Design and Synthesis of Novel Symmetric Diaminofluorene Prolinamide Analogues As Potent Hepatitis C Virus Inhibitors

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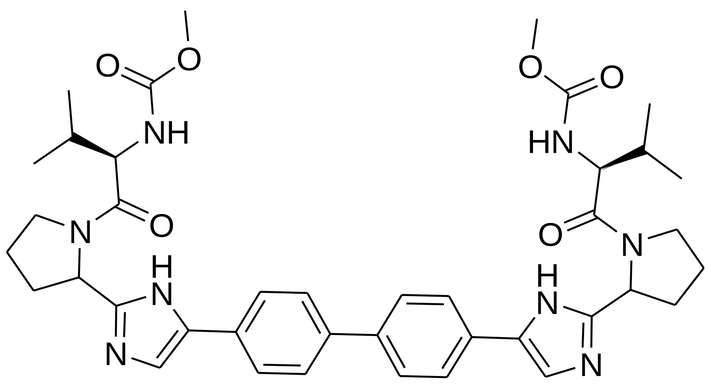
Abstract

Hepatitis C virus (HCV) is a major health challenge worldwide, since the discovery of NS5A direct acting antivirals; pharmaceutical researchers turned their attention to the design, synthesis, and optimization of novel NS5A inhibitors, outside the chemical space of commercially available drugs. This study describes the discovery of highly potent hepatitis C virus (HCV) NS5A inhibitors based on symmetrical prolinamide derivatives of diaminofluorene. Modification on the diaminoflourene backbone included the use of (*S*)-prolinamide or its isostere(*S,R*)-piperidine-3-caboxamide both bearing *S, R*-phenylglycine with different terminal carbamate groups. Compound 26 (Diethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-(((9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl)) bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate) exhibited potent inhibitory activity against HCV genotype (GT) 1b (EC_{50}= 40 pM and an approximate selectivity index of > 2.8 x10^6) and a high selectivity on (GT) 1b versus GT 4a. Interestingly, it exhibited a significant effect on GT 3a (EC_{50} = 1.2 nM) as well. The SAR analysis revealed that a picomolar inhibitory activity is attained with the use of *S*-prolinamide capped with *R*-phenylglycine residue bearing a terminal alkyl carbamate group.

Introduction

Hepatitis C virus (HCV) infection represents a disease of significant global impact that afflicts around 170 million people worldwide. In 2014, the first NS5A inhibitor daclatasvir (DCV) was FDA approved it was reported as a potent anti-HCV agent, especially in Genotype-1.[1] Daclatasvir bears a central biaryl core unit linked to an imidazole and proline moiety, while a terminal capping group of *L*-valine methylcarbamate is used.[2] The major drawback of DCV appears to be a relatively low genetic barrier to resistance. Pharmaceutical industry developed novel NS5A inhibitors with both symmetric and asymmetric cores, the novel analogues had pan genotypic activity, better safety profile and are less prone to resistance.



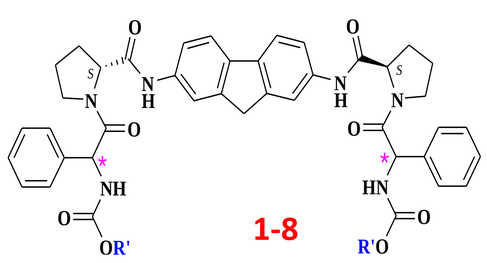
DCV

References

1-Cohen, J., The scientific challenge of hepatitis C. *Science* **1999**; *285* (5424), 26-30.

2-Belema M, Meanwell NA. Discovery of daclatasvir, a pan-genotypic hepatitis C virus NS5A replication complex inhibitor with potent clinical effect. J Med Chem. **2014** ; *57*(12):5057-5071.

Results and Discussion



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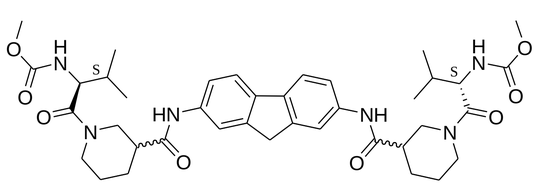

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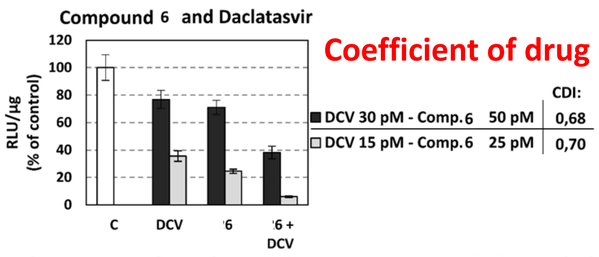
Table 1: Activity, Cytotoxicity and selectivity if the synthesized compounds on Con-1 Genotype 1b replicon assay

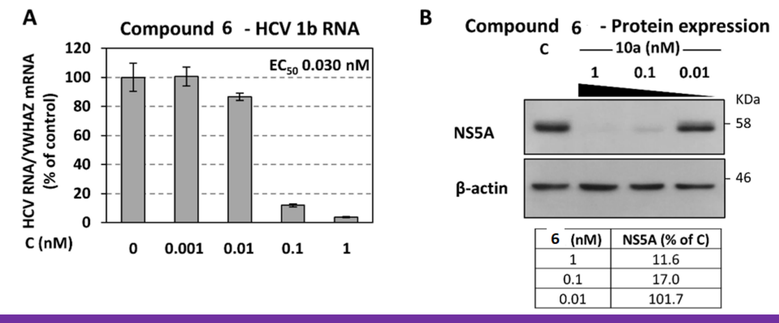
Cpd	Chirality*	R′	HCV replicon 1b		
			EC_{50} (nM)	CC_{50} (nM)	SI_{50}
1	*S*	$-CH_3$	8.50	87,510	10,298
2	*S*	$-CH_2CH_3$	7.49	>100,000	>13,349
3	*S*	$-C_4H_9$	2.25	>100,000	>44,444
4	*S*	$-CH_2C_6H_5$	1.12	>100,000	>89,606
5	*R*	$-CH_3$	0.142	95,390	669,874
6	*R*	$-CH_2CH_3$	0.036	>100,000	>2,779,322
7	*R*	$-C_4H_9$	4.03	>100,000	>24,795
8	*R*	$-CH_2C_6H_5$	94.55	>100,000	>1,058
9	*S*	---	342.20	>100000	>292
DCV	---	---	0.027	17,700	655,556

Fig. 1: Drug-drug interaction studies of compound (6) with the approved HCV inhibitor DCV.



Coefficient of drug interaction (CDI≈0.7)

Fig. 2: Validation of compound (6) activity by determining HCV RNA and NS5A protein levels with reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or western blot analysis.



Conclusions

- The 2,7-diaminofluorene-*S*-prolinamide core analogues exhibited better activity over the 2,7-diaminofluorene-(*S, R*)-piperidine-3-caboxamide core.
- Derivatives with the *R*-phenylglycine showed higher potency over the *S*-phenylglycine
- Compound (6) exhibited potent inhibitory activity against HCV genotype (GT) 1b (EC_{50}= 40 pM and ~ 3x10^6 selectivity index).
- Compound (6) showed high selectivity on GT 3a (EC_{50} = 1.2 nM).

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