이학석사 학위논문

Development of benzidine and diaminofluorene prolinamide derivatives as potent hepatitis C virus NS5A inhibitors

새로운 벤지딘 및 다이아미노플루오렌 유도체 구조의 C형 간염 바이러스 저해제 개발에 관한 연구

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선 민 경

CONTENTS

Abstract

Development of benzidine and diaminofluorene prolinamide derivatives as potent hepatitis C virus NS5A inhibitors

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To come up with hepatitis C virus (HCV) NS5A inhibitors, we designed a series of highly potent inhibitors based upon the modification of known inhibitor structures. We synthesized symmetrical prolinamide derivatives of benzidine and diaminofluorene. The structure-modification allowed us to form a library of potent HCV NS5A inhibitors. After optimizing the benzidine prolinamide skeleton, we developed novel inhibitors possessing meta-substituted benzidine core structures that presented the most potent anti-HCV activities. Furthermore, through a battery of studies including hERG ligand binding assay, CYP_{450} binding assay, rat plasma stability test, human liver microsomal stability test, and pharmacokinetic studies, the identified compounds **12**, **14**, **15**, **28** and **29** are found to be nontoxic, and are expected to be effective anti-HCV therapeutic agents.

Keywords: HCV, NS5A inhibitor, benzidine, diaminofluorene, structure-activity relationship
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I. Introduction

HCV infection has been reported in almost 170 million people all over the world (approximately 2.4% of the world population), including about 5 million people in the United States (US) with another 3 to 4 million added population worldwide annually [1-5]. It has been calculated that near 70-80% of those infected with HCV will proceed to chronic hepatitis. If HCV-inected patients ignore the infection, it may result in liver cirrhosis and hepatocellular carcinoma, with eventual death (1-5%) [6, 7]. Generally, therapies for HCV patients have comprised hypodermic injections of pegylated interferon-α (PEG-IFN-α) in combination with oral doses of ribavirin (RBV). But this interferon-based therapy has a restricted sustained virologic response (SVR), especially in patients infected with genotype 1 HCV. In 2011, the US Food and Drug Administration (FDA) licensed Boceprevir (Merck) and Telaprevir (Vertex Pharmaceuticals and Johnson & Johnson) as anti-HCV NS3/4A protease inhibitors in combination with PEG-IFN-α and RBV for G-1-infected cases [9-13]. Recently, advantageous result is expected from the launching of direct acting antivirals (DAAs) into the therapeutic regimen, and their possible limitations include a limited genetic barrier, which may be causing to drug-resistant mutants through long term treatment [10, 14-19]. Thus, the finding of effective and safe antiviral molecules towards various targets of HCV gene is highly required [20-24].

A polyprotein from the HCV genome is consisted of nearly 3,200 amino acids, which includes three parts of structural proteins (E1, E2, and core) and six parts of nonstructural proteins (NS2, NS3, NS4A-4B, and NS5A-5B) [25-28]. Among the nonstructural proteins, NS5A has been known to have a direct function in virus assembly, viral replication, virus persistence, virion production and pathogenesis [29,30]. The three major domains in NS5A are domain I (37 to 213 residues), containing a zinc binding motif required for viral RNA replication, domain II (250 to 342 residues), which interacts with NS5B and cellular proteins, and lastly domain III (356 to 447 residues), which

plays a role in infectious virus assembly, but not in RNA replication [31-39].

In 2010, daclatasvir (**1**), a novel NS5A inhibitor, was shown to have strong anti-HCV activity, particularly in the case of the HCV G-1 infection. The US FDA approved this unprecedented class of inhibitor in 2014 [40-44]. The effective concentration (EC_{50}) of daclatasvir was reported to be two-digit picomolar (pM) range in *in vitro* assay. Also this treatment diminished HCV RNA levels by an average of $3.3\log_{10}$ without any toxicity with a single 100 mg dose in clinical trials [45]. This outcome encouraged many pharmaceutical companies and research groups to focus on the development of new type of inhibitors targeting NS5A [46-55]. These days, there are a number of candidates in this class of inhibitors: ABT-267, GS-5885, GSK-2336805, MK-4882, MK-8742, ACH-2928, ACH-3102, BMS-346, BMS-665, BMS-824393, EDP-239, AZD-7295, IDX-719, PPI-461, and PPI-1301 (Figure 1) [56-63]. Very recently, interferon-free multi-class drug cocktails (daclatasvir and asunaprevier, Harvoni® and Viekira Pak®) have been reported as the most favorable therapy [64]. Daclatasvir has a middle biaryl-core unit connected to an imidazole and proline part and a methoxycarbonylated L-valine part as a capping group (Figure 2) [65]. In 2012, Schinazi and coworkers published on simple NS5A inhibitors including a biphenyl moiety with few changes at the end parts [66, 67].

Recently, our group published a new class of NS5A inhibitors represented by BMK-20113, which has benzidine core(A) and L-proline (B) linked as an amide groups and diverse capping groups (C) [68]. Herein we report further advances in antiviral activities through the introduction of novel structural modifications to the backbone of BMK-20113, namely substituted benzidines and diaminofluorene derivatives.

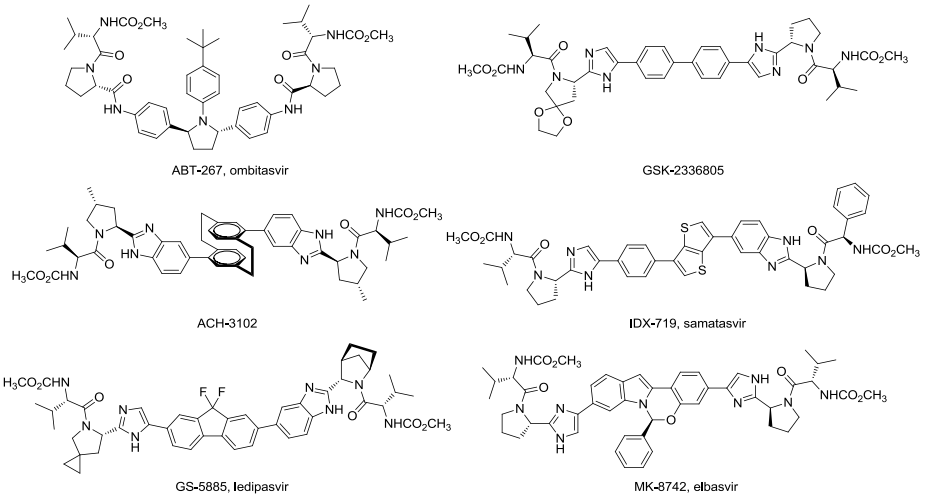


Figure 1. Structure of NS5A inhibitors

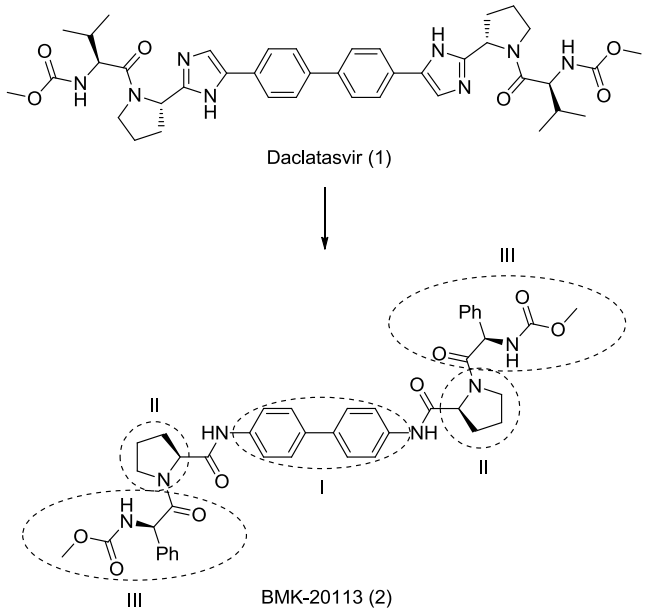
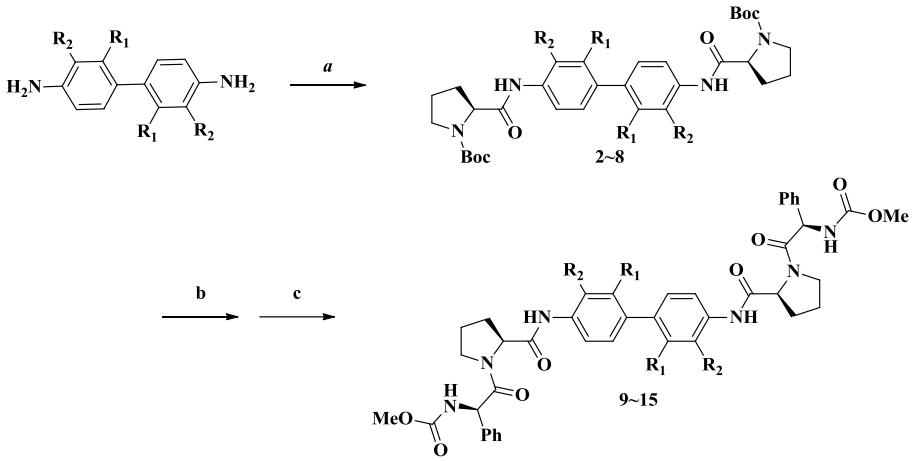


Figure 2. Strategy for designing HCV NS5A inhibitors

II. Result and Discussion

1.1 Chemistry



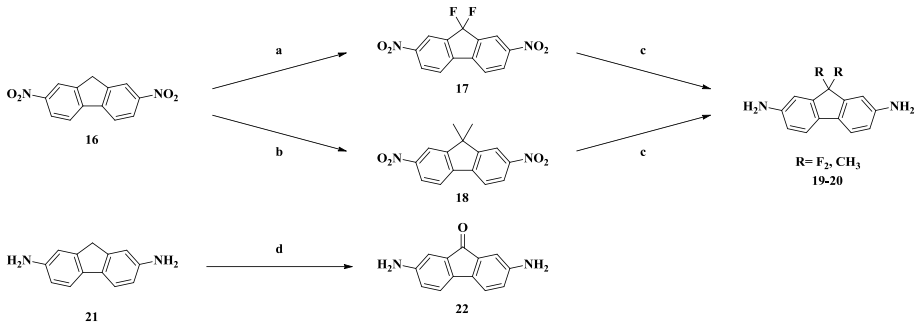
		R1	R2
2	9	H	H
3	10	H	CH_3
4	11	CH_3	H
5	12	CF_3	H
6	13	F	H
7	14	Cl	H
8	15	Br	H

[a]Reagents and conditions: (a) N-Boc-L-Proline, EDCI, DCM, 92-97%; (b) TFA, DCM;
(c) Methoxycarbonyl phenylglycine(capping group), EDCI, DIPEA, DCM, 32-73% (2 steps).

Scheme 1. Synthesis of benzidine derivatives and prolinamide skeleton[a]

Our first trial for structure-based design approach is centered on the benzidine core through the utilization of various substituted benzidine compounds. Structural modifications were carried out using *o*- or *m*-substituted benzidine derivatives, such as *o*-methyl, *m*-methyl, *m*-trifluoromethyl, *m*-fluoro, *m*-chloro, and *m*-bromo benzidines by employing the standard peptide coupling reaction using1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) in dichloromethane (DCM). Through chromatographic column chromatography, almost pure *N*-Boc-protected prolinamide products **2-8** were obtained in excellent yields (92-97%). The Boc protecting groups of the secondary amine site were deprotected with dilute trifluoroacetic acid (TFA) in DCM [69]. Then *N*-methoxycarbonylphenylglycine capping group was coupled with the free

amine with the aid of EDCI and *N*,*N*-diisopropylethylamine (DIPEA) in DCM. Capping groups were incorporated and the final products **19-15** were obtained in low to good yields (32-73%) (Scheme 1).

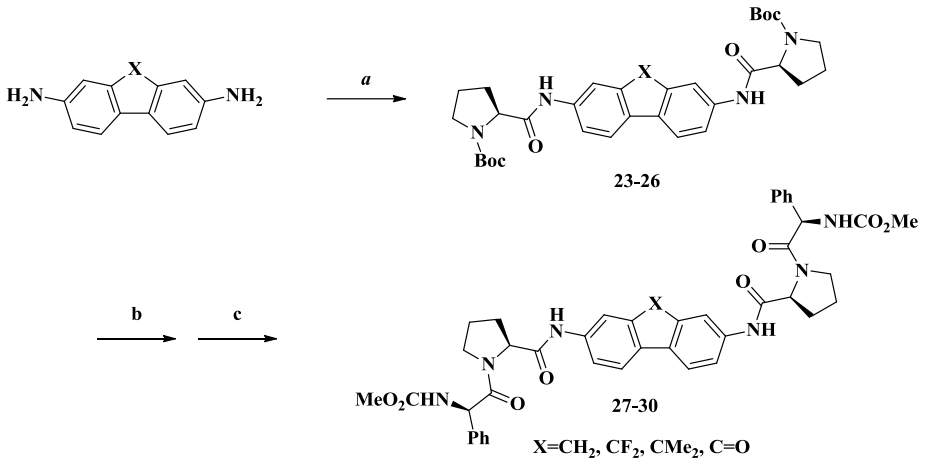


aReagents and conditions: (a) N-fluorobenzenesulfonimide, LiHMDS in THF (1.00 M solution), THF, 86%; (b) iodomethane, NaOt-Bu, DMF, 80%; (c) Fe_3O_4, $NH_2NH_2H_2O$, DMF, 99%; (d) Cs_2CO_3, DMSO, 76%.

Scheme 2. Synthesis of fluorene-2,7-diamine derivatives[a]

For the synthesis of the fluorene core structures, we needed to prepare 2,7-diaminofluorene derivatives (Scheme 2). Compound **16** was treated with 1.00 M lithium hexamethyldisilazide (LiHMDS) followed by *N*-fluorobenzenesulfonimide (NFSI) in tetrahydrofuran (THF) to provide di-fluorinated product **17** in 86% yield.

Dimethylated dinitrofluorene (**18**) was synthesized through the treatment of 2,7-dinitrofluorene with NaO*t*-Bu followed by iodomethane in DMF to furnish the expected 9,9-dimethyl-2,7-dinitro-9*H*-fluorene **18** in 80% yield [70]. Both **17** and **18** were simply reduced to diamine groups with hydrazine monohydrate in the presence of commercially available Fe_3O_4 nanoparticles as a recyclable heterogeneous nanocatalyst for hydrogenation of nitro group reduction [71]. Extremely high yields (99%) of the desired diamino products **19** and **20** were obtained. Moreover, the diaminofluoreone compound **22** was oxidized to the 9*H*-fluorene-2,7-diamine in the presence of Cs_2CO_3 as a strong base in DMSO to give a good yield (76%) [72]. (Scheme 2).



		X
23	27	CH_2
24	28	CF_2
25	29	CMe_2
26	30	C=O

[a]Reagents and conditions: (a) N-Boc-L-Proline, EDCI, DCM, 91-97%; (b) TFA, DCM; (c) Methoxycarbonyl phenylglycine(capping group), EDCI, DIPEA, DCM 50-66% (2 steps).

Scheme 3. Synthesis of fluorene-containing prolinamide derivatives[a]

For the formation of final inhibitor compounds in the fluorene series, various 2,7-diaminofluorene intermediates were prepared using the method described in Scheme 1. We obtained *N*-Boc-protected compounds **23-26** in high yields (91-97%). Removal of the Boc groups and coupling of the L-phenylglycine derivative gave us the anticipated fluorene derivatives **27-30** in moderate yields (50-66%) (Scheme 3).

1.2 SAR Study

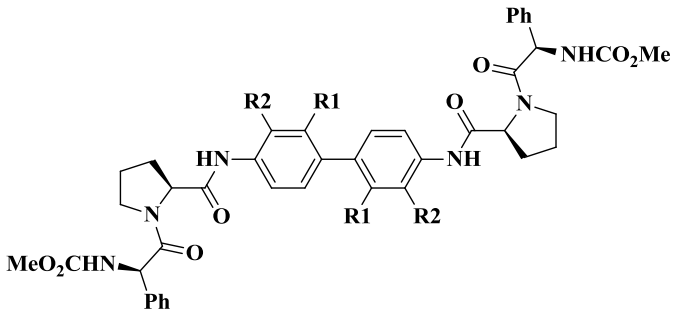


Table 1. Structure-activity relationships of inhibitors containing substituted benzidine derivatives against HCV type 2a and type 1b.

Entry	Compound	R_1	R_2	HCVCC EC_{50} (type2a) (nM)	Replicon EC_{50} (type 1b) (nM)	Cytotoxicity (μM)
1	**1**	H	H	0.26	0.028	>25
2	**10**	H	CH_3	193	7.4	>25
3	**11**	CF_3	H	0.025	0.0025	>25
4	**12**	CH_3	H	0.32	0.035	>25
5	**13**	F	H	0.16	0.005	>25
6	**14**	Cl	H	0.01	0.007	>25
7	**15**	Br	H	0.01	0.007	>25

With the structure of L-prolinamide and phenylglycine carbamates at both ends, we proceeded the SAR studies of substituted benzidine derivatives through variation of the middle biaryl core part. First, we synthesized the *ortho*-methylbenzidine derivative **10**, which exhibited low activities against HCV NS5A (EC_{50} of GT-2a and GT-1b: 193 nM and 7.4 nM, respectively; Table 1, entry 2). However, we observed that a series of *meta*-substituted benzidine derivatives **11-15** have higher anti-HCV inhibitory activities. The EC_{50} of CF_3-substituted compound **11** in GT-2a and GT-1b were 25 and 2.5 pM's, respectively (Table 1, entry 3), and the CH_3-substituted compound **12**

showed 320 and 35 pM's, respectively (Table 1, entry 4). After recognizing the enhanced inhibitory effect of the *meta*-substituted compounds, we investigated inhibitors having a heteroatom substitution in the *meta*-position of the benzidine core [73-76]. Three inhibitors containing *meta*-halogen substituted benzidine centers (compounds **13, 14** and **15** respectively) showed great antiviral activities. EC_{50} values against GT-2a and GT-1b were 160 and 5 pM's, respectively, for compound **13** (Table 1, entry 5) and 10 and 7 pM's for compounds **14** and **15** (entries 6 and 7, respectively).

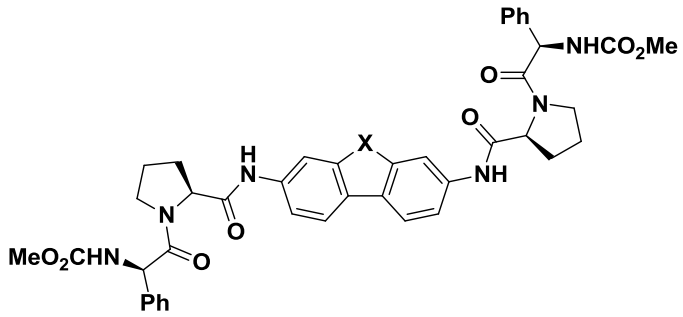


Table 2. . Structure-activity relationship studies of inhibitors containing a fluorene skeleton against HCV type 2a and type 1b.

Entry	Compound	X	HCVCC EC_{50} (type 2a) (nM)	Replicon EC_{50} (type 1b) (nM)	Cytotoxicity (μM)
1	**27**	CH_2	5.6	0.009	>25
2	**28**	CF_2	0.059	0.003	>25
3	**29**	CMe_2	0.02	0.004	>25
4	**30**	C=O	4.3	0.004	>25

When we investigated the viral inhibitory activities of compounds **9-15**, *meta*-substituted benzidine prolinamides exhibited highly potent HCV inhibiton activities. Thus, we predicted that the activity of *m-,m*-connected benzidine, i.e., fluorene derivatives would show high potency in the antiviral assays. In accordance with the positive results of the previous study of a

fluorenyl core structure NS5A inhibitors [62], we studied inhibitors with a fluorenyl core. We were glad to observe that these products also exhibited extremely high potencies, especially in the replicon genotype-1b assay (Table 2, entries 1-4).

Inhibitory activities (EC_{50}) of compound **27** containing the parent fluorene were 5.6 nM and 9 pM's against GT-2a and GT-1b, respectively (Table 2, entry 1). The flurorenone-containing inhibitor **30** exhibited a similar potency to compound **27;** EC_{50} of GT-2a and GT-1b were 4.3 nM and 4 pM, respectively (Table 2, entry 4). Furthermore, substituents at the methylene position of the fluorene core showed a substantial increase in antiviral activity against the GT-2a gene [77]. The inhibitory activity of compound **29** with a dimethyl-substituted fluorene moiety was 2,800-fold higher against the GT-2a gene than that of compound **27** containing the parent fluorene (EC_{50} of GT-2a and GT-1b: 20 and 4 pM's, respectively; Table 2, entry 3). Replacement of the dimethyl with difluorine as in **28** showed a similar effect (EC_{50} of GT-2a and GT-1b: 59 and 3 pM's, respectively; Table 2, entry 2) [78]. None of the inhibitors listed in Table 1 and Table 2 were cytotoxic at 25 μM.

1.3 Biology test

Table 3. Results of hERG ligand binding assay[a]

Entry	Compound	% Inhibition (10 μM)
1	**12**	7.98±4.49
2	**14**	<1
3	**15**	5.80±3.22
4	**28**	39.1±2.93
5	**29**	25.7±4.90
6	Control (astemizole)	99.9
[a]Fluorescence polarization assay		

Since the compounds **12, 14**, **15**, **28** and **29** exhibited high inhibitory activities both in the HCV GT-2a and the replicon (GT-1b) systems (Table 2, entries 3, 6, 7, and Table 3, entries 3, 4, respectively), we proceeded to carry out further evaluation of these elect compounds [79]. First, to evaluate any probable cardiac toxicity, a hERG ligand binding assay was performed, which examines inhibition of the inner rectifying voltage-gated fluorescence polarization potassium ion channel encoded by hERG gene [80,81]. Compared to astemizole, which was used as a control (99.9% inhibition at 10 μM), the values of compounds **12,14**, **15**, **28** and **29** (7.98%, 1%, 5.80%, 39.1% and 25.7% inhibition at 10 μM, respectively) suggested that these inhibitors bound poorly to the hERG membrane preparations, reflecting minimal possible cardiac toxicity (Table 3).

Table 4. Stability in rat plasma (% remaining)

Entry	Compound	0.5 h (%)	1 h (%)	2 h (%)
1	**12**	93	99	99
2	**14**	65	61	63
3	**15**	72	75	70

Next, when the compounds were tested against rat plasma, our data showed a high *in vitro* stability for compound **12**, which was unscathed after 2 hours (99% of the compound remained; Table 4, entry 1), whereas after incubating for 30 min, compounds **14** and **15** had degraded slightly (Table 4, entries 2 and 3). Consequently, it was necessary to carry out additional stability tests.

Table 5. Human liver microsomal stability (% remaining)

Entry	Compound	0.5 h (%)
1	**12**	79
2	**14**	97
3	**15**	96
4	**28**	92
5	**29**	95

In the human liver microsomal stability test, compound **12** was diminished 21% in the plasma after 0.5 h (Table 5, entry 1). Compounds **14**, **15**, **28,** and **29** had lower levels of degradation after 0.5 h (Table 5, entries 2, 3, 4, and 5, respectively). These results indicate that these promising compounds had high microsomal stability in the human liver [82,83].

Table 6. EC_{50} of compounds (μM) against various subtypes of CYP_{450}[a]

Entry	Compound	1A2	2C9	2D6	2C19	3A4
1	**12**	78.8	0.660	>100	4.64	4.64
2	**14**	>100	0.0877	4.94	0.724	2.46
3	**15**	23.9	0.116	41.2	1.04	3.99
5	**28**	>100	0.277	>100	13.9	0.976
4	**29**	>100	0.269	>100	2.94	1.03

[a] Standard inhibitors for each CYP_{450} subzymes: 1A2: α-naphthoflavone, 2C9: sulfaphenazole, 2D6: quinidine, 2C19: miconazole 3A4: ketoconazole.

Next, we investigated CYP_{450} inhibitory profiles to evaluate any drug-drug

interactions possible with the selected compounds **12, 14**, **15**, **28** and **29**. In general, high EC_{50} values for the CYP_{450} enzyme inhibitor indicate decreased liability for drug-drug interactions [84,85]. Our selected compounds exhibited low inhibition profiles for various CYP_{450} isoforms. CYP 1A2, 2D6, 2C19, and 3A4 were rather weakly inhibited in the presence of compounds **12, 14**, **15**, **28** and **29**, while the inhibition was higher for CYP 2C9 (0.660, 0.0877, 0.116, 0.269, and 0.277 μM respectively; Table 6). However, there should still be a large safety margin when we compare these inhibitions to their anti-viral activities [86]. In summary, compounds **12, 14**, **15**, **28** and **29** do not significantly inhibit these five representative CYP enzymes (Table. 6) [87].

Table 7. Pharmacokinetics features of selected inhibitors in rats[a]

Entry	Compound	Administration route	Dose (mg/kg)	C_{max} (μmol/mL)	$t_{1/2}$ (h)	F (%)
1	**12**	P.O.	10	0.44±0.14	3.80±0.11	11.74
2	**14**		10	0.11±0.07	2.34±0.37	3.12
3	**15**		10	0.17±0.07	6.37±3.39	4.99
4	**28**		10	0.39±0.20	1.68±0.43	7.6
5	**29**		10	0.98±0.02	4.45±4.02	13.8
6	**12**	IV	5	13.76±4.1	2.90±0.62	
7	**14**		5	16.63±2.57	0.88±0.34	
8	**15**		5	15.88±1.62	1.55±0.25	
9	**28**		5	18.64±19.4	0.90±0.02	
10	**29**		5	13.26±1.13	1.62±0.78	
[a] Vehicle : 5% DMSO/10% Solutol/85% HPBCD (20%, w/v), n = 3						

Studies on the pharmacokinetics (PK) of 5 selected compounds were carried out in rats, with the vehicles consisting of 5% DMSO, 10% solutol, and 85% (2-hydroxypropyl)-β-cyclodextrin (HPBCD) (Table 7). Despite high potencies in *in vitro* studies of HCV inhibition, the selected compounds provoked some concern due to the symmetrical natures of their structures and their high molecular weights, which might lead to undesirable PK properties [88-94].

Our data showed that the highest maximum concentration in plasma (C_{max}) was obtained with compound **29** (0.98 μmol/mL) through oral administration, and compound **28** (18.64 μmol/mL) through intravenous administration (Table 7, entries 4 and 10, respectively). The half-lives ($t_{1/2}$) of these two compounds upon p.o. administration were 1.7 to 6.4 h, and those in IV administration were 0.9 to 2.9 h, respectively. When we checked the oral bioavailability (F%), their low solubility and high lipophilicity contributed to a moderate oral absorption and bioavailability [95,96].

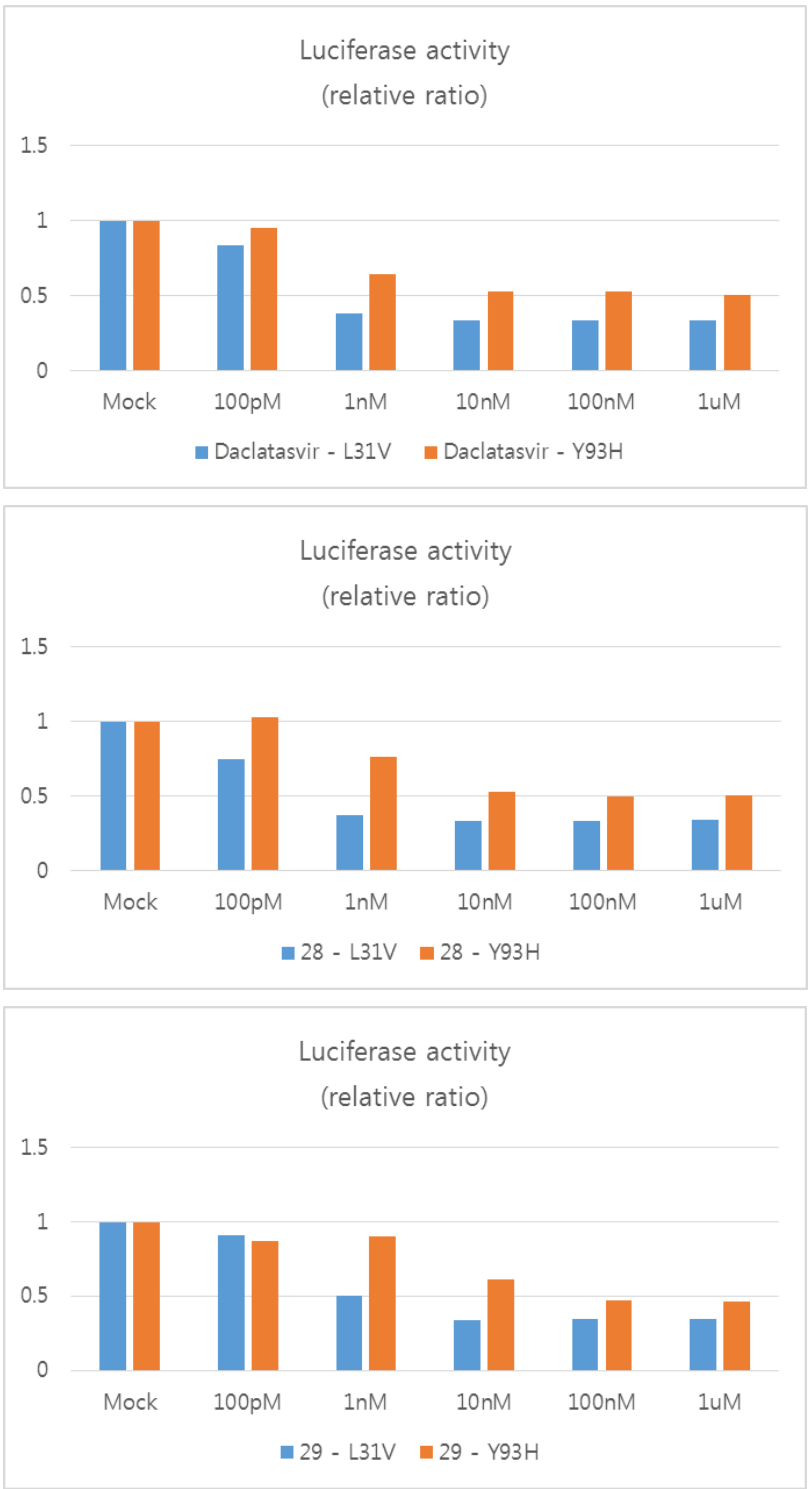


Figure 3. Resistance profiles of inhibitors daclatasvir, 28, and 29. Measurements were carried out in triplicate.

Then, we tested some of our selected inhibitors (**28** and **29**) against daclatasvir resistant mutants (L31V and Y93H) as described in Materials and Method [19]. *In vitro* transcribed resistant mutant RNAs (L31V and Y93H) were individually transfected to Huh7.5.1 cells [97,98]. After 4 h transfection, the culture media were replaced with DMEM media containing serially diluted compounds. By comparing the luciferase activities of **28** and **29** with those of daclatasvir, we concluded that **28** had a higher activity than **29** and a similar anti-viral potency to daclatasvir, to single mutant viruses. Therefore, we concluded that **28** could be a solution to the resistance problems of current HCV drugs.

Table 8. Cytotoxicity of 28 in eukaryotic cells[a]

Compound	IC_{50} (μM)				
	VERO	HFL-1	L929	NIH 3T3	CHO-K1
28	>100	>100	>100	>100	>100

[a] VERO: African green monkey kidney cell line; HFL-1: human embryonic lung cell line; L929: NCTC clone 929, mouse fibroblast cell line; NIH 3T3: mouse embryonic fibroblast cell line; CHO-K1: Chinese hamster ovary cell line.

Then, we tested the cytotoxicity of compound **28** against some eukaryotic cells [99]. The cell counting kit-8 assay method was used to measure the survival rate of the cells by using WST-8 tetrazolium salt. Hydrophilic WST-8 turns into orange formazan dye by dehydrogenase in cell lines. The number of living cells is determined by the activated dehydrogenase, which indicates the eukaryotic cell survival rate. At the maximal concentration (100 μM) used in the experiment, compound **28** showed less than 50% inhibition (the limit) in monkey kidney, human embryonic lung, mouse fibroblast, and hamster ovary cell line (Table 8). Thus, we concluded that **28** is nontoxic and safe in the tested eukaryotic cell lines.

Table 9 Bacterial reverse mutation assay of 28[a]

Tester strain	Compound	Dose (μg/plate)	Revertant colonies/plate (Mean ± SD) [Factor][b]	
			Without S-9 mix	With S-9 mix
TA-98	Vehicle control	0	18 ± 2	28 ± 3
	28	200	23 ± 5[1.3]	30 ± 2[1.1]
TA-100	Vehicle control	0	117 ± 10	126 ± 19
	28	200	106 ± 3[0.9]	115 ± 8[0.9]
	Positive control	Dose (μg/plate)	Without S-9 mix	With S-9 mix
TA-98	2-Nitrofluorene	1	187 ± 14[10.2]	*ND*
	Benzo[a]pyrene	2	*ND*	171 ± 10[6.1]
TA-100	Sodium azide	1	728 ± 15[6.2]	*ND*
	Benzo[a]pyrene	2	*ND*	521 ± 28[4.1]
[a] (Mean± SD, n = 3)				
[b] No. of revertant colonies in the treated plate/No. of revertant colonies in the vehicle control plate				
ND : Non Determined				

Next, we investigated the possibility that compound **28** might have genetic toxicity and checked its possibility using the Ames test [100,111]. For this purpose, we used *Salmonella typhimurium* strains TA-98 and TA-100 that require several amino acids for growth to test whether compound **28** functions as a mutagen. Growth of TA-98 and TA-100 strains in the presence of the compound of interest in the media lacking histidine indicates that the compound is a potential mutagen inducing substitution, addition, and/or deletion of DNA. The result is considered to be positive when the number of colonies on the compound-treated plate increases double or more of those on the vehicle control plate. When we tested 200 μg/plate of **28**, the number of revertant colonies on the compound-treated plate was not increased compared with that on the vehicle-treated plate. And the number was much lower than

those on 4 positive control compound-treated plates (Table 9). Therefore, compound **28** is considered to be non-genotoxic.

III. Conclusion

In conclusion, we developed a series of extremely potent HCV NS5A inhibitors using new benzidine and fluorene prolinamide derivatives as core structures. Several of them have high potencies that produce inhibition even at the single digit pM level. Through the SAR studies using a variety of benzidine, proline, and fluorene derivatives with a phenyl glycine capping group, we were able to identify inhibitors possessing excessively high inhibitory activities. Among the new inhibitors, compounds **12, 14, 15, 28** and **29** were chosen for further evaluation since they were the most potent. A synergistic effect with the NS5B polymerase inhibitor was seen with compound **12**. Moreover, subsequent studies demonstrated that these compounds have a desirable low cardiac toxicity, high human microsomal stability, and limited drug-drug interaction possibilities. Compound **28** was revealed to be non-toxic for eukaryotic cells and genetically non-toxic by the Ames test. The results of this study suggest that compound **28** is a highly potent and safe lead warranting further study as a potential HCV drug candidate. Further research on other pharmacological properties of the selected inhibitors and new structure of the HCV NS5A inhibitors is currently under progress in our laboratory.

IV. Experimental

Chemical Studies

General Chemical Methods

The ^{1}H and ^{13}C NMR-spectra were measured with an Agilent 400-MR DD2 Magnetic Resonance System (400 MHz) and a Varian/Oxford As-500 (500 MHz) Spectrophotometer. The signals were reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet) and chemical shifts were measured as parts per million (δ values) from tetramethylsilane as an internal standard at probe temperature in $CDCl_3$ or DMSO-D_6 for neutral compounds. Reactions that needed anhydrous conditions were carried out in flame-dried glassware under a positive pressure of dry N_2 using standard Schlenk line techniques. Evaporation of solvents was performed at reduced pressure using a rotary evaporator. TLC was performed using silica gel 60F254 coated on an aluminum sheet (E. Merck, Art.5554). Chromatogram was visualized by UV-lamp (Vilber Lournat, VL-4LC) and/or colorized with following solutions: (a) 20% ethanolic phosphomolybdic acid (PMA), (b) potassium permanganate solution, and (c) 2% ninhydrin ethanolic solution. Column chromatography was performed on silica gel (Merck. 7734 or 9385 Kiesel gel 60), and the eluent was mentioned in each procedure. High resolution mass spectra (HRMS) were recorded on a ThermoFinnigan LCQ™ Classic, Quadrupole Ion-Trap Mass Spectrometer. HPLC analyses were carried out on an Agilent HP1100 system (Santa Clara, CA, USA), composed of an auto sampler, quaternary pump, photodiode array detector (DAD), and HP Chemstation software. The separation was carried out on a C18 Vydac 218TP54 column 250 x 4.6 mm i.d. (5 µm particle size) with 0.1% TFA in water (A), acetonitrile (B), as a mobile phase at a flow rate of 1 mL/min at 20 ℃. Method: 100% A and 0% B (0 min), 0% A and 100% B (10 min), 0% A and 100% B (20 min), 100% A and 0% B (22 min), 100% A and 0% B (25

min). All materials were purchased from a commercial supplier and used without further purification unless otherwise noted.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((3,3'-dimethyl-[1,1'-biphenyl]-4,4' diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (3).

A mixture of *N*-Boc-L-proline (9.47 g, 44.0 mmol), EDCI (9.97 g, 52.0 mmol), and *ortho*-tolidine (4.25 g, 20.0 mmol) in CH_2Cl_2 (30 mL) was stirred at ambient temperature for 2 h. The resulting residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with 1.0 N aq HCl solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Without any purification, **3** was obtained as a solid (11.3 g, 93%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.35 (d, 2H), 7.51 (s, 2H), 7.48-7.42 (m, 4H), 4.34 (m, 2H), 3.45 (m, 2H), 3.55 (m, 2H), 2.17 (s, 6H), 2.16 (m, 2H), 1.94-1.81 (m, 6H), 1.42-1.37 (app br s, 18H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.4, 153.3, 138.5, 135.4, 132.2, 128.3, 125.3, 124.0, 78.5, 59.9, 46.6, 31.4, 28.1, 23.3, 17.9. HRMS: Anal. calcd. for $[M+H]^+$ $C_{34}H_{46}N_4O_6$: 607.3490; found 607.3480.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((2,2'-bis(trifluoromethyl)-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (4). Yield 4.3 g (96%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.44 (d, 2H), 8.21 (s, 1H), 8.14(s, 1H), 7.92-7.82 (dd, 2H), 7.33(m, 2H), 4.26 (m, 2H), 3.50-3.38 (m, 4H), 2.22 (m, 2H), 1.94-1.80 (m, 6H), 1.42 (s, 9H), 1.30 (s, 9H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.9, 171.6, 153.8, 153.2, 139.0, 132.0, 131.0, 128.19, 128.15, 127.9, 124.9, 122.1, 121.2, 121.0, 116.34, 116.28, 116.2, 116.1, 78.7, 78.6, 60.5, 60.1, 54.1, 46.6, 46.4, 31.0, 29.9, 27.8, 27.7, 24.0, 23.3. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -57.37. HRMS: Anal. calcd. for $[M+H]^+$ $C_{34}H_{40}F_6N_4O_6$: 715.2925; found 715.2919.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((2,2'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (5). Yield 2.75 g (96%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.97 (s, 2H), 7.57-7.41 (m, 4H), 6.97 (app br s, 2H), 4.26 (m, 2H), 3.42-3.35 (m, 4H), 2.21

(m, 2H), 1.97 (s, 6H), 1.89-1.78 (m, 6H), 1.40-1.31 (app br s, 18H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.4, 171.0, 153.6, 153.2, 138.0, 138.0, 135.69, 135.65, 129.52, 120.48, 120.47, 120.4, 120.3, 116.7, 116.5, 78.6, 78.4, 60.3, 60.0, 46.7, 46.5, 31.3, 31.1, 30.2, 28.1, 28.0, 23.9, 23.3, 19.7. HRMS: Anal. calcd. for $[M+H]^+$ $C_{34}H_{46}N_4O_6$: 607.3490; found 607.3489.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((2,2'-difluoro-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (6). Yield 2.6 g (93%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.33 (s, 2H), 7.72-7.68 (m, 2H), 7.46-7.36 (m, 4H), 4.28-4.19 (m, 2H), 3.45-3.32 (m, 4H), 2.22 (m, 2H), 1.99-1.79 (m, 6H), 1.40-1.29 (app br s, 18H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 172.0, 171.6, 160.1, 157.7, 153.6, 153.1, 140.5, 140.4, 131.58, 131.55, 117.0, 116.9, 115.1, 115.0, 106.2, 106.0, 78.74, 78.57, 60.4, 60.1, 46.7, 46.6, 31.0, 30.2, 28.1, 28.0, 24.0, 23.4. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -113.53. HRMS: Anal. calcd. for $[M+H]^+$ $C_{32}H_{40}F_2N_4O_6$: 647.2398; found 647.2887.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((2,2'-dichloro-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (7). Yield 2.8 g (95%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.31 (app br s, 2H), 7.97-7.87 (m, 2H), 7.63-7.52 (m, 2H), 7.29-7.25 (m, 2H), 4.29-4.20 (m, 2H), 3.47-3.41 (m, 2H), 3.38-3.32 (m, 2H), 2.25-2.19 (m, 2H), 1.92-1.82 (m, 6H), 1.41-1.31 (app br s, 18H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.9, 171.5, 153.6, 153.1, 139.9, 132.5, 132.0, 131.6, 119.2, 117.6, 78.7, 78.5, 60.4, 60.1, 46.7, 46.5, 31.3, 31.0, 30.1, 28.1, 28.0, 23.9, 23.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{32}H_{40}Cl_2N_4O_6$: 647.2398; found 647.2394.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((2,2'-dibromo-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (8). Yield 990 mg (92%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.26 (s, 2H), 8.12-8.01 (m, 2H), 7.66-7.55 (m, 2H), 7.23 (app br s, 2H), 4.26-4.20 (m, 2H), 3.39-3.35 (m, 4H), 2.22 (m, 2H), 1.89-1.81 (m, 6H), 1.40-1.30 (app br s, 18H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 172.0, 171.6, 153.7, 153.1, 139.9, 135.9, 131.4, 123.0, 122.3, 122.2, 118.2, 118.1, 78.8, 78.6, 60.5, 60.2,

46.8, 46.6, 31.0, 30.2, 28.2, 28.0, 24.0, 23.4. HRMS: Anal. calcd. for $[M+H]^+$ $C_{32}H_{40}Br_2N_4O_6$: 735.1387; found 735.1373.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (10). Compound **3** (144 mg, 0.238 mmol) in CF_3CO_2H (1 mL) and CH_2Cl_2 (1 mL) was stirred at room temperature for 5 h. The volatile component was removed in vacuo, EDCI (119 mg, 0.620 mmol), and **capping group**(L-Phenylglicine (100 mg, 0.572 mmol) were added in batches over 4 min to a solution of *i*-Pr_2NEt (208 L, 1.192 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred at room temperature for 75 min. The residue was divided between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. A silica gel mesh was prepared from the residue and submitted to flash chromatography (silica gel: EtOAc/hexane as eluent) to provide **10** as a white solid (77 mg, 41%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.31 (s, 2H), 7.74 (d, 2H), 7.54-7.23 (m, 16H), 5.52 (d, 2H), 4.52 (m, 2H), 3.85 (m, 2H), 3.52 (s, 6H), 3.18 (m, 2H), 2.28 (s, 6H), 2.00-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.1, 168.8, 156.1, 137.1, 136.5, 135.4, 132.2, 128.6, 128.2, 128.1, 128.0, 125.2, 123.9, 60.6, 56.8, 51.6, 46.9, 29.1, 24.3, 17.9. HRMS: Anal. calcd. for $[M+H]^+$ $C_{44}H_{48}N_6O_8$: 789.3606; found 789.3597.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((2,2'-bis(trifluoromethyl)-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (11). Yield 145 mg (32%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.29 (s, 2H), 8.21 (d, 2H), 7.83 (m, 2H), 7.75 (d, 2H), 7.43-7.05 (m, 12H), 5.51 (d, 2H), 4.41 (m, 2H), 3.85 (app br s, 2H), 3.54 (s, 6H), 3.20 (app br d, 2H), 2.06-1.82 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.26, 168.75, 156.42, 139.30, 137.16, 132.79, 131.36, 128.89, 128.35, 128.25, 125.07, 122.88, 121.81, 116.37, 61.05, 56.97, 51.87, 47.22, 29.45, 24.51. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -57.28. HRMS: Anal. calcd. for $[M+H]^+$ $C_{44}H_{42}F_6N_6O_8$:

897.3041; found 897.3046.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((2,2'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (12). Yield 292 mg (73%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.84 (s, 2H), 7.73 (d, 2H), 7.59 (s, 2H), 7.48-7.14 (m, 12H), 6.87 (d, 2H), 5.51 (d, 2H), 4.42 (m, 2H), 3.84 (m, 2H), 3.55 (s, 6H), 3.20 (m, 2H), 1.98 (s, 6H), 1.97-1.78 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.2, 168.4, 156.1, 137.9, 137.1, 135.8, 135.8, 129.58, 128.62, 128.1, 127.9, 120.5, 116.7, 60.7, 56.8, 51.7, 47.0, 29.4, 24.3, 19.8. HRMS: Anal. calcd. for $[M+H]^+$ $C_{44}H_{48}N_6O_8$: 789.3606; found 789.3605.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((2,2'-difluoro-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (13). Yield 134 mg (40%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.43 (s, 2H), 7.74-7.71 (m, 3H), 7.46-7.11 (m, 15H), 5.51 (d, 2H), 4.43 (m, 2H), 3.83 (m, 2H), 3.54 (s, 6H), 3.19 (m, 2H), 2.05-1.77 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.8, 168.4, 160.0, 158.0, 156.1, 140.5, 137.2, 131.6, 128.6, 128.5, 128.1, 127.9, 127.6, 117.1, 115.1, 106.3, 106.1, 60.8, 56.7, 51.7, 47.0, 29.3, 24.3. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -73.45. HRMS: Anal. calcd. For $[M+H]^+$ $C_{42}H_{42}F_2N_6O_8$: 797.3105; found 797.3112.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((2,2'-dichloro-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (14). Yield 146 mg (44%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.14 (s, 2H), 7.97-7.91 (m, 2H), 7.75 (d, 2H), 7.65-7.55 (m, 2H), 7.43-7.12 (m, 12H), 5.51 (d, 2H), 4.39 (m, 2H), 3.84 (m, 2H), 3.55 (s, 6H), 3.20 (m, 2H), 2.06-1.79 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.8, 168.5, 156.2, 139.9, 137.1, 132.6, 132.1, 131.7, 128.6, 128.1, 127.9, 119.3, 117.7, 60.8, 56.73, 51.68, 47.0, 29.3, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{42}H_{42}Cl_2N_6O_8$: 829.2514; found 829.2518.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((2,2'-dibromo-[1,1'-biphenyl]-4,4'-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (15). Yield 110 mg (42%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.13 (s, 2H), 8.32-8.12 (m,2H), 7.92-7.61 (m, 4H), 7.41-7.13 (m, 12H), 5.51 (d, 2H), 4.39 (m, 2H), 3.84 (m, 2H), 3.55 (s, 6H), 3.52 (m, 2H), 3.19 (m, 2H), 2.05-1.81 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.7, 168.5, 156.2, 139.8, 137.1, 135.9, 131.3, 128.6, 128.1, 127.9, 123.0, 122.3, 118.1, 60.8, 56.7, 51.7, 47.0, 29.3, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{42}H_{42}Br_2N_6O_8$: 917.1504; found 917.1521.

9,9-Difluoro-2,7-dinitro-9*H*-fluorene (17). 2,7-Nitro-9*H*-fluorene (100 mg, 0.39 mmol) and *N*-fluorobenzenesulfonimide (NFSI) (369 mg, 1.17 mmol) were dissolved in DMF and cooled to -20°C. LiHMDS (1.0 M in THF, 1.17 mL, 1.17 mmol) was added dropwise over 5 min. After an additional 2 h at 0°C, TLC indicated the completion of the reaction, and the excess base was quenched by addition of MeOH. The suspension was filtered over Celite and concentrated in vacuo. A silica gel mesh was prepared from the residue and submitted to flash chromatography (silica gel: CH_2Cl_2/hexane as eluent) to provide **17** as a yellow solid (98 mg, 86%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 8.63 (d, 2H), 8.53 (d, 1H), 8.56 (d, 1H), 8.36 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 149.1, 142.5, 138.5, 129.2, 144.3, 120.8, 119.6. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -110.3.

9,9-Dimethyl-2,7-dinitro-9*H*-fluorene (18). A mixture of 2,7-nitro-9*H*-fluorene (100 mg, 0.39 mmol) and NaO*t*-Bu (75 mg, 0.78 mmol) were dissolved in DMF at ice bath under N_2. Then, CH_3I (49 L, 0.78 mmol) was slowly added to the mixture, and stirred for 2 h. The solution was poured into water and a precipitate was formed. The product was filtered, washed with water, and air-dried. Without any purification, **18** (89 mg, 80%) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 8.59 (d, 2H), 8.33 (m, 4H), 1.60 (s, 6H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 156.2, 148.1, 142.7, 123.6, 122.9, 118.7, 47.9, 25.6.

9,9-Difluoro-9*H*-fluorene-2,7-diamine (19). A magnetic stirrer bar, Fe_3O_4 (purchased from Aldrich, 4 mg, 0.015 mmol), and DMF (0.5 mL) were added to an oven-dried Schlenk tube, and the mixture was sonicated in an ultrasound bath for 1 min under argon. Compound **18** (22 mg, 0.075 mmol) and hydrazine monohydrate (29 L, 0.60 mmol) were then added to the mixture. The reaction mixture was stirred at 80°C under an argon atmosphere until the reaction was completed. After magnetic separation of the catalyst, the organic layer was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Without any purification, **19** (17 mg, 98%) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 7.19 (s, 1H), 7.17 (s, 1H), 6.8 (d, 2H), 6.61 (d, 1H), 6.59(d, 1H), 5.3 (s, 4H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 148.0, 137.3, 137.1, 127.7, 123.7, 119.8, 116.45, 109.1. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -106.7. HRMS: Anal. calcd. for $[M+H]^+$ $C_{13}H_{10}F_2N_2$: 233.0885; found 233.0885.

9,9-Dimethyl-9*H*-fluorene-2,7-diamine (20). Yield of 69 mg (98%) was obtained as a yellow solid. ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 7.21 (s, 1H), 7.19 (s, 1H), 6.6 (d, 2H), 6.47 (d, 1H), 6.45(d, 1H), 4.9 (s, 4H), 1.3 (s, 6H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 153.5, 146.7, 128.4, 118.6, 112.6, 108.5, 45.6, 27.7. HRMS: Anal. calcd. for $[M+H]^+$ $C_{15}H_{16}N_2$: 225.1386; found 225.1383.

2,7-Diamino-9*H*-fluoren-9-one (22). A mixture of 9H-fluorene-2,7-diamine (294 mg, 1.5 mmol) and Cs_2CO_3 (1.5 g, 4.5 mmol) in DMSO (7 mL) was stirred under an atmosphere of air. When TLC showed that no starting material remained, the solution was poured into water and a precipitate was formed. The product was filtered, washed with water, and air-dried. Without any purification, **22** (239 mg, 76%) was obtained as a solid. 1H NMR (DMSO-d6, δ = 2.5 ppm, 400 MHz): 7.10 (s, 1H), 7.08 (s, 1H), 6.70 (d, 2H), 6.57 (d, 1H), 6.57 (d, 1H) 5.30 (s, 4H). 13C NMR (DMSO-d6, δ = 39.52 ppm, 100 MHz): 194.9, 148.2, 134.6, 133.3, 119.9, 118.6, 109.7. HRMS: Anal. calcd. for [M+H]+ $C_{13}H_{10}N_2O$: 211.0866; found 211.0867.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (23). A mixture of *N*-Boc-L-proline (323 mg, 1.5 mmol), EDCI (312 mg, 1.63 mmol), and 2,7-diaminofluorene (123 mg, 0.63 mmol) in CH_2Cl_2 (2 mL) was stirred at ambient temperature for 2 h. The resulting residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with 1.0 N aq HCl solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Without any purification, **23** was obtained as a solid (359 mg, 97%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.0 (d, 2H), 7.69 (d, 2H), 7.72 (d, 2H), 7.57-7.51 (m, 2H), 4.31-4.21 (m, 2H), 3.88 (s, 2H), 3.45-3.37 (m, 4H), 2.23 (m, 2H), 1.94-1.80 (m, 6H), 1.41 (app br s, 9H), 1.29 (app br s, 9H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 171.4, 153.2, 143.5, 137.6, 136.3, 119.5, 118.0, 116.1, 78.6, 78.5, 60.4, 46.6, 31.1, 28.2, 28.0, 24.0, 23.4. HRMS: Anal. calcd. for $[M+H]^+$ $C_{33}H_{42}N_4O_6$: 591.3177; found 591.3168.

(2*S*,2'*S*)-Di-*tert*-butyl 2,2'-(((9,9-difluoro-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (24). Yield 28 mg (95%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.29 (app br s, 2H), 8.06 (s, 1H), 8.02 (s, 1H), 7.69-7.66 (m, 4H), 4.30-4.25 (m, 1H), 4.21-4.18 (m, 1H), 3.45-3.41 (m, 2H), 3.37-3.34 (m, 2H), 2.23-2.16 (m, 2H), 1.92-1.80 (m, 6H), 1.40 (app br s, 9H), 1.27 (app br s, 9H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 174.3, 173.9, 171.9, 171.5, 153.6, 153.1, 139.4, 133.56, 133.49, 122.8, 122.7, 121.2, 114.5, 114.4, 78.8, 78.6, 60.5, 59.3, 58.6, 46.6, 46.2, 46.1, 31.0, 30.3, 28.2, 28.0, 27.9, 27.7, 24.0, 23.4. ^{19}F NMR (DMSO-d_6, 377 MHz,): δ -108.9, -109.0. HRMS: Anal. calcd. for $[M+H]^+$ $C_{33}H_{40}F_2N_4O_6$: 627.2989; found 627.2997.

(2*S*,2'*S*)-di-*tert*-butyl 2,2'-(((9,9-dimethyl-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (25). Yield 201 mg (97%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.10 (app br s, 2H), 7.84 (s, 1H), 7.79 (s, 1H), 7.66 (d, 2H), 7.51 (t, 2H), 4.31-4.28 (m, 1H), 4.24-4.21 (m, 1H), 3.44-3.40 (m, 2H), 3.37-3.31 (m, 2H), 2.21-2.16 (m, 2H), 1.95-1.80 (m, 6H), 1.40 (app br s, 9H), 1.34 (s, 6H), 1.28 (app br s,

9H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 174.3, 173.9, 171.4, 171.0, 153.6, 153.2, 138.1, 138.0, 133.7, 133.6, 119.7, 118.4, 118.2, 113.8, 113.6, 78.6, 78.5, 60.4, 60.0, 46.6, 46.1, 42.2, 42.1, 31.0, 30.3, 28.2, 28.1, 27.9, 27.1, 24.0, 23.4. HRMS: Anal. calcd. for $[M+H]^+$ $C_{35}H_{42}N_4O_6$: 619.3490; found 619.3496.

(2*S*,2'*S*)-di-*tert*-butyl 2,2'-(((9-oxo-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-1-carboxylate) (26). Yield 131 mg (91%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.24 (s, 2H), 7.91 (m, 2H), 7.91 (m, 2H), 7.62 (m, 2H), 4.26-4.16 (m, 2H), 3.46-3.40 (m, 2H), 3.37-3.31 (m, 2H), 2.24-2.16 (m, 2H), 1.94-1.76 (m, 6H), 1.40 (app br s, 9H), 1.27 (app br s, 9H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 192.9, 171.9, 171.4, 153.6, 153.1, 139.6, 138.8, 134.2, 125.0, 121.1, 115.0, 78.8, 78.6, 60.5, 60.1, 46.6, 46.1, 31.0, 30.2, 28.2, 28.0, 24.0, 23.4. HRMS: Anal. calcd. for $[M+H]^+$ $C_{33}H_{40}N_4O_7$: 605.2970; found 605.2980.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (27). A mixture of biphenyl **23** (300 mg, 0.51 mmol) in CF_3CO_2H (2 mL) and CH_2Cl_2 (2 mL) was stirred at room temperature for 5 h. The volatile component was removed in vacuo. EDCI (253 mg, 1.3 mmol) and **capping group** (255 mg, 1.3 mmol) were added in batches over 4 min to a solution of *i*-Pr_2NEt (441 L, 2.5 mmol) in CH_2Cl_2 (2 mL), and the reaction mixture was stirred at room temperature for 75 min. The residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. A silica gel mesh was prepared from the residue and submitted to flash chromatography (silica gel: EtOAc/hexane as eluent) to provide **27** as a white solid (198 mg, 50%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.92 (s, 2H), 7.91 (s, 2H), 7.75-7.69 (m, 4H), 7.56 (d, 2H), 7.44-7.13 (m, 10H), 5.52 (d, 2H), 4.43 (m, 2H), 3.88-3.83 (m, 2H), 3.55 (s, 6H), 3.21 (m, 2H), 2.04-1.78 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.2, 168.5, 156.2, 143.6, 137.5, 137.2, 136.4, 128.7, 128.1, 127.9, 119.6, 118.1, 116.2,

60.8, 56.8, 51.7, 47.0, 36.7, 29.4, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{43}H_{44}N_6O_8$: 773.3293; found 773.3296.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((9,9-difluoro-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (28). Yield 18 mg (52%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.14 (s, 2H), 8.05 (s, 2H), 7.77 (d, 2H), 7.71 (s, 4H), 7.43-7.10 (m, 10H), 5.51 (d, 2H), 4.39 (m, 2H), 3.85 (m, 2H), 3.55 (s, 6H), 3.21 (m, 2H), 2.06-1.79 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.6, 168.5, 156.2, 139.3, 137.3, 137.0, 128.6, 128.4, 128.1, 127.9, 127.6, 122.8, 121.2, 114.5, 60.8, 56.7, 51.6, 47.0, 29.3, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{43}H_{42}F_2N_6O_8$: 809.3105; found 809.3109.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((9,9-dimethyl-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (29). Yield 135 mg (53%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 9.96 (s, 2H), 7.86 (s, 2H), 7.74 (d, 2H), 7.69 (d, 2H), 7.51 (dd, 2H), 7.43-7.10 (m, 10H), 5.52 (d, 2H), 4.43 (m, 2H), 3.83 (m, 2H), 3.55 (s, 6H), 3.20 (m, 2H), 2.05-1.78 (m, 8H), 1.41 (s, 6H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 170.1, 168.3, 156.1, 153.7, 138.0, 137.2, 133.7, 128.6, 128.1, 127.8, 119.8, 118.3, 113.7, 60.7, 56.7, 51.6, 47.0, 46.5, 29.3, 27.2, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{45}H_{48}N_6O_8$: 801.3606; found 801.3611.

Dimethyl ((1*R*,1'*R*)-((2*S*,2'*S*)-2,2'-(((9-oxo-9*H*-fluorene-2,7-diyl)bis(azanediyl))bis(carbonyl))bis(pyrrolidine-2,1-diyl))bis(2-oxo-1-phenylethane-2,1-diyl))dicarbamate (30). Yield 12 mg (66%). ^{1}H NMR (DMSO-d_6, δ = 2.5 ppm, 400 MHz): 10.10 (s, 2H), 7.91 (s, 2H), 7.76 (t, 4H), 7.64 (d, 2H), 7.43-7.10 (m, 10H), 5.51 (d, 2H), 4.39 (m, 2H), 3.85 (m, 2H), 3.55 (s, 6H), 3.20 (m, 2H), 2.05-1.79 (m, 8H). ^{13}C NMR (DMSO-d_6, δ = 39.52 ppm, 100 MHz): 192.8, 170.6, 168.5, 156.2, 139.5, 138.8, 137.1, 134.2, 128.6, 128.1, 127.9, 125.0, 121.1, 115.0, 60.8, 56.7, 51.7, 47.0, 29.3, 24.3. HRMS: Anal. calcd. for $[M+H]^+$ $C_{43}H_{42}N_6O_9$: 787.3086; found 787.3089.

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국문초록

새로운 벤지딘 및 다이아미노플루오렌 유도체 구조의 C형 간염 바이러스 저해제 개발에 관한 연구

2010년 BMS(Bristol-Myers Squibb)사에서 개발한 biaryl계 HCV NS5A Inhibitor (BMS-790052, Daclatasvir)가 매우 높은 활성으로 HCV NS5A를 저해하는 것으로 Nature 지에 발표되었다. 이에 Daclatasvir의 imidazole을 amide 작용기로 치환하여 Benzidine으로부터 3단계 반응을 통하여 Benzidine prolinamide 구조의 신규 저해제를 개발하였다. 이후 구조-활성 관계를 통하여 새로운 biaryl계와 fluorene 유도체 화합물들을 합성하였으며 최적화된 화합물 선정하고자 하였다. 이를 토대로 하여 타 기관과 협업을 통한 저해제의 바이러스 저해 활성 측정, 전임상 후보물질 도출을 위한 물성조사, 약동역학 테스트 및 초기 독성 조사하였다. 그 결과 **12, 14, 15, 28** and **29**과 같은 독성이 없고 기대한 항-HCV 약물로써 고활성을 가지는 새로운 C형간염 저해제를 합성하였다.