Antiviral Chemistry and Chemotherapy
2018, Vol. 26: 1–8

DOI: 10.1177/2040206618756430
journals.sagepub.com/home/avc
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Current and future use of nucleo(s)tide prodrugs in the treatment of hepatitis C virus infection

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Abstract

This review describes the current state of discovery of past most important nucleoside and nucleotide prodrugs in the treatment of hepatitis C virus infection as well as future potential drugs currently in discovery or clinical evaluation. I highlight first generation landmark prodrug compounds which have been the foundations of incremental improvements toward the discovery and approval milestone of Sofosbuvir. Sofosbuvir is the first nucleotide prodrug marketed for hepatitis C virus treatment and the backbone of current combination therapies. Since this approval, new nucleotide prodrugs using the same design of Sofosbuvir McGuigan prodrug have emerged, some of them progressing through advanced clinical trials and may become available as new incremental alternative hepatitis C virus treatments in the future. Although since Sofosbuvir success, only minimal design efforts have been invested in finding better liver targeted prodrugs, a few novel prodrugs are being studied and their different modes of activation may prove beneficial over the heart/liver targeting ratio to reduce potential drug–drug interaction in combination therapies and yield safer treatment to patients. Prodrugs have long been avoided as much as possible in the past by development teams due to their metabolism and kinetic characterization complexity, but with their current success in hepatitis C virus treatment, and the knowledge gained in this endeavor, should become a first choice in future tissue targeting drug discovery programs beyond the particular case of nucleos(t)ide analogs.

Keywords

Hepatitis C virus, nucleoside analogs, nucleotide analogs, prodrugs, NS5B

Date received: 21 September 2017. accepted: 13 December 2017

Introduction

According to a recent report,[1] in 2015 globally, an estimated 71 million people were living with chronic hepatitis C infection accounting for 1% of the world population, with only 20% knowing their infection status. Mortality was still increasing and an estimated 1.75 million new HCV infections occurred worldwide in 2015. Infection with HCV becomes chronic in most infected persons and a person may be infected with HCV for as long as 30 years or more before developing any clinical symptoms of disease and 20% or more develop life-threatening end-stage chronic liver disease, such as cirrhosis or hepatocellular carcinoma. In 2015, HCV led to 411,000 deaths.

The research for more effective HCV treatments has developed and advanced significantly in the recent years and the focus on direct-acting antiviral agents (DAAs) and specially nucleotide prodrugs having a broad genotypic coverage and high barrier to resistance have emerged as the best promise for backbone combination to eradicate HCV in the next decade.

Nucleo(s)tide prodrugs are pharmacologically inactive modified analogs able to be transformed in vivo to their parent nucleo(s)tide via metabolic or chemical processes occurring in the body. For the purpose of clarity, I will here use under the generic "prodrug" term only "carrier prodrugs" (covalently bound chemical entity releasing the "drug" by hydrolytic cleavage at the target site) and not bioprecursors

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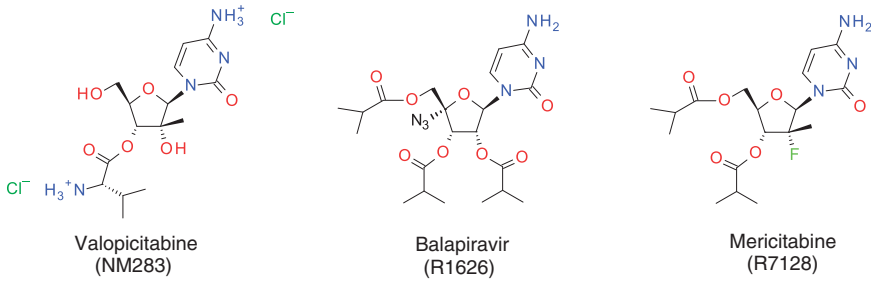


Figure 1. First clinical stage nucleoside prodrugs. (a) Valopicitabine (NM283), (b) Balapiravir (R1626), and (c) Mericitabine (R7128).

(chemical entity metabolized into the pharmacologically active entity) as defined elsewhere.[2]

This review will cover the different main current and future prodrugging strategies used with the more significant reported active nucleo(s)tides which landmark the field of HCV. Comprehensive reviews of nucleos(t)ide prodrugs have been reported elsewhere.[3–9]

A-nucleoside prodrugging

Both nucleosides and nucleotides can be prodrugged depending on the shortcoming properties one wants to overcome.

Nucleoside prodrugging is performed on a nucleoside that can be efficiently metabolized to its active triphosphate (TP) in order to overcome bioavailability or tissue targeting shortcomings. The more common oral bioavailability issues are usually due to lack of

- permeation through biological membranes (lipophilicity is too low) and the prodrug design will mask or counterbalance the polar functions of the parent nucleoside (e.g. *iso*butyryl esters of Balapiravir), or taking advantage of amino acid active transport (e.g. L-valine ester of Valopicitabine);
- solubility, which is less common with nucleoside analogs, but can be mitigated by prodrugging with polar or ionizable pro-moieties (e.g. valine esters);

Addressing tissue targeting topics can be more complex as the required enzymatic pro-moiety cleavage in a specific tissue can be different from one parent nucleoside analog to another as well as species dependent.

The first nucleoside prodrug evaluated in clinical trials for HCV was NM283 from Idenix (Figure 1), a 3′-L-valine ester of its parent nucleoside NM107 setting the scene in HCV therapies with the most used 2′C-Me sugar modification. The valine ester substituent was chosen to improve poor bioavailability of NM107 when given orally.[10] NM283 was shown in later clinical phases to be not stable enough in the gastrointestinal (GI) tract, leading to GI side effects and was discontinued.[11]

Other first generation prodrugs followed with Balapiravir and Mericitabine, both tri- and di-*iso*butyryl esters, respectively, of their corresponding nucleoside (Figure 1).

None of these first generation nucleoside prodrugs led to sufficient clinical benefit to allow approval of a simple nucleoside prodrug, because daily dose normalized viral load reductions were too low (Table 1).[25]

B-nucleotide prodrugging

On the other hand, nucleotide prodrugging is usually performed to overcome 5′-monophosphorylation problem or to improve liver targeting. As opposed to nucleoside prodrugging, the advantage in HCV activity of a 5′-monophosphate prodrug can be demonstrated in cell culture experiments as shown in Table 2.

1. SATE-phosphoramidate prodrugs

The first clinical proof of concept for such kind of nucleotide prodrugs was reported by Idenix with the discovery of IDX184 (Scheme 1).[40,41] Other nucleotide prodrugs were then reported based on the 2′C-Me well-known sugar backbone with different prodrug moieties giving various improvements over the parent nucleoside.

IDX184 is a benzylamine/"SATE" phosphoramidate prodrug which benefits from a thioester enzymatic cleavage liberating the corresponding carboxylic acid and the 2-thioethyl side chain which undergoes self emulating cleavage.[42] While ethylene sulfide was proposed as a cleavage metabolite, it has been shown that this metabolite was not found in vivo, but glutathione adduct was instead formed.[43] The benzylamine phosphoramidic acid is further cleaved by a phosphoramidase to yield the 5′-monophosphate.[31] Further metabolism by cellular kinases gives the active corresponding TP (Scheme 1).

IDX184 improved dramatically the clinical dose efficiency as over a two weeks once a day 100 mg dose

Table 1. Clinical dose efficiency of HCV nucleoside and nucleotide prodrugs.

Prodrug	Daily dose	Viral load reduction (log_{10}) at end of treatment[a]	Dose normalized viral load reduction (log_{10}/g)
NM283[12–15] (Valopicitabine)	800 mg	1.2 (2 wk treatment)	1.5
R1626[16] (Balapiravir)	3000 mg (1500 mg bid)	1.2 (2 wk treatment)	0.4
R7128[17] (Mericitabine)	3000 mg (1500 mg bid)	2.7 (2 wk treatment)	0.9
IDX184[18]	100 mg	2.7 (2 wk treatment)	27.0
GS-7977 (Sofosbuvir)[19,20]	400 mg	4.7 (1 wk treatment)	11.8
BMS-986094[21]	100 mg	2.53 (1 wk treatment)	25.3
AL-335[22] (Adafosbuvir)	800 mg	4.00 (1 wk treatment)	5.0
ACH-3422[23]	700 mg	3.4 (1 wk treatment)	4.9
IDX21437 (MK-3682/Uprifosbuvir)[24]	300 mg	4.23 (1 wk treatment)	14.1

HCV: hepatitis C virus.
[a]Genotype 1 patients.

Table 2. HCV activity in cell culture experiments.

Compound	EC_{50} (μM)[a]	Prodrug EC_{50} improvement[b]	CC_{50} (μM)	SI
NM283[26]	7.600	0.3	>100	>13
Balapiravir[27]	1.100	1.2	>1 000	>909
Mercicitabine[28–30]	0.850	0.7	>100	>118
IDX184[31]	0.203	16	>75	>370
Sofosbuvir[16,32,33]	0.092	>1087	>100	>1087
GS-0938[34]	0.144	67	>100	>694
BMS-986094[20]	0.010	580	7	700
IDX19368[35]	0.160	36	>100	>613
AL-335[36]	0.075	NR	>100	>1333
ACH-3422[37]	0.050	NR	>25	>500
MIV-802[38]	0.045	>1111	>100	>2222
IDX21437[39]	56.800	1	>100	>2

HCV: hepatitis C virus; NR: Not reported.
[a]Genotype 1b replicon assay.
[b]Fold change EC_{50} nucleoside/EC_{50} prodrug activity (improvement of the prodrug versus parent nucleoside).

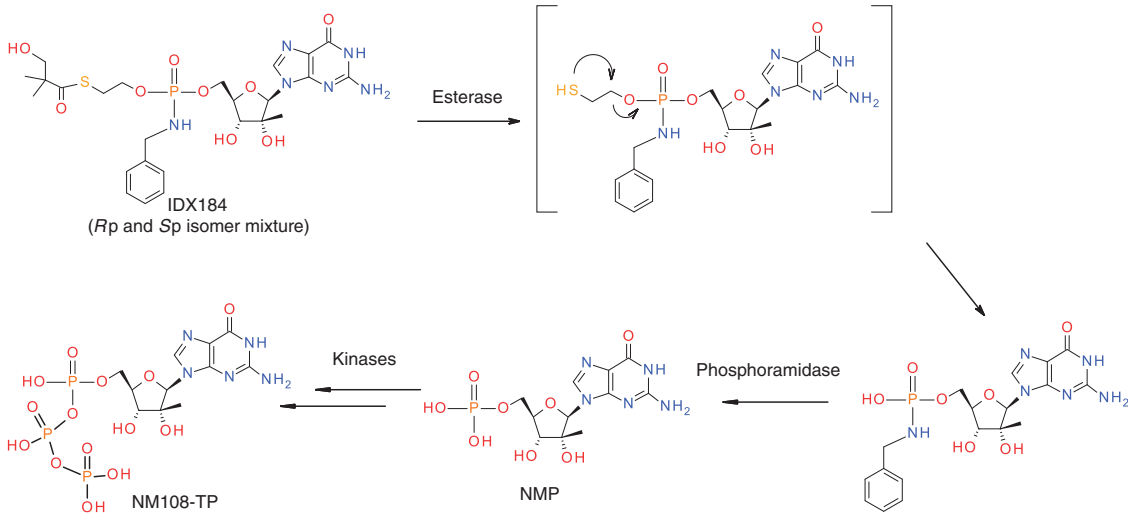
treatment, HCV viral load reduced by 2.7 log[11] giving one of the highest viral load reduction efficiency per gram of drug at 27 (Table 1). However, IDX184 suffered from dose-limited absorption as seen in the dose escalation nonlinearity Cmax.[44] IDX184 clinical development was stopped as a consequence of BMS-986094 severe cardiac side effects, both compounds sharing the same active Nuc-TP in vivo (vide infra).

2. McGuigan prodrugs

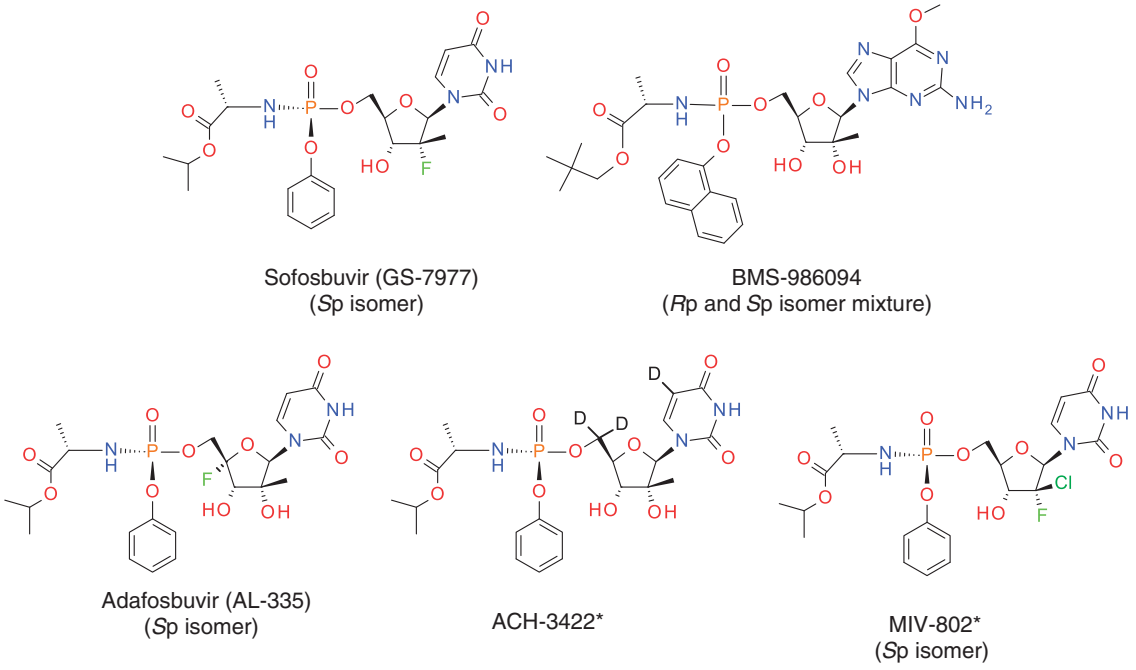
GS-7977 (Sofosbuvir) is a McGuigan phosphoramidate prodrug (L-alanine/phenol) originally developed by Pharmasset and is to date the only nucleotide prodrug which has received approval for HCV treatment in December 2013.[47] After the clinical proof of concept of GS-7977, other groups have used similar McGuigan prodrug as exemplified by BMS-986094, AL-335, ACH-3422, and MIV-802 to reduce the development risk associated with the metabolites formed by the pro-moieties (L-alanine and phenol). The cleavage and release of these pro-moieties in vivo have been well characterized for GS-7977 or other analogs bearing the same prodrug stereochemistry.[48–51]

The first step involves hydrolysis of the carboxylic ester by cathepsin A (Cat A) and carboxylesterase 1 followed by an intramolecular cyclization of the carboxylate on the phosphorus atom, displacing the phenolate and followed by water hydrolysis of the unstable cyclized intermediate to yield the alanyl phosphoramidic acid metabolite which is further hydrolyzed by the enzyme hHint 1 to the nucleoside-monophosphate (NMP). In the case of GS-7977, this NMP is then phosphorylated by UMP-CMP kinase to its nucleoside-diphosphate (NDP), and final phosphorylation by Nucleoside DiPhosphate Kinase (NDPK) affords its nucleoside-triphosphate (Scheme 2).

BMS-986094 is a McGuigan prodrug that was designed to improve in vitro activity in the replicon assay owing to an increase of the lipophilicity by using a naphthol in place of the usual phenol, substituting the shorter *iso*propyl ester with a *neo*pentyl and by removing a hydrogen bond donor on the guanine base with a 6 methoxy analog. These structural modifications improved the replicon EC_{50} with activities as low as 10 nM but with a cytotoxic value CC_{50} of 7 μM giving a selectivity index (toxicity/activity) of 700 (Table 2).[52] BMS-986094 phase II clinical trial was stopped due to a fatal cardiac adverse effect that was characterized further as a mitochondrial toxicity mainly due to its TP and to a lesser extent to its



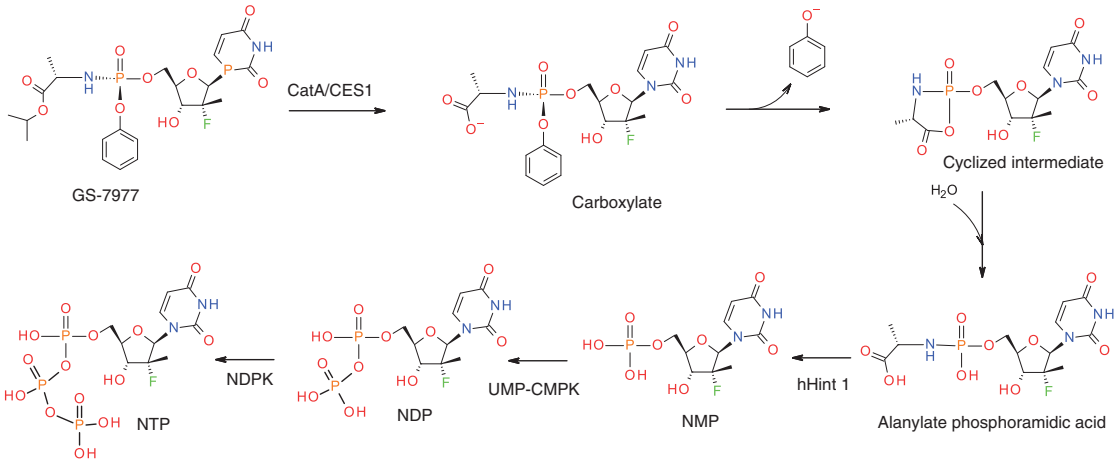
Scheme 1. SATE IDX184 nucleotide prodrug and its proposed decomposition pathway.



*potential structure from corresponding references[30, 31, 32]

Figure 2. McGuigan phosphoramidate nucleotide prodrugs. (a) Sofosbuvir (GS-7977) (Sp isomer), (b) BMS-986094 (Rp and Sp isomer mixture), (c) Adafosbuvir (AL-335) (Sp isomer), (d) ACH-3422*, and (e) MIV-802* (Sp isomer). *Potential structure from Deshpande,[31] Kalayanov et al.,[45] and Andersson.[46]

prodrug moieties.[53] The effect of *neo*pentyl ester prodrug of BMS-986094 in place of the *iso*propyl ester present in Sofosbuvir can be clearly seen in a previous study by McGuigan et al., where these two esters were synthesized with the same nucleoside backbone and tested.[20] The *iso*propyl ester analog of BMS-986094 proved to be over 14 times less toxic in the Huh7 cells, so some of the toxicity of BMS-986094 can be attributed to its *neo*pentyl ester modification. It has also been reported by Deval et al., with another comparable pair of compounds by making the BMS-986094-monophosphate prodrug on Sofosbuvir nucleoside. The Sofosbuvir-modified hybrid had an increase in the cell toxicity assay of Huh7 and U937 cells compared to Sofosbuvir.[21]



Scheme 2. McGuigan prodrug metabolism.

Three other McGuigan prodrugs still in clinical development are AL-335, ACH-3422, and MIV-802 for which little preclinical data have been reported but for which the HCV replicon activity is similar or slightly better than Sofosbuvir (Table 2). The early virologic load decrease in patients is much less efficient than Sofosbuvir (Table 1) for the first two more advanced candidates (AL-335 and ACH-3422), and it was recently announced that AL-335 would not be developed further in combination.[54] Although one cannot exclude that MIV-802 or ACH-3422 could potentially progress further in combination with other DAAs.

3. Cyclic phosphotriester (CPO) prodrugs

The 3′,5′-CPO prodrug structural unit shows possible significant improvements on the medicinal chemistry perspective, allowing smaller molecular weight and therefore better ligand efficiency as well as lower number of rotational bonds which, with the former property, may both provide enhanced passive diffusion through cell membrane. Both GS-0938 and IDX19368 (Figure 3) are actually double prodrugs as they bear the ethoxy masking group on the 6-guanine base position allowing a better solubility of these guanosine derivatives. The in vivo metabolism was studied in the case of GS-0938 and is described in Scheme 3. It involves a first oxidative cleavage by cytochrome (CYP3A4), followed by opening of the cyclic 3′,5′-phosphodiester (CPOH) by phosphodiesterase, the last step being the hydrolysis of the 6-ethoxy guanine prodrug by adenosine deaminase-like protein 1.[55]

4. D-amino acid based aryl-phosphoramidate prodrugs (PON)

IDX21437 is a D-amino acid phosphoramidate prodrug of the well-established HCV active 2′-β-modified

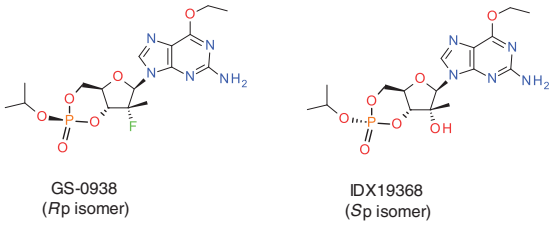
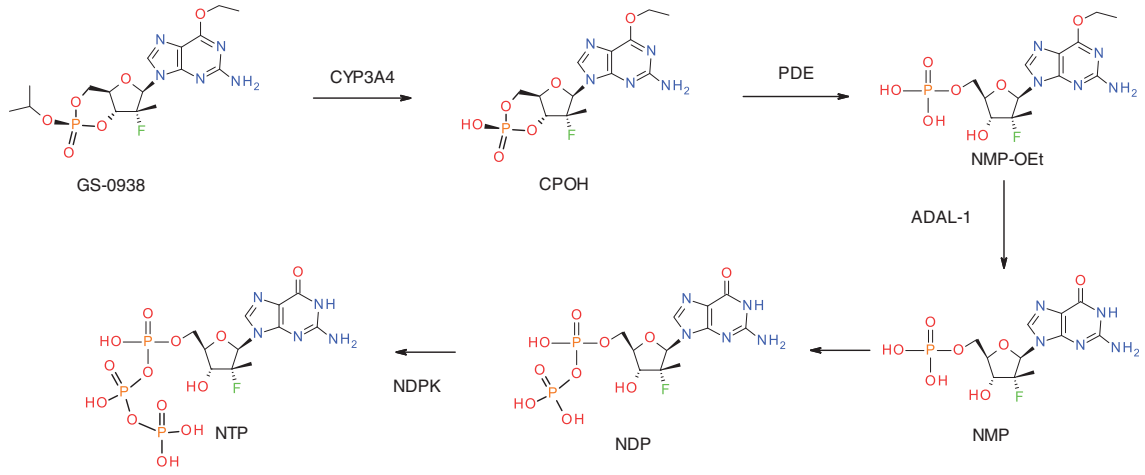


Figure 3. Clinical and preclinical 3′,5′-CPO prodrug. (a) GS-0938 (*Rp* isomer) and (b) IDX19368 (*Sp* isomer).

ribonucleoside family. As seen in Table 2, it has a very different profile in cell culture experiments, compared to other clinical candidates, due to the unnatural amino acid configuration part of its prodrug, giving a lack of activity in the HCV replicon system and would therefore not be viewed by classical medicinal chemists as a promising compound. But actually, this compound displayed an unexpectedly good in vivo profile in regards to its ability to form high levels of its corresponding active TP in animal liver, the target organ for HCV. The metabolism of IDX21437 was reported and proved to require a different enzymatic system for the initial cleavage compared to McGuigan prodrug Cat A involvement (Scheme 4).[39] The different enzymes involved in the metabolism of D-amino acid phosphoramidate is supposed to be responsible for the better liver to heart selectivity, as D-alanylate phosphoramidic acid metabolite was not observed in heart cells.[44] Currently, IDX21437 (now MK-3682) is progressing in phase II combination studies.

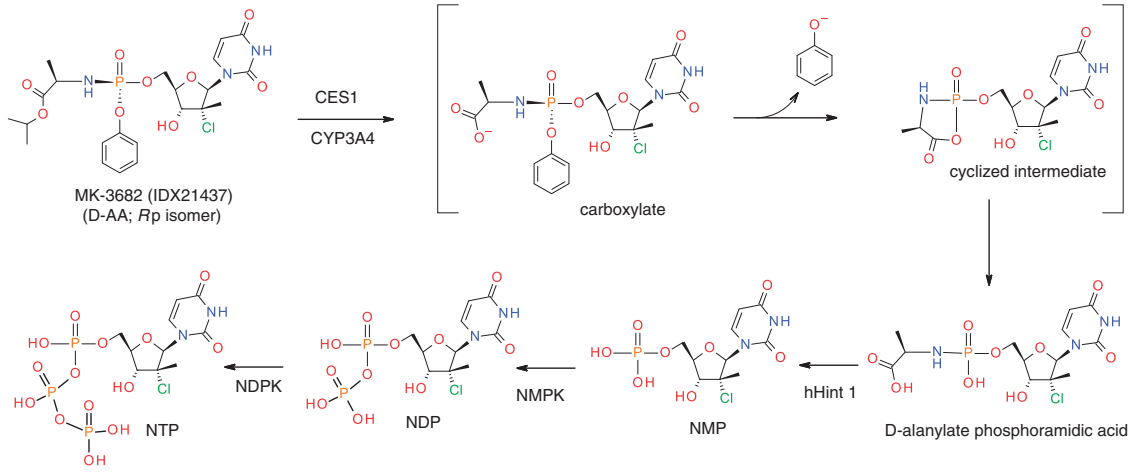
5. Other miscellaneous prodrugs

Other HCV nucleotide prodrugs were reported in early discovery studies as CC-1845 from Cocrystal, for which the structure is unknown but likely



4. D-aminoacid based Aryl-Phosphoramidate prodrugs (PON)

Scheme 3. CPO prodrug metabolism.



Scheme 4. D-amino acid phosphoramidate prodrug IDX21437 and its proposed metabolism.

a McGuigan prodrug of 2′C-Me-2,6,-disubstituted purine analog. However, recently the company has declared that preclinical studies indicated higher than acceptable toxicity and have now switched to a backup compound CC-2850.[56]

Conclusion

From the first nucleosides through the first generation of their prodrugs to the second generation of nucleotide prodrugs demonstrating increasing added value of liver targeting in HCV, no new simple nucleosides or their prodrugs would be further developed but favoring their nucleotide prodrugs as can be seen by the latest candidates in discovery or ongoing clinical evaluation. With the knowledge gathered by the different metabolism pathways of pro-moieties, future nucleotide prodrugs will be designed toward more elaborated and tissue targeted drugs with single or multiple prodrugs and possible combinations of the above well characterized and main classes of prodrugs as can be already seen in recent patent applications in the HCV and other disease areas. I can envision for the future of HCV nucleos(t)ide drugs better liver targeting based on more specific liver metabolism, compared to other tissues as exemplified by IDX21437, rather than first path metabolism effect as observed in the earlier *per os* prodrug design. HCV nucleotide drug discovery has been a tremendous scientific emulation for the last 15 years and will be able to serve as a foundation case

for other disease area nucleos(t)ide prodrug development as well as, more broadly, prodrug targeting example for other class of drugs in the future.

Acknowledgments

The author wishes to thank Dr Gilles Gosselin for his invaluable assistance in reviewing this manuscript.

Declaration of conflicting interests

The author is an employee of Idenix, an MSD company.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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