrimidines (HAPs), sulfamoylbenzamides (SBAs), sulfamoylpyrroloamides (SPAs), and glyoxamoylpyrroloamides (GLPs) (Figure 1).

CAMs allow exposure of cccDNA to degrading enzymes, preventing cccDNA formation during de novo infection [10†] and are hypothesized to have a higher barrier to drug resistance than NAs [11]. CAMs also recently demonstrated the ability to cause capsid disassembly [12†]. Thus, this approach should be beneficial both for the development of novel antivirals and for combination
therapy with NAs so that multiple mechanisms are targeted simultaneously to perhaps induce synergy.

**Phenylpropenamides (PPAs) and heteroaryldihydropyrimidines (HAPs)**

PPAs and HAPs represent two of the most studied chemical classes of CAMs [13]. PPAs, discovered in 1998 [14], are thought to act as a molecular adhesive that facilitates an assembly active state [15], driving capsid assembly at a rate high enough to exclude the pgRNA complex and resulting in the production of empty capsids. PPAs inhibited wild type and lamivudine-resistant HBV strains in vitro (Compound AT-61, and its optimized version, AT-130, inhibited viral replication with EC_{50} values of 1200 and 130 nM, respectively [19]), but clinical trials are necessary to confirm utility [14,20].

HAPs, in contrast, form aberrant, nonfunctional capsid particles [16], because they interfere with the direct interaction of the termini of the viral genome [17] and change the geometry of the contacts between capsid protein dimers at the quaternary level [18*]. Optimization efforts of HAPs have led to a number of potent analogs, such as BAY 41-4109 (EC_{50} = 50 nM) [21] and GLS-4 (EC_{50} = 12 nM) [22,23]. A summary of these efforts can be found in a recent review [24**].

Interestingly, PPAs and HAPs both bind to the same hydrophobic pocket formed at the dimer–dimer interface near the C-termini of HBV core protein subunits. This results in large-scale allosteric conformational changes that are slightly varied by the respective classes, thereby destabilizing the HBV core protein and increasing capsid assembly rate.

In contrast, the last three years have seen a strong interest in the discovery and development of novel CAM classes (Table 1), which include SBAs, SPAs and GLPs. The remainder of this review will provide a brief overview of this literature.

**Benzamides (BAs) and sulfamoylbenzamides (SBAs)**

BAs were discovered at the Blumberg Institute as the result of a high-throughput screening of 26,900

| Table 1 |
| Reports on Novel HBV CAMs, Published Between 2015–2018 |

<table>
<thead>
<tr>
<th>Class</th>
<th>Company/university</th>
<th>Publication date</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>SBA</td>
<td>Janssen Sciences Ireland</td>
<td>09/2015</td>
<td>[25**,26]</td>
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<td></td>
<td>Emory University</td>
<td>06/2017</td>
<td>[27**]</td>
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<td></td>
<td>Drexel University, Blumberg Institute, and Arbutus Biopharma</td>
<td>08/2018</td>
<td>[28]</td>
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<tr>
<td>BA</td>
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<td>05/2017</td>
<td>[29**]</td>
</tr>
<tr>
<td>SPA</td>
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<td>04/2016</td>
<td>[30]</td>
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<tr>
<td></td>
<td>05/2018</td>
<td>[31]</td>
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<td>Cyclized SPA</td>
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<td>01/2017</td>
<td>[32]</td>
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<tr>
<td>GLP</td>
<td>Janssen Sciences Ireland</td>
<td>01/2015</td>
<td>[33**]</td>
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<td></td>
<td>Emory University</td>
<td>09/2017</td>
<td>[34*,35*]</td>
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<tr>
<td>Substituted GLP</td>
<td>Gilead</td>
<td>06/2018</td>
<td>[36]</td>
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<tr>
<td>Dihydropodibenzon [b,f][1,4] thiazepine-8-carboxamide</td>
<td>Assembly Biosciences, Indiana University Research and Technology</td>
<td>03/2018</td>
<td>[37]</td>
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<td>Tricyclic 4-pyridone-3-carboxylic acid</td>
<td>Hoffmann-La Roche</td>
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<td>Jansen R&amp;D Ireland</td>
<td>06/2018</td>
<td>[40,41]</td>
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<tr>
<td>N-phenyl-carboxamide</td>
<td>05/2018</td>
<td>[39]</td>
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compounds. Lead optimization efforts of the initial BA hits disclosed in 2017, resulted in BA-38017 (Figure 2a), which inhibits HBV replication with an EC_{50} value of 160 nM and induces the formation of empty capsids [29**].

The early SBA lead, DVR-23 (Figure 2a), first disclosed in 2013, inhibits a viral replication step before DNA synthesis to form empty capsids similar to the PPA series [42]. Since their discovery, various optimization efforts were conducted by the Blumberg Institute and collaborators, the R&D groups at Janssen and the Schinazi group at Emory University.

The substituents on Rings A and B of the SBA scaffold (see Figure 1 for designation of ring labels) were explored by the Blumberg Institute as a continuation of their original patent [28]. Several compounds exhibited EC_{50} values below 1 μM against HepDES19 cells. Janssen scientists reported the anti-HBV activity of their SBA library, with EC_{50} values in the range of 59 μM–50 nM in HepG2.2.15 cells. (Figure 2b) [26].

The Schinazi group published their further optimization efforts on the SBA class in 2017 (Figure 2c) [27**]. While the study did not result in improved activities, it elucidated several SAR trends. They conducted molecular modeling to show that a substituted amino group positions in a narrow, hydrophobic, solvent-exposed tunnel, which does not form specific interactions with the HBV capsid protein, and several small, hydrophobic substitutions are well tolerated. A cycloalkyl group gave the best result among the substituted amino groups. Benzyl-sulfonamide-substituted, amino acid-substituted and disulfonamide-substituted compounds were found to be much less potent due to a steric clash. The exchange of the original aniline moiety with substituted benzyl amines resulted in loss of activity. The N-arylamido group is an important moiety because reversing the positions of the NH and C=O functionalities of the amide, N-methylation and substitution with a sulfamoyl moiety diminished the activity significantly. Similarly, rigid bicyclic derivatives exhibited no activity. However, reversing the positions of the NH and SO_2 functionalities of the sulfonamide group largely preserved the potency.

**Figure 2**

Key emerging sulfamoylarylamides and glyoxamoy/pyrrolamides.
A study conducted at Janssen described the low metabolic stability (<10% remaining after 15 min exposure to human liver microsomes, HLM) and low solubility observed in the SBAs [25**]. However, introduction of a 4-fluoro substituent on Ring A, with either branched aminooalkyls or tetrahydrofurans on Ring B, enhanced metabolic stability (Figure 2b). The optimized compound [74% remaining after 15 min (HLM), improved solubility and EC50 = 120 nM (HBV DNA HepG2.2.15)] showed moderate plasma clearance, good oral bioavailability and promising in vivo reduction of cccDNA in genotype D HBV-infected chimeric mice with humanized liver. Janssen reported further biological profiling and revealed that their compounds have a dual mode of action, that is, interfering with both the assembly and disassembly of the HBV cspid and preventing access of de novo hepatocytes to viral genetic material [43].

Sulfamoylpyrrolamides (SPAs)

Janssen’s SAR studies also extended to furan, pyrrole, pyridine, and thiophene substitutions for the phenyl Ring B (Figure 2d–f). Among the sulphanamoylthiophenamides, several potent compounds with EC50 values reaching 32 nM were identified (Figure 2d) [44]. Further exploration revealed that conversion of Ring B to a pyrrole ring improved the potency by 10-fold more than that of sulphanamoylthiophenamides (Figure 2e), establishing a new class of inhibitors, the sulfamoylpyrrolamides (SPAs) [30]. In addition to eliciting a decrease in total viral RNA and pgRNA levels, SPAs regulate multiple steps of the HBV life cycle including HBeg biogenesis [31*]. In 2017, Vendeville et al. at Janssen released their findings on cyclized SPAs, which maintained low nanomolar antiviral activity (Figure 2f) [32]. At present, no additional information regarding the safety, metabolic stability, or in vitro and in vivo studies is available.

Glyoxamoylpyrrolooxamides (GLPs)

GLPs are the newest class of highly potent CAMs, which resulted from the substitution of the sulfonylamide group with a glyoxamide in the SPA series (Figure 2g–i). Janssen, Emory University and Gilead Sciences are currently pursuing the development of these scaffolds, but there has been limited disclosure of these compounds’ profiles, other than their selective inhibition of the HBV viral load, capsid formation and reduction of cccDNA at low nanomolar concentrations.

Vandyck et al. were the first to file a patent application covering the GLP class of compounds [33*]. The active compounds have EC50 values of 2 nM, and the series maintained similar substitutions on Rings A and B, as in the previous series (Figure 2g). The Schinazi group focused their efforts on GLAs and soon after, reported scaffolds with picomolar EC50 values (Figure 2h) [34*]. GLP-26 exhibited Hep AD38 HBV DNA EC50 = 3 nM, and reduced cccDNA by >90% at 1 μM [35*]. Unlike the issues with metabolic stability presented by the SBA class, GLP-26 exhibited promising in vivo data, with a T1/2 of >24 hours (in dog and human plasma) and a T1/2 = 7.6 hours (in HLMs). Dual combination of GLP-26 with Entecavir resulted in a strong, synergistic antiviral effect. Furthermore, these compounds disrupt already-formed capsids [35*].

Gilead Sciences’ recently published patent application describes exploration of substituting pyroles with 2,3-dihydro-1H-pyrrolizines and 1,1a,6,6a-tetrahydrocyclopenta[b]pyrrolizines (Figure 2i) [36]. Both of these series exhibited low nanomolar HBV inhibition and T1/2 values ranging from 4 to 16 hours. Interestingly, introduction of a 1,2,3-triazole branch off of the 3,3-difluorocyclobutylazane ring led to a fivefold increase in potency (EC50 = ~2–4 nM) when compared to structures with a 1-methylsubstituted 3,3-difluorocyclobutylazane ring (EC50 = ~60–100 nM).

Development of novel classes of CAMs

Aside from the structurally related SBAs, SPAs and GLAs, the past three years have seen an emergence of structurally novel classes of HBV antivirals. Assembly Biosciences filed a patent application for novel dihydrodibenzothiophene-8-carboxamides (Figure 3a) [37]. The percent of cccDNA viral load was measured at 10 μM for this series, which suggested promising activity. Additionally, a published patent application by Hoffmann-La Roche Inc. reports tricyclic 4-pyridone-3-carboxylic acids (Figure 3b) [38], whose EC50 values were found to be in the mid-nanomolar concentrations. Researchers at Janssen are currently developing N-phenyl-carboxamidine derivatives, which also have mid-nanomolar to low-nanomolar EC50 values against cccDNA (Figure 3c) [40,41]. Further biological profiling has not yet been disclosed for these scaffolds.

Summary and future directions

Current HBV therapies suppress viral replication but are ineffective as a cure for the disease as they do not eradicate cccDNA, which provides for viral persistence. This review highlights recent advances in the development of CAMs to suppress HBV viral load and decrease cccDNA formation. As studied in various cell lines SPAs and GLPs demonstrate promising in vitro and in vivo activities, but more focus on efficacy and safety results is warranted. Examining the utility of new molecules is limited to clinical trials as there is a lack of cell cultures that support multiple rounds of HBV infection, and are limited in the study of viral spread. Furthermore, all of the cell cultures produce extremely low quantities of cccDNA which is another limitation that needs to be addressed in the future [45]. However, with the emergence of novel CDMS studies of the nucleocapsid assembly process, experiments using CAMs could highlight whether these modulators interfere with the actual
assembly or with the self-correction process of nucleocapsids. Furthermore, the application of CAMs in combination with existing antiviral agents could identify a tandem mechanism to block cccDNA formation to achieve a clinical cure.

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**Conflict of interest statement**

Nothing declared.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


12. Qazi S, Schlucksup CJ, Rittichier J, VanNieuwenhze MS, Zlotnick A: An assembly-activating site in the hepatitis B virus capsid protein can also trigger disassembly. *ACS Chem Biol* 2018, 13:2114-2120. Site mutation of the dimer–dimer interface of the HBV core proteins was used to examine the effect of covalently linking small molecules to them. These ligands triggered a destabilization conformation causing capsid disassembly in a dose-dependent manner.


HAP and SBA are likely to have distinctive resistance profiles due to differences in a unique hydrophobic subpocket that is occupied by the thiazole group of the representative HAP.


This review summarizes the recent advances in the discovery and development of novel anti-HBV small molecules.


Efforts toward the hit-to-lead optimization of the SBA class and further profiling of the mode of action of JN0J632.


Molecular modeling and SAR studies around the SBA scaffold elucidate key features necessary to achieve potent HBV DNA inhibition.


First report to demonstrate that the benzamide class promote the formation of empty capsids through specific interactions with the HBV core.


Antiviral profiling of SBAs in differentiated HepaRG cells revealed inhibition of de novo establishment of cccDNA.


First disclosure of GLP inhibitors of HBV DNA.


Extensive SAR studies around the GLP scaffold to achieve potent analogues.


Disclosure of further profiling of a promising GLP.


