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REVIEW ARTICLE

Successes in antiviral drug discovery: a tribute to Nick Meanwell

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Abstract

Drug discovery is a difficult task, and is even more challenging when the target evolves during therapy. Antiviral drug therapy is an excellent example, exemplified by the evolution of therapeutic approaches for treatment of hepatitis C and HIV-1. Nick Meanwell and his colleagues made important contributions leading to molecules for treatment of hepatitis C and HIV-1, each with distinct mechanisms of action. This review summarizes the discovery and impact of these drugs, and will highlight, where applicable, the broader contributions of these discoveries to medicinal chemistry and drug discovery.

Keywords Antiviral drug discovery · NS5A · NS5B · NS3A protease · Hepatitis C · HIV-1

Introduction

Antiviral drug discovery in 2023 is a highly active therapeutic endeavor with substantial value to human health as a result of the Covid-19 pandemic. It is likely to remain important for the foreseeable future, and one in which lessons learned from the past can be applied. Two characteristics of viruses combine to make this task especially challenging: viruses are parasites that reproduce within living cells and they will mutate in ways that can improve viral survivability and thereby reduce efficacy of therapeutics. Four successful case histories in this field highlight where Dr. Meanwell and colleagues made seminal contributions and provide notable problem solving examples to multiple approved antivirals with distinct mechanisms of action for treatment of hepatitis C (HCV) and HIV-1 infections. Their work is a part of large body of research that resulted in a cure for HCV, and where HIV-1 is now a chronic, rather than fatal, disease that can be managed.

Hepatitis C is an RNA flavivirus that was discovered in 1975 and characterized in 1989 [1]. Infection with this virus affects millions worldwide and can have severe effects on liver function, including cancer, making an effective therapy a substantial goal [2]. Several nonstructural proteins (NS) were identified in the viral genome, and among these two proteins, NS3/4A and NS5B RNA dependent RNA polymerase attracted interest because both are essential for virus replication, their respective druggable functions as a serine protease and an RNA dependent RNA polymerase, respectively benefited from a wealth of existing knowledge [3, 4]. Two complications quickly became apparent that made drug discovery more challenging. Seven different genotypes of the virus (GT1-7) were identified, with distinct distribution throughout the world and cell-based culture methods needed to be developed to accurately reflect the viral life cycle [5, 6]. Ideally one would prefer a single suitably safe compound with comparable efficacy against all seven genotypes, recognizing that as an alternative, a compound with activity against the predominant genotypes would be acceptable. Screening methodology using cell-based systems was problematic, and as a result, those interested in discovery of NS3 and/or NS5 enzyme inhibitors relied on target-based techniques amenable for high throughput screening (HTS), with NS3 protease the first target to be investigated.

Bristol Myers carried out an NS3 protease inhibitor HTS screen and found no hits. A parallel, a cell-based screen using bovine viral diarrhea virus (BVDV), a related virus that could be cultured allowing for a dual assessment of mammalian cell cytotoxicity, also failed to furnish hits that could be validated in biochemical assays, forcing the team to adopt a design approach based on peptide and nonpeptide compounds known to inhibit serine proteases [7]. Data from peptidic inhibitors revealed key binding pockets on NS3,

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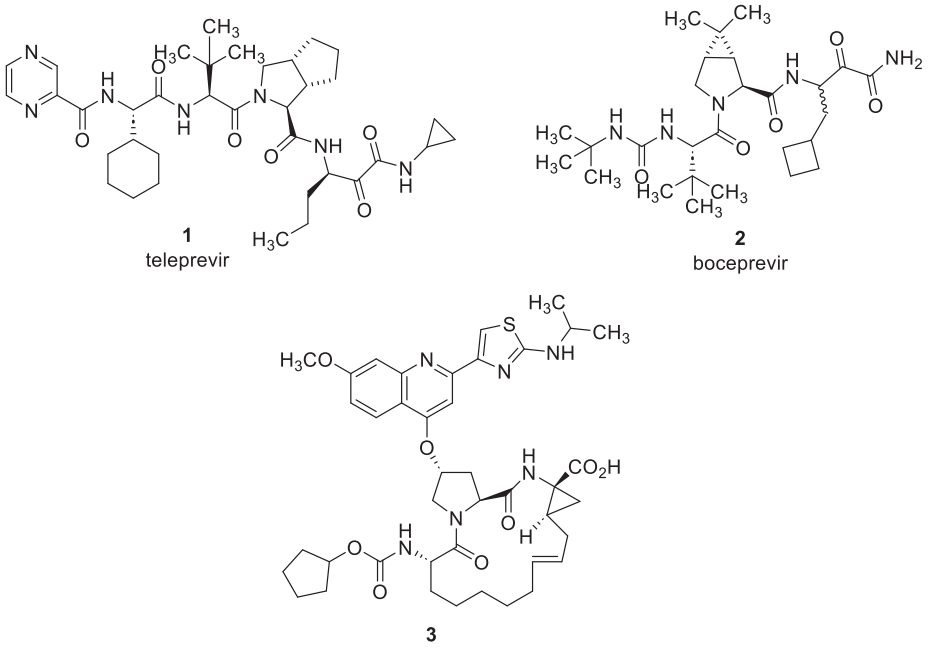
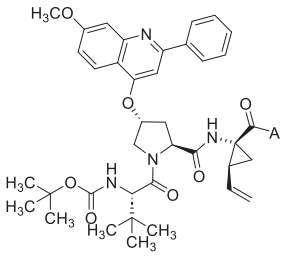


Fig. 1 First generation HCV NS3A Protease Inhibitors

that amino acid binding pockets surrounding the peptide cleavage site were unlike mammalian serine proteases and revealed the enzyme was subject to product inhibition [8]. Incorporation of known serine protease inhibitor moieties such as α-keto amides, coupled with modification of these peptide inhibitors furnished first generation NS3 protease inhibitors such as teleprevir (**1**) and boceprevir (**2**, Fig. 1) that were approved for use in the US in combination with pegylated interferon α and ribivarin [9, 10].

These initial NS3 protease inhibitors required frequent high doses, had to be administered for several months and efficacy was limited by frequent viral mutation. NS3 inhibitor discovery was stimulated by a report that a macrocycle discovered at Boehringer Ingelheim, **3** (ciluprevir, BILN-2160, Fig. 1) was a potent inhibitor (EC_{50} 1.2 nM) with excellent selectivity versus mammalian serine and cysteine proteases, good oral bioavailability and antiviral activity in a phase 1 trial [11]. More importantly, virus levels in plasma were reduced to undetectable within 48 hours after the first dose in GT-1 infected patients, a feature that was difficult to achieve with existing combination therapy.

Careful analysis of the crystal structure revealed opportunities for design of novel NS3 protease inhibitors based on macrocycle **3**. One particular feature was explored by the BMS group, namely application of an acylsulfonamide to replace the carboxylic acid to occupy the S1 pocket and extend into the S1' pocket on the enzyme to enhance potency. Acylsulfonamides are known to have similar acidity to carboxylic acids, and in early model compounds showed promise in both enzymatic and cellular assays. As shown in Fig. 2, acylsulfonamide **6** demonstrates a significant improvement in both enzymatic and cellular activity [12].



4 A = OH, IC_{50} 54 nM, EC_{50} 550 nM
5 A = $NHSO_2CH_3$, IC_{50} 36 nM, EC_{50} 600 nM
6 A = $NHSO_2$-cC_3H_5, IC_{50} 1 nM, EC_{50} 4 nM

Fig. 2 Acylsulfonamide-based NS3A Protease Inhibitors

This early proof of principle example provided the impetus to continue to develop this lead. SAR in the S1' pocket was carefully investigated, where it was discovered that a cyclopropyl group was favored (Figs. 2, 6). As a part of this work, the team also developed an efficient novel synthesis of the requisite sulfonamide building block. In spite of low bioavailability and rapid clearance in rat and dog, **6** concentrated in rat liver, and interestingly had good in vitro metabolic stability in microsomal preparations.

Careful structure-based analysis aimed at simultaneously identifying important enzyme-inhibitor interactions, coupled with a need to reduce molecular weight revealed that the quinoline ring could be modified to an isoquinoline and the phenyl group could be eliminated to furnish a comparably potent NS3 protease inhibitor (**7**, Fig. 3) [12] that achieved a significant increase in AUC and liver concentration following intraduodenal dosing. Liver uptake was considered an important feature in profiling because the virus replicates in this organ. This analog was extensively profiled in safety and

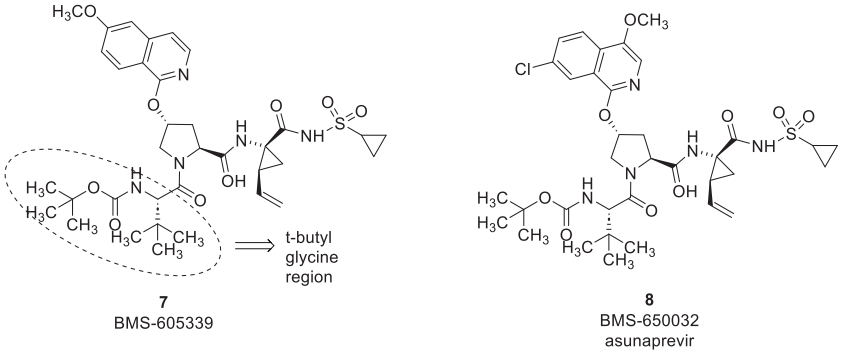


Fig. 3 BMS NS3 protease HCV clinical candidates

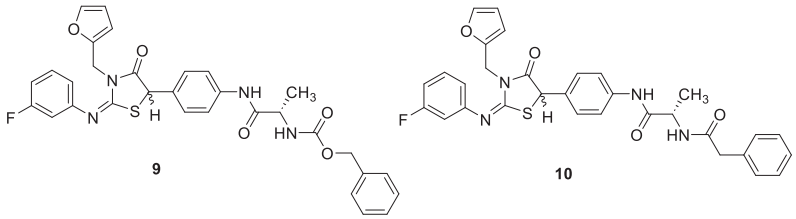


Fig. 4 Early NS5A hit and lead

pharmacokinetic studies in which a profile emerged that merited human clinical evaluation. Four doses (10, 30, 60 and 120 mg) in a single ascending dose study were administered to healthy volunteers. Plasma levels increased with dose, with a terminal half-life between 4 and 8 h. In phase Ib studies, three doses (10, 50 and 120 mg) were evaluated in HCV-infected patients. A dose-response was noted in all patients at each dose; at the highest dose a mean 1.8 $\log_{10}$ IU/mL decrease in virus levels was measured 12 h after dosing. However, some receiving drug also displayed mild bradycardia, junctional rhythm disturbances and PR-interval prolongation were noted. These cardiovascular effects resulted in termination of further clinical studies, and established a need to include detailed study of such effects preclinically. This resulted in use of a Langendorff isolated heart assay at a high concentration of a candidate. This assay evaluates heart rate, heart rhythm and contractility intervals in an intact, perfused organ. Careful analysis of data from this complex, low throughput assay led to the hypothesis that the methoxy substituent in **7** played a key role in these cardiovascular effects.

As a result of this analysis, extensive SAR investigations in the isoquinoline ring and in the t-butyl glycine region in **7** were carried out to identify potent, selective, orally bioavailable candidates with an appropriate level of cardiac safety [13]. The team observed that the t-butyl glycine region of **7** was best left unchanged and that minor changes to the isoquinoline ring provided the best combination of protease inhibition, cellular activity, pharmacokinetics and safety. These studies furnished **8** (Fig. 3, BMS-650032) which was ultimately approved for use in Japan as asunaprevir in combination with daclatasvir for treatment of HCV genotype 1 infection [14].

Treatment of HCV infections evolved rapidly during the early 2010s, with the discovery that direct-acting antivirals might prove to be curative. In 2012, a phase IIa study using **8** and an NS5A replication complex inhibitor daclatasvir (**18**, Fig. 6 below) showed 36% of patients treated with this combination achieved a sustained virologic response after 12 and 24 weeks of therapy, without the need to add ribivarin or interferon [15]. The significance of this observation was cited as a "watershed moment" in a commentary associated with the publication [16]. Approval of sofosbuvir, an inhibitor of HCV NS5B polymerase, developed by Gilead, that in combination with ribivarin, as curative therapy, with a twelve week course of therapy made this goal a reality for hepatitis C patients [17]. This demonstrated that safe, effective HCV treatment could be achieved in a shorter period of time and stimulated research into other viral targets that could be combined with sofosbuvir to reduce the length of therapy further and/or reduce the incidence of resistance.

The BMS team employed cell-based phenotypic assays to screen for leads because such assays, while more complex, permit the discovery of hits that act on targets that require this complexity [18]. To help identify hits with HCV selectivity, BMS included the BVDV virus investigated in the discovery of asunaprevir, coupled with cell viability determination to establish if hits were comparatively non-toxic to mammalian cells and acted by a distinct mechanism. This led to the discovery of **9** (Fig. 4), a hit with an EC_{50} of ~750 nM that was selective for HCV versus BVDV and showed no mammalian cell toxicity up to 50 µM. SAR studies led to a more potent analog **10** (EC_{50} 5 nM) that was used in viral mutation experiments to confirm that NS5A was the protein target [19].

The iminothiazolidinone ring in these compounds underwent oxidative degradation in solution and the impurities were carefully characterized by NMR and mass spectrometry. During the research to understand this process, the team recognized that **10** decomposed in HCV cell culture to two major products that demonstrated antiviral activity. Incubation of **9** in HCV cell culture medium on a larger scale allowed for purification and structure elucidation of the products. The most active of the two was a dimeric structure in which a new carbon–carbon bond formed between the two thiazolidinone rings at the epimeric methine position. Subsequent testing of this compound showed it more potent than **10**, with an EC_{50} of 0.6 nM. The team hypothesized that improved potency was associated with dimerization, and this concept was applied during exploration of chemically stable templates [19].

The challenge associated with implementing this in drug design required creative thinking to replace the complex heterocyclic structure with lower molecular weight alternatives, while keeping in mind the extensive SAR that was available during the optimization campaign leading to **10**. Comparison of two alternatives, one using a stilbene replacement (**11**) and a second based on a bi-benzyl scaffold (**12**, Fig. 5), and incorporating a proline-based replacement for the benzyl amide showed that there was a substantial difference in activity, with the stilbene nearly 350-fold more potent using a GT-1b variant of HCV [19].

Evaluation of **11** against various known genotypes of HCV showed that it was significantly less active against the GT-1a variant, which was the most prevalent North American subtype, with substantial distribution worldwide. Replacement of the stilbene and the need to identify alternatives to the anilide moieties because of their possible hydrolytic instability and mutagenic potential of the resulting metabolites were key tasks for the medicinal chemistry team. Earlier SAR demonstrated that the amide functions on the pyrrolidine ring were important contributors to HCV potency. This was applied in optimization of **11** to discover analogs that retained the stilbene and anilides to provide compounds with acceptable pharmacokinetic properties and GT-1a activity with the expectation that favorable candidates lacking these known liabilities would follow.

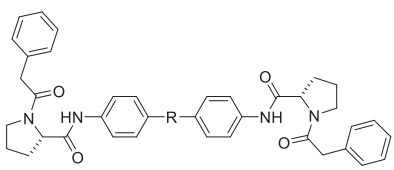


Fig. 5 Dimeric NS5A inhibitors

Progression from **11** to daclatasvir went through the alkyne analog **13** and benzimidazole **14** (Fig. 6) that showed a similar activity differential against GT-1a and -1b. These analogs removed the stilbene and anilide liabilities, respectively. To address the substantial difference in 1a versus 1b potency, the alkyne was replaced with a biphenyl, and the benzimidazole was dissected into a separate imidazole ring leading to **15** that provided a highly potent candidate versus both genotypes. Pharmacokinetic examination of **15** showed poor absorption, thought to be associated with high molecular weight. De-symmetrization, reflected in **16**, furnished a molecule with modest bioavailability in rats and mice and improved bioavailability in dogs, accompanied by reduced GT-1a potency. While investigating this aspect of SAR, a new, symmetrical compound, **17**, restored both potency and pharmacokinetics. Replacement of the alanine fragment in **17** with valine furnished daclatasvir **18** [20].

Daclatasvir demonstrated potent antiviral activity against all wild-type HCV genotypes and selected examples of drug-resistant variations. Equally important, **18** furnished liver levels suitable for efficacy in preclinical species, a promising observation for this disease. In early clinical studies, a rapid and durable effect on viremia was measured at doses as low as 1 mg, resulting in as much as a 1.8 $\log_{10}$ IU/mL decrease in viral load. Higher doses provided correspondingly greater effects, extending up to 48 h post dose [21]. This rapid, direct antiviral effect occurred more quickly than with other approved NS3A or RNA-dependent RNA polymerase inhibitors. Subsequent experiments revealed that this potent and long-lasting effect was due to an unanticipated effect on viral assembly [22]. When used as a single agent, resistance to daclatasvir rapidly developed, leading to exploration of various combination therapies. Fortunately, the discovery that a mechanistically distinct direct acting antiviral, sofosbuvir (**19**, Fig. 7) [23], a prodrug that acts as a defective substrate for NS5B leading to chain termination demonstrated excellent activity in combination therapy as well as a low likelihood of resistance. The two agents could be effectively combined as shown by others [24].

The coronavirus pandemic stimulated exploration of existing direct-acting antiviral agents for efficacy. Daclatasvir in combination with sofosbuvir was investigated in Covid-19 patients in both open label and placebo controlled studies. A large (1083 patient) study in Iran showed that this combination offered no benefit versus placebo on the time to hospital discharge or survival [25]. Another smaller trial of 55 patients with mild Covid infection were studied for symptomatic relief. There was no difference in symptom relief or hospitalization after seven days. After one month on the combination, there was a significant decrease in fatigue and dyspnea in patients receiving the combination [26].

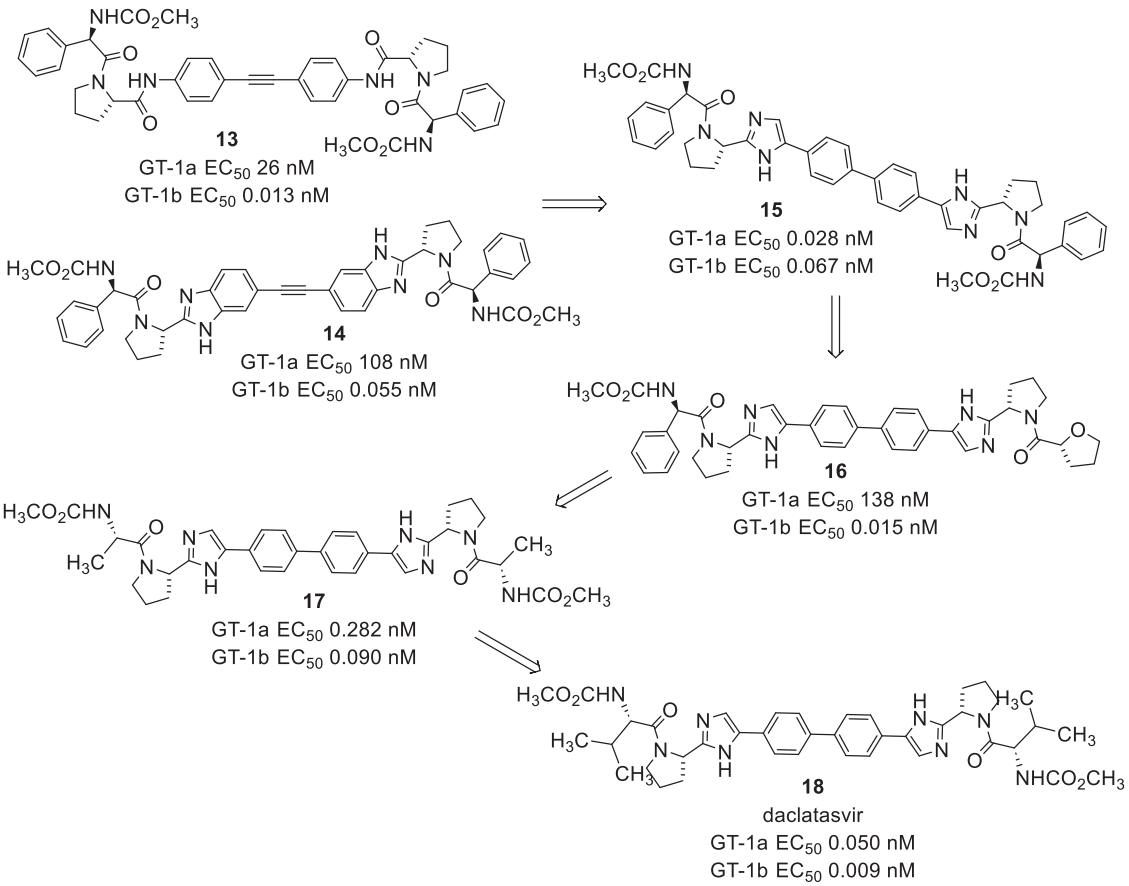


Fig. 6 Lead optimization leading to daclatasvir

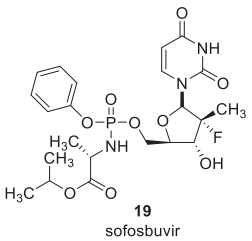


Fig. 7 Sofosbuvir

The team at BMS continued to investigate discovery of additional anti-HCV targets because it was clear that combination therapy, involving as many as three mechanistically different agents, for HCV was a key. As noted above, BMS demonstrated proof of principle that a combination of an NS3A protease inhibitor with an NS5A replication inhibitor was curative. This was amplified by observation that sofosbuvir, an NS5B inhibitor, displayed a comparatively lower incidence of resistant HCV mutants. To complement and extend these ongoing investigations, attention was directed toward discovery of allosteric inhibitors of NS5B. Structure-activity relationships in an indole-based chemotype showed that this scaffold bound allosterically to NS5B, e.g., **20** [27], allowing for differentiation relative to sofosbuvir. The complex structure could be simplified to improve pharmaceutical properties such as water solubility, resulting in **21**, with a seven-fold reduction in anti-HCV activity (Fig. 8). Optimization was assisted by structure-based design that revealed key interactions and conformational information on how potential inhibitors interacted with the protein [27]. Introduction of polar functional groups into the benzazepine ring improved both pharmaceutical properties and potency, exemplified by **22**. Potency was improved further by appending the amide substituent in **20** to **21**, affording **23**. Pharmacokinetic examination of **22** and **23** showed that oral bioavailability and clearance of the more complex structure were poor compared to the much simpler analog **22**.

Continued structure-activity studies of **22** revealed that the endocyclic amide could be replaced by an exocyclic version, with the benefit of adding a vector for rapid exploration of amide functionality. A racemic morpholine amide **24** (Fig. 9) had good potency (~200 nM), with poor pharmacokinetic properties that were likely due to rapid formation of an acyl glucuronide. Polar replacements were examined to help provide improved water solubility and

after some work, an acylated sulfonyl urea functional group was found to be suitable, as in **25**. It was also discovered that a methoxy substituent on the phenyl ring was an additional contributor to improved pharmacokinetics and potency. A further improvement in potency was observed when the alkene was cyclopropanated and the diastereomers separated to furnish **26**. However, during profiling, PXR activation, a marker for CYP3A4 induction, was observed in morpholine amide analogs. This issue was addressed in two ways: by adding alkyl groups to the morpholine and by exploring more polar amides such as **27**. The latter was favored because this also improved water solubility. The pharmacokinetics of **27** were promising, furnishing liver levels of approximately 7.5 μM following a 10 mg/kg dose in rats, and there was no PXR activation. Final optimization involved fine-tuning the piperazine ring to include study of bridged analogs. A two carbon bridge nearer the amide carbonyl (**28**) proved to be measurably more potent

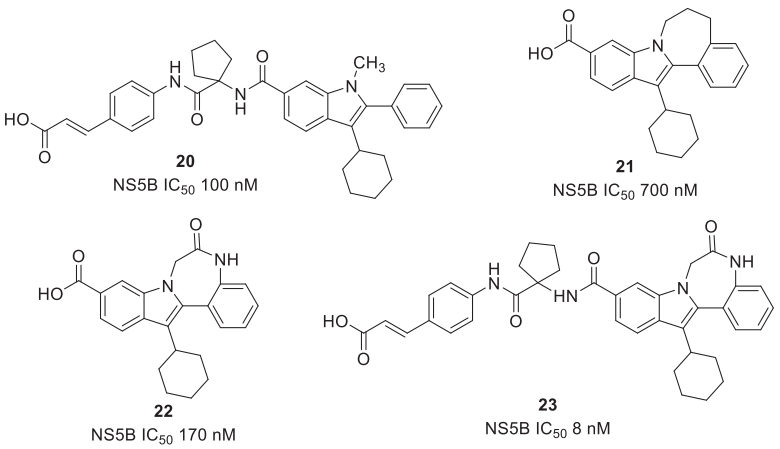


Fig. 8 Early allosteric NS5B leads

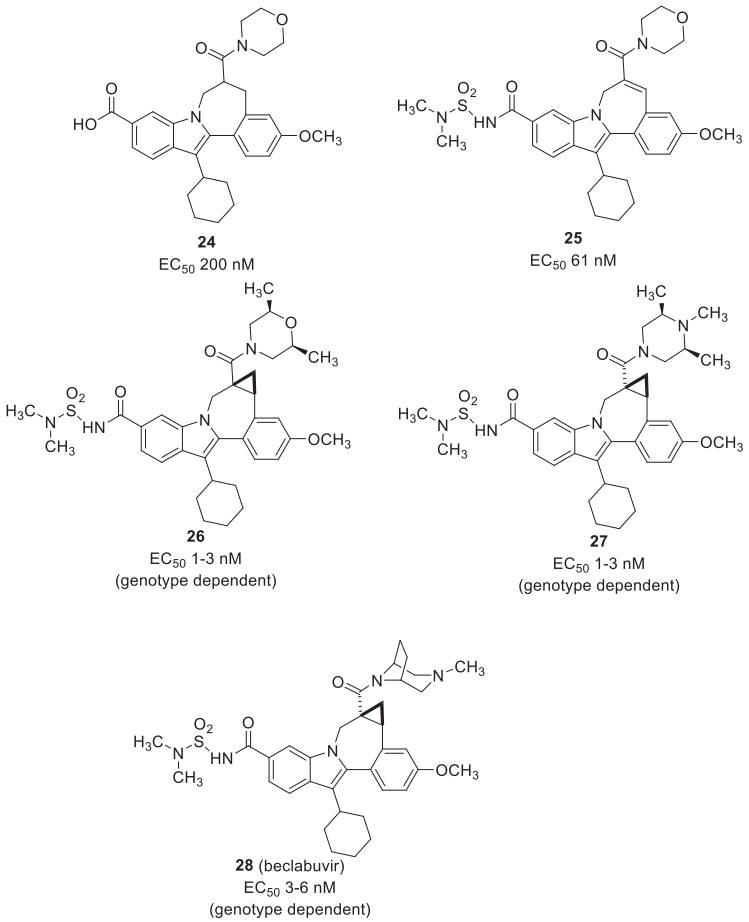


Fig. 9 Evolution leading to beclabuvir

compared to a regioisomer with the bridge further away. The pharmacokinetics, PXR and genotypic activity profile of **28** all were very promising, leading to advancement into clinical evaluation and ultimately approval as beclabuvir.

The final example of successful antiviral drug discovery targeted a different virus, HIV-1. When Acquired Immune Deficiency Syndrome was first identified in 1981, the causative organism was unknown and laboratories around the world began an intensive search with a focus on viruses [28]. The virus was definitively identified by two groups working independently in the US and France [28]. This set off a worldwide effort to treat this previously fatal disease. The advent of antiretroviral therapy that initially targeted reverse transcriptase, followed by HIV-1 protease made the disease treatable, but not curable [29, 30]. However, like HCV, treatment required multiple drugs with distinct mechanisms of action to reduce the likelihood of resistance and prolong life.

The team at BMS undertook this challenge to identify a candidate with a new mechanism of action targeting the requisite association of the virus via a surface glycoprotein, gp120, with CD4, a receptor on cell surfaces that is needed for viral entry. A high-throughput phenotypic screen revealed a simple indole glyoxamide hit as an attachment inhibitor (AI, **29**, Fig. 10) that showed high selectivity for HIV-1 compared to a range of other viruses, including HIV-2, a simian immunodeficiency virus and bovine viral diarrhea virus mentioned previously. Optimization of **29** furnished a clinical candidate **30** with modest antiviral efficacy (less than a 2 log reduction in viral load) at high dose (800–1600 mg), with a limited spectrum of activity against a panel of HIV genotypes [31]. There was a clear need to improve on this initial candidate to make this mechanism of action a viable contributor to anti-HIV treatment.

Aqueous solubility of **30** was one important issue associated with this project. Existing structure activity relationships suggested a solution by increasing the polarity of the indole fragment and a series of azaindoles were studied. Implementation required development of synthetic methodology to enable not only the synthesis of desired substituted azaindoles, but also allow for installation of the essential glyoxamide moiety. Two different solutions were devised for different azaindoles, allowing the examination of nitrogen at each position [32, 33]. Each of these four azaindoles had at least a twenty-fold improvement in water solubility relative to the indole parent as hoped. All of them retained good antiviral activity, with low mammalian cell toxicity and no hERG inhibition. After examining, then discarding representative 4- and 7-aza derivatives, attention was focused on 6-azaindoles that furnished **30**.

While work on the azaindoles was underway, parallel effort studied a range of C7 substituents in the original indole chemotype. It was discovered that secondary amides were of particular interest and incorporation of heterocycles, e.g., **31** and **32** (Fig. 11) provided highly potent (picomolar) anti-HIV agents. These compounds proved to be unsuitable for progression due to metabolic stability and/or cell permeability issues. However the information was directly applied to 6-azaindole derivatives in which hypothetically preferred conformational flexibility was enforced by intramolecular hydrogen bonding, as shown in partial structure **33**.

As an alternative to amides, direct heterocycle substitution at C7 was investigated, with the aim of maintaining the key hydrogen bonds to enforce a coplanar arrangement of this substituent as shown in Fig. 10. Examples are shown in Fig. 12 and in general this group of AI compounds tended to show improved pharmacokinetic properties and an

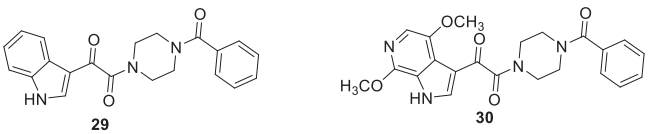


Fig. 10 HTS hit and initial AI clinical candidate

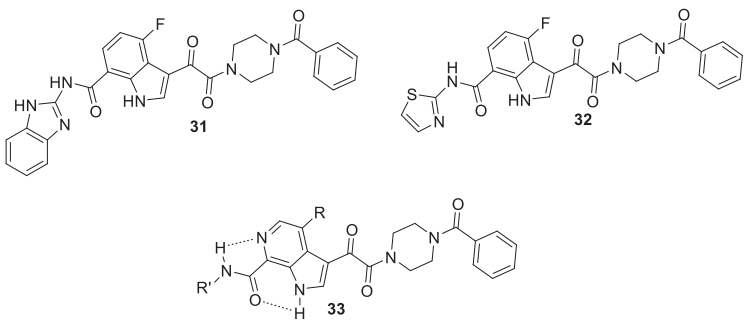


Fig. 11 C7 indole amides and conformational restriction

improved spectrum of antiviral activity compared to others. More detailed binding experiments were carried out with **36** using **30** as a comparator because **36** demonstrated activity against HIV isolates from patients resistant to HIV protease therapy. These studies revealed an unusual biphasic profile that showed **36** bound twenty-fold more tightly and with slower dissociation rate [34, 35].

The promising profile of these heterocyclic AIs was being developed in conjunction with clinical evaluation of **30** with pharmacokinetic and clinical information from this work providing a guide for an improved attachment inhibitor. A high fat meal was required to maintain plasma levels of **30** above the minimal levels needed to maintain anti-HIV efficacy. This was thought to be due to the poor aqueous solubility of **30** that limited dissolution and absorption from the GI tract. The aqueous solubility of **34** and **36** was somewhat improved, however it remained less than ideal, leading to an effort to identify a suitable prodrug form that would solve this issue. The team took advantage of existing experience with phosphate prodrugs that reliably improved aqueous solubility for several drugs, including phenytoin, two different anticancer compounds an antifungal and another antiviral agent.

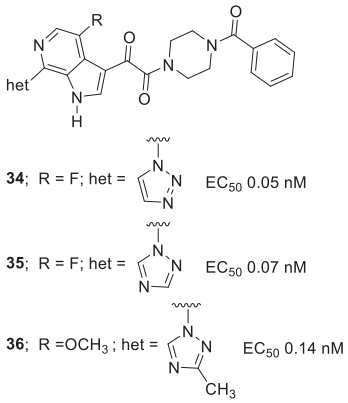


Fig. 12 Heterocyclic attachment inhibitors

Using the indole nitrogen, acid labile phosphate phosphate esters (**37**) were readily prepared (Fig. 13) and showed rapid acid-mediated hydrolysis, to furnish the corresponding acid **38**. Alkaline phosphatase-mediated phosphate cleavage followed by spontaneous decomposition of the hydroxymethyl intermediate **39** led to the active compound. Pharmacokinetic studies demonstrated rapid formation and dose-related increases in plasma levels of the active compound, even in the absence of food.

In preclinical studies, the phosphate prodrug derived from **36** delivered parent drug effectively to plasma in a dose-related fashion that was not affected by food, a profile largely reproduced in clinical studies. Concerns over release of formaldehyde from the prodrug were addressed based on the anticipated doses and established safety of related phosphate prodrugs that were already marketed and in use. A suitable salt form of **38** was identified and the compound advanced to clinical development. Phase III studies were undertaken in multi-drug resistant HIV patients who were receiving additional anti-HIV medications. Viral load was reduced at eight

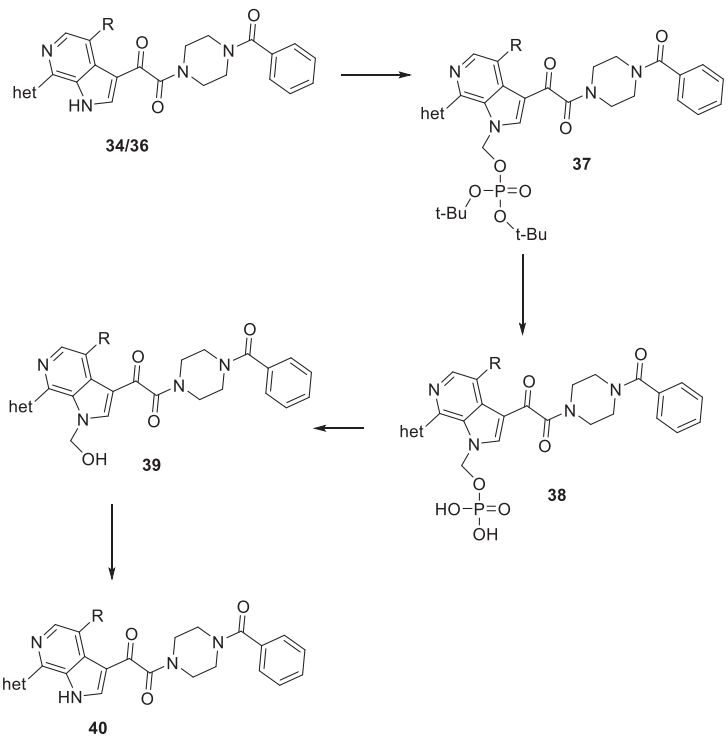


Fig. 13 Prodrug discovery of temsavir

days in patients receiving **38**, and at 48 weeks 54% of those in the treatment group had lower viral loads compared to patients in the placebo group. These results were sufficient to warrant approval for use and provide a novel mechanism of action for treatment of this disease.

Summary

The foregoing examples in successful antiviral drug discovery are a tribute to the skills and creativity of Nicholas Meanwell and his colleagues. This review is not intended to be a complete discussion of each project because that information is readily available elsewhere by those who were directly involved. In each case, there were distinct questions and issues that required solutions. It is important to keep in mind this work was carried out with pressing time constraints to get safe, effective agents to patients who urgently needed them. There are multiple lessons in this work that those in drug discovery, both chemists and biologists, can learn and adopt to their individual work. These include to use of appropriate experiments to create new molecules and evaluate their activity in complex systems, as well as to understand molecular features that play a key role in that activity.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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