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Novel 4-Thiazolidinones as Non-Nucleoside Inhibitors of Hepatitis C Virus NS5B RNA-Dependent RNA Polymerase

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In continuation of our efforts to develop new derivatives as hepatitis C virus (HCV) NS5B inhibitors, we synthesized novel 5-arylidene-4-thiazolidinones. The novel compounds **29**–**42**, together with their synthetic precursors **22**–**28**, were tested for HCV NS5B inhibitory activity; 12 of these compounds displayed IC_{50} values between 25.3 and 54.1 μM. Compound **33**, an arylidene derivative, was found to be the most active compound in this series with an IC_{50} value of 25.3 μM. Molecular docking studies were performed on the thumb pocket-II of NS5B to postulate the binding mode for these compounds.

Keywords: Antiviral agents / Hepatitis C / HCV NS5B polymerase / Molecular modeling / 4-Thiazolidinones

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Introduction

Hepatitis C virus (HCV) was identified in 1989 as the causative agent for non-A, non-B hepatitis infection [1] and currently has an estimated prevalence of 180–200 million people worldwide [2]. HCV infection is the principle cause of mortality related to hepatocellular carcinoma and is a leading indication for liver transplantation. HCV is also marked by its lymphotropic characteristics with patients developing cryoglobulinemia, B-cell non-Hodgkin's lymphoma, and monoclonal gammopathies [3].

To date, there is no prophylactic vaccine available against HCV. HCV vaccine development has proved particularly challenging due to HCV diversity and antiviral host immunity, resulting in only a few vaccine candidates being tested in phase I/II trials [4]. Treatment of HCV has been an unmet clinical need as efficacy of the most widely used pegylated interferon-alpha (PEG-IFN-alpha) plus ribavirin (RBV) treatment regimen is limited against various HCV genotypes and in addition associated with drawbacks of specificity and toxicity.

Following identification of HCV as an enveloped, positive-stranded RNA virus with 9.6 kb genome encoding three structural (Core, E1, and E2) and seven nonstructural (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [5–7], development of small molecule directly acting antivirals (DAA) against HCV yielded two NS3-4A protease inhibitors: boceprevir (Victrelis®) and telaprevir (Incivek®). Upon clinical use of approved boceprevir and telaprevir in combination with Peg-IFN-α and RBV for the treatment of genotype 1 chronic hepatitis C in adult patients, the effectiveness of anti-HCV therapy has increased to 70% from 40 to 50%. However, since HCV exhibits genetic heterogeneity and a high mutation rate, four new DAA treatment options have been suggested; HCV NS3 protease inhibitors simeprevir (Olysio®) and faldaprevir together with Peg-IFN-α and RBV for the treatment of genotype 1 patients; nucleotide NS5B polymerase inhibitor sofosbuvir (Sovaldi®) plus RBV with and without Peg-IFN-α for the treatment of

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Abbreviations: AP, allosteric pocket; **HCV,** hepatitis C virus; **NI,** nucleoside inhibitor; **NNI,** non-nucleoside inhibitor; **PP-I,** palm pocket-I; **RdRp,** RNA-dependent RNA polymerase; **SP,** subpocket; **TP,** thumb pocket.

genotypes 1–6 patients and genotypes 1–4 patients, respectively, sofosbuvir together with NS5A replication inhibitor daclatasvir for genotypes 1–3 patients [8–10].

Another DAA approach against HCV has focused on targeting its NS5B RNA-dependent RNA polymerase (RdRp), which catalyzes HCV RNA replication and has no functional equivalent in the host [11, 12]. NS5B, comprising 531 amino acid residues, is folded into characteristic fingers, palm, and thumb subdomains of the polymerase protein family. Extensive crystallographic studies has facilitated the identification of the binding sites for nucleoside triphosphate substrate and validation of the five allosteric pockets on NS5B. Thumb pocket-I (TP-I) and TP-II are located in the thumb subdomain, palm pocket-III (PP-III) and PP-IV are located in the palm subdomain and PP-V is located in the finger subdomain [13–15].

Small molecule inhibitors binding to allosteric pockets of NS5B are considered to have the advantage of being more selective in addition to demonstrating fewer off-target side effects compared to nucleoside or nucleotide analogs as there are no homologous cellular enzymes, which bind to these allosteric inhibitors [16, 17]. Non-nucleoside allosteric inhibitors of HCV RdRp have been reported to include diverse chemical scaffolds as benzo-1,2,4-thiadiazines, 1,5-benzodiazepines, benzo[*de*]-isoquinolines, pyrazolo[1,5-*a*]pyrimidines, acrylic acid, anthranilic acid, indole-2-carboxylic acid, rhodanine, amino-substituted isothiazole, benzofuran, 2-oxy-6-fluorobenzamide, piperazine-2-carboxamide, indole, and phenylalanine [18]. Clinically validated drugs (Fig. 1) such as telaprevir (VX-222), ABT-072, tegobuvir (GS-9190), filibuvir (PF 868554), setrobuvir (ANA598), and VCH-916 hold promise as non-nucleoside NS5B polymerase inhibitors of HCV [19].

4-Thiazolidinones have been reported to possess a wide range of biological activities including antiproliferative, antibacterial, antifungal, and antimycobacterial functions [20–24]. These compounds are also known to target cyclooxygenase (COX) enzymes thus making thiazolidinones excellent candidates to develop as anti-tumor and anti-inflammatory agents [23, 24]. Further, thiazolidinone compounds have been shown to inhibit the HIV-1 reverse transcriptase (HIV-RT) [25–27]. The therapeutic potential of the thiazolidinone scaffold against HCV NS5B was first reported employing 2′,4′-difluoro-4-hydroxybiphenyl-3-carboxylic acid[2-(5-nitro-2-furyl/substituted phenyl)-4-thiazolidinone-3-yl]amides [21, 28]. Subsequently, 2,3-diarylthiazolidinones were reported to exhibit significant differences in activity against HCV RdRp and HIV-RT [27, 29–31].

Existing literature reports also presents similar chemotypes carrying thiazolone [1, 16, 32], 2-thioxo-1,3-thiazolidinone (rhodanine) [33, 34], and iminothiazolidinone (prominently BMS 824) [35] scaffolds as inhibitors of HCV NS5B. Recently, we reported 2-[[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]-imino]-5-(2,6-dichlorobenzylidene)-1,3-thiazolidin-4-one and 2-[[5-(4-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino]-5-(2,6-dichlorobenzylidene)-1,3-thiazolidin-4-one as HCV NS5B inhibitor leads with IC_{50} value of 5.6 and 19.8 μM, respectively [36]. In continuation of our efforts to develop anti-HCV NS5B inhibitors, we synthesized new derivatives of thiazolidinones bearing modifications at the aryl moiety of 5-aryl-1,3,4-thiadiazole and replacement of the arylidene core with 2,6-dichloro or 3-fluoro substituted benzylidene groups (Fig. 2). The synthesized compounds were evaluated for their *in vitro* NS5B RdRp inhibitory potential followed by cellular antiviral toxicity profiling. Further binding interactions of the most active compound with NS5B were elucidated through docking study.

Results and discussion

Chemistry

The target compounds were synthesized using a stepwise reaction protocol (Scheme 1). Thiosemicarbazones **1**–**7** were obtained by reaction of thiosemicarbazide and appropriate aldehyde. 2-Amino-1,3,4-thiadiazole derivatives **8**–**14** were obtained by oxidative cyclization of thiosemicarbazones **1**–**7** in the presence of ferric chloride. A suggested mechanism for this reaction is depicted in Scheme 2. According to the proposed cyclization mechanism, reaction is initiated by deprotonation, forming a radical at thiosemicarbazone N2. After resonance of this radical, the thiol radical attacks toward

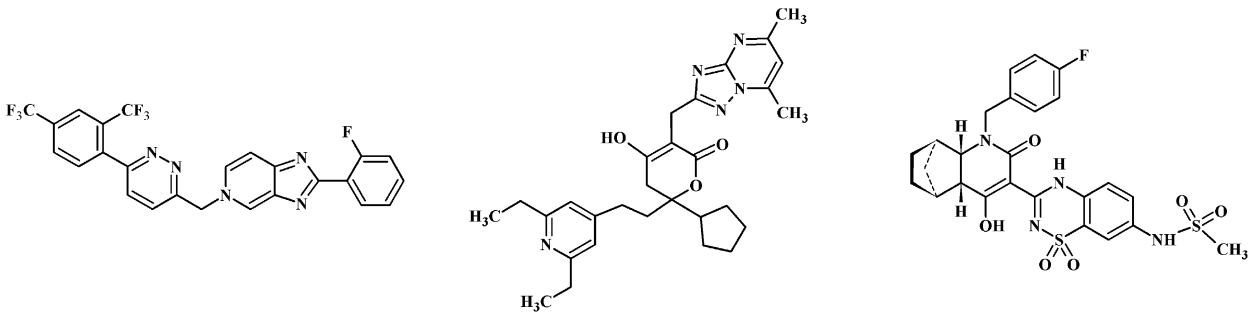


Figure 1. Small molecule inhibitors of HCV NS5B on clinical trials.

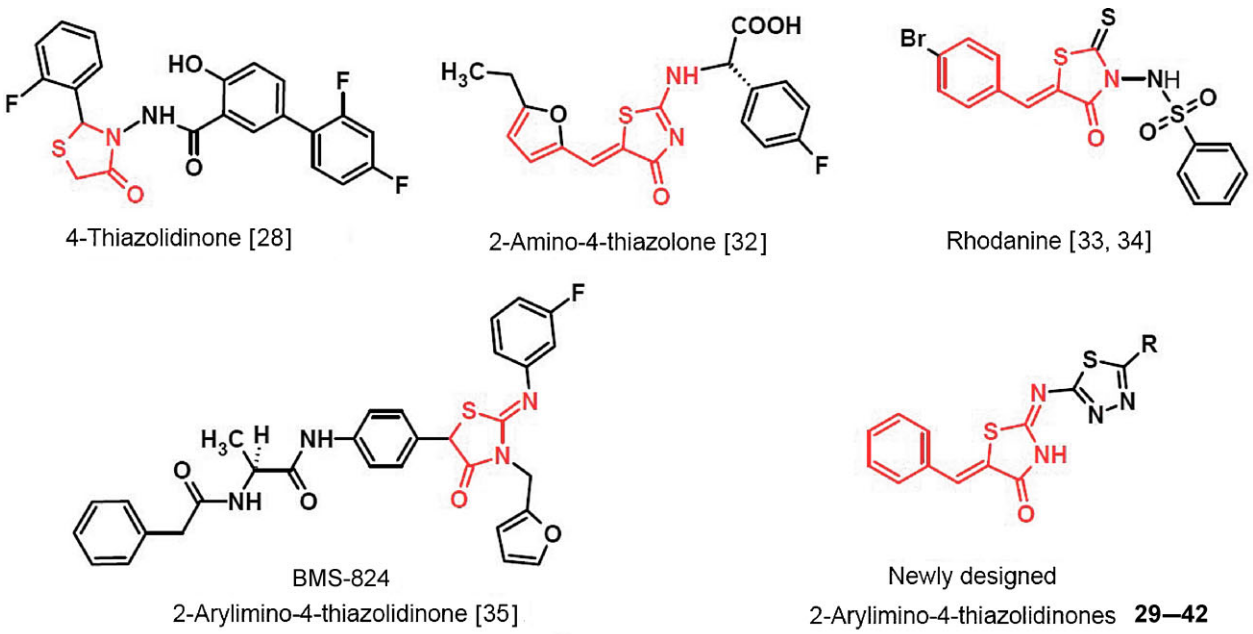
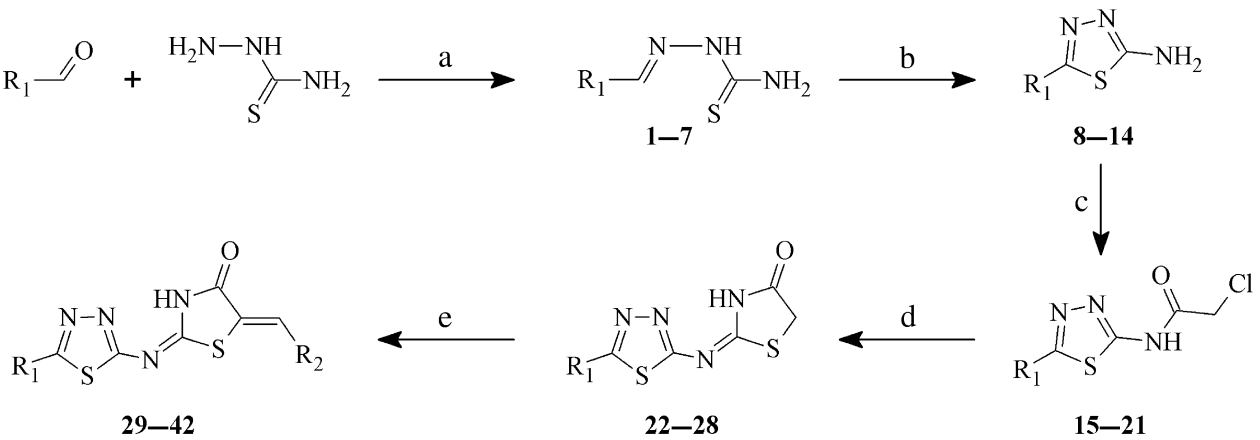


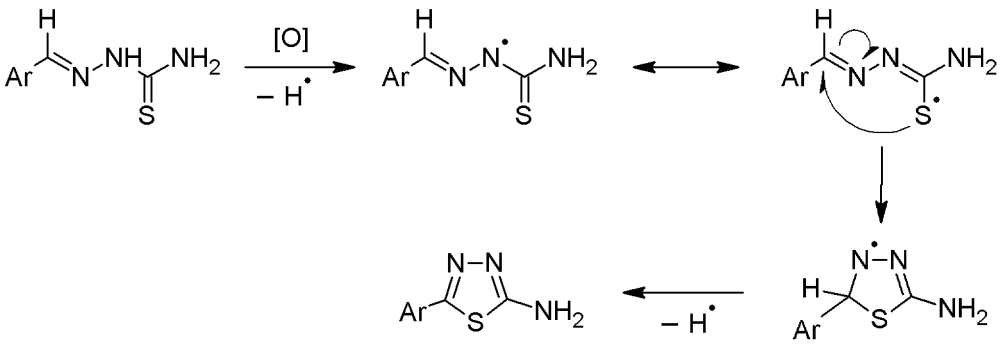
Figure 2. Structures of existing 4-thiazolidinones, 2-thioxo-4-thiazolidinones, 4-thiazolones, and the newly designed compounds **22–42**.



Scheme 1. Synthetic route to compounds **1–42**. *Reagents and conditions*: (a) Ethanol, acetic acid, reflux; (b) $FeCl_3$, ethanol, reflux; (c) $ClCH_2COCl$, TEA, DCM, rt; (d) NH_4SCN, ethanol/dioxane, reflux; (e) R_2-CHO, CH_3ONa, methanol, reflux.

azomethine carbon to form the ring and expulsion of another hydrogen radical finally yields 5-aryl-1,3,4-thiadiazole-2-amines **8–14** [37]. Syntheses of 2-chloro-*N*-[5-(aryl)-1,3,4-thiadiazol-2-yl]acetamides **15–21** were carried out by the reaction of the appropriate amines with chloroacetylchloride in the presence of TEA. Cyclization of chloroacetamides **15–21** in the presence of ethanolic solution of ammonium thiocyanate in dioxane afforded 2-{[5-(substituted phenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-ones **22–28**. In earlier studies, 1,3-thiazolidin-4-ones were obtained by the reaction of chloroacetamides with ammonium thiocyanate in ethanol [24, 38–41] or acetone [42], or DMF (under MW irradiation) [43].

The target compounds **29–42** were obtained by the reaction of 4-thiazolidinones **22–28** with commercially available aldehydes in the presence of sodium methoxide. This method was different from earlier studies in which 4-thiazolidinones were reacted with aldehydes in acetic acid medium buffered



Scheme 2. The mechanism of 1,3,4-thiadiazole ring closure via oxidative cyclization of thiosemicarbazones **1–7**.

with sodium acetate or in ethanol and piperidine. However, use of sodium methoxide in methanolic medium instead of sodium acetate in acetic acid provided shorter reaction times [44].

All synthesized compounds were checked for purity using TLC, HPLC-UV/DAD, and elemental analyses. The new compounds **12**, **16**, **17**, **19**, **21–24**, **26**, **28**, and **29–42** were characterized by their melting points and spectral data (IR, ^{1}H NMR, and MS) and ^{13}C NMR spectra were recorded for compounds **22–24**, **26–28**, **31**, **37**, and **41**. Absorption bands at 1715 cm^{-1} were attributed to the C=O stretching band of 2-chloro-*N*-[5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide **19**, which was not seen in 5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazole-2-amine **12** IR spectrum. In the NMR spectrum of **12** N–H protons were determined at 7.38–7.64 ppm with aromatic protons. However, the N–H proton of **19** was described at 13.22 ppm. After the cyclization of this compound **19**, we obtained 2-{[5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one **26** for which N–H proton was determined at 12.69 ppm and CH_2 protons were determined at 4.40 ppm.

Absorption bands at 1738–1709 cm^{-1} were attributed to the C=O stretching bands of 1,3-thiazolidine-4-one compounds **22–28** and provided confirmatory evidence for ring closure [21, 44]. Another support for thiazolidinone ring closure was the detection of signals at 4.15–4.40 ppm, due to the presence of endocyclic –S–C**H**$_2$ protons [45, 46]. NH protons of compounds **22–28** were determined at 12.43–12.69 ppm accounting for a lactam proton but not for an imine proton, which is expected to exhibit lower chemical shift values [40, 47]. ^{13}C NMR data of the thiazolidinones **22–28** have also supported the carbon framework [21, 36, 48]. The carbonyl carbon of the thiazolidinone ring appeared at about 174 ppm, the signal of the thiazolidinone C_5 appeared at 36 ppm and the signal at about 167–171 ppm was attributed to C_2 of thiazolidinone ring. Disappearance of –S–CH_2 signals in the ^{1}H NMR spectra of 5-arylidene-1,3-thiazolidinone-4-ones **29–42** indicates that active methylene group of 4-thiazolidinones reacted with selected aldehydes. N–H protons of compounds were determined at 13.16–13.23 ppm, but for compounds **31**, **32**, **34–36**, and **42** exchanged with deuterium in DMSO-d_6. Compounds **29–42** exhibited Ar–C**H**=C resonances at 7.75–7.78 ppm separately within the aromatic peaks range of 7.33–7.81 ppm. It is known that the 5-arylidene-1,3-thiazolidinones may exist as *E*/*Z* geometrical isomers due to the rotational restriction about C=C double bond. In *Z* isomer, the methine proton resonates at higher chemical shift values due to the deshielding effect of the adjacent C=O group while it resonates at lower chemical shifts due to comparatively less deshielding effect of the sulfur in *E* isomer [22, 38, 47, 49]. To this respect, we decided that 5-arylidene-1,3-thiazolidin-4-ones **29–42** were the *Z* isomers.

While the carbonyl carbon of the thiazolidinone ring appeared at 174 ppm, C_2 of thiazolidinone ring for compounds **29–42** appeared with aromatic carbons. Methylidene carbon of arylidene moiety showed resonance around 120–130 ppm in accordance with the literature [36, 44, 50].

High-resolution electrospray ionization (ESI) mass spectra (HRMS) of compounds **32** and **39** confirmed their molecular weights and empirical formulae, with less than 5 mmu bias between calculated and experimental *m*/*z* values of molecular ions. Low-resolution ESI mass spectra of compounds **22–39** and **41** were recorded in either positive or negative ionization mode. The LC–MS/MS (ESI) analysis of the synthesized compounds gave correct molecular ion peaks corresponding to $(M+H)^+$ in positive ionization and $(M-H)^-$ in negative ionization mode in each case.

Biological activity

Inhibition of NS5B RdRp activity by compounds 22–42

The anti-NS5B RdRp activity of the target compounds **22–42** (Table 1) was determined against recombinant NS5BCΔ21 1b in a primer-dependent elongation assay as described previously [28, 51]. Preliminary screening to identify candidate NS5B inhibitors was first conducted at 50 μM compound concentration and those exhibiting ≥50% inhibition of NS5B RdRp activity at this concentration were further investigated for IC_{50} value evaluation. This investigation led to the identification of 12 compounds satisfying this criterion, while nine compounds exhibited no inhibition or ≤50% inhibition

Table 1. Anti-HCV effects in cell-based assays, anti-NS5B RdRp activity, and calculated lipophilicity values of compounds 22–42.

Compound	Lab. ID code	R_1	R_2	Cell viability (%)[a]	Huh7/ Rep-Feolb inhibition (%)[b]	NS5B RdRp Inhibition (%)[c]	IC_{50} (μM)	log *P*[d]
22	KUC110142	3-Pyridyl	–	99 ± 8	NI	6 ± 5	ND	0.81
23	KUC110132	2-Fluorophenyl	–	90 ± 5	NI	NI	ND	1.66
24	KUC110136	3-Fluorophenyl	–	71 ± 1	25 ± 2	NI	ND	1.70
25	KUC110124	2-Chlorophenyl	–	71 ± 8	NI	9 ± 1	ND	2.29
26	KUC110128	2-Chloro-6-fluorophenyl	–	68 ± 2	34 ± 3	NI	ND	2.45
27	KUC110116	2,4-Dichlorophenyl	–	85 ± 6	NI	NI	ND	2.78
28	KUC110120	2,6-Dichlorophenyl	–	68 ± 2	40 ± 4	31 ± 2	ND	2.78
29	KUC110144	3-Pyridyl	3-Fluorophenyl	45 ± 4	69 ± 4	71 ± 2	47.3 ± 2.4	3.09
30	KUC110134	2-Fluorophenyl	3-Fluorophenyl	32 ± 2	74 ± 2	79 ± 5	44.6 ± 3.9	3.81
31	KUC110138	3-Fluorophenyl	3-Fluorophenyl	28 ± 1	79 ± 3	86 ± 1	34.8 ± 2.8	3.85
32	KUC110126	2-Chlorophenyl	3-Fluorophenyl	36 ± 3	89 ± 3	55 ± 5	ND	4.24
33	KUC110130	2-Chloro-6-fluorophenyl	3-Fluorophenyl	28 ± 2	97 ± 2	83 ± 1	25.3 ± 1.7	4.33
34	KUC110118	2,4-Dichlorophenyl	3-Fluorophenyl	ND	ND	ND	ND	4.66
35	KUC110122	2,6-Dichlorophenyl	3-Fluorophenyl	30 ± 3	97 ± 2	84 ± 3	37.2 ± 10.2	4.76
36	KUC110143	3-Pyridyl	2,6-Dichlorophenyl	29 ± 3	92 ± 1	74 ± 9	49.5 ± 2.9	4.01
37	KUC110133	2-Fluorophenyl	2,6-Dichlorophenyl	26 ± 1	97 ± 1	80 ± 1	54.1 ± 10.6	4.82
38	KUC110137	3-Fluorophenyl	2,6-Dichlorophenyl	30 ± 10	77 ± 6	80 ± 2	50.5 ± 2.3	4.74
39	KUC110125	2-Chlorophenyl	2,6-Dichlorophenyl	41 ± 2	88 ± 4	88 ± 2	40.2 ± 4.0	5.07
40	KUC110129	2-Chloro-6-fluorophenyl	2,6-Dichlorophenyl	33 ± 1	84 ± 5	90 ± 1	38.5 ± 4.2	5.35
41	KUC110117	2,4-Dichlorophenyl	2,6-Dichlorophenyl	23 ± 2	58 ± 4	91 ± 1	42.5 ± 1.9	5.59
42	KUC110121	2,6-Dichlorophenyl	2,6-Dichlorophenyl	26 ± 1	88 ± 4	90 ± 1	41.5 ± 1.7	5.70

[a] Huh7 and [b] Huh7/Rep-Feo1b cells were treated with the indicated compounds for 48 h for cell viability (100 μM) and anti-HCV activity (50 μM) evaluations. Cells treated with equal amounts of DMSO served as control. The data represents the average ± SD of three independent experiments in duplicate.

[c] NS5B RdRp inhibition was determined *in vitro* at 50 μM concentration of the indicated compound. The IC_{50} values were determined from dose–response curves employing 8–12 concentrations of the compound in duplicate in two independent experiments. Curves were fitted to data points using nonlinear regression analysis and IC_{50} values interpolated employing Calcusyn software. NI, no inhibition; ND, not determined. Compound **34** was not soluble at 5 mM concentration and not included in the screening.

[d] log *P* values were calculated using ALOGPS 2.102 log *P*/log *S* calculation software (http://www.vcclab.org).

of NS5B RdRp activity at this concentration. Of these, compounds **22–28** bearing 1,3-thiazolidine-4-one moiety displayed 6–31% inhibition whereas their corresponding arylidene derivatives **29–42** exhibited 71–91% NS5B RdRp inhibitory activity. Compounds **29–42** were further screened for their NS5B inhibition potency and yielded IC_{50} values between 25.3 and 54.1 μM. Among these, compound **33** with IC_{50} value of 25.3 μM was the most active of the arylidene derivatives. Together this data suggests that arylidene moiety attached to the 1,3-thiazolidine-4-one ring may be essential for anti-HCV NS5B activity.

High throughput cell-based anti-HCV screening of compounds

The parental Huh7 cells were employed for screening the effect of the compounds on cell viability while the Huh7/Rep-Feo1b replicon cells served as reporters for the anti-HCV activity of the compounds in cell-based assay [52, 53]. This investigation yielded a clear cut trend, with the 1,3-thiazolidine-4-one moiety bearing compounds **22–28** exhibiting ≥68% cellular viability at 100 μM concentration in contrast to ≤45% cellular viability observed with the corresponding arylidene derivatives **29–42** (Table 1). This data suggests that toxicity of 1,3-thiazolidine-4-one compounds **29–42** increased by integration of arylidene core.

Furthermore, there appeared to be an inverse correlation between the effect of the compounds on cellular viability and their antiviral efficacy. Thus, compounds **22**, **23**, and **25** with ≥85% cellular viability exhibited no effect on HCV RNA replication at 50 μM concentration. The remaining four 1,3-thiazolidine-4-one bearing compounds displayed ≥68% cellular viability but ≤40% inhibition of HCV RNA replication at the aforementioned concentrations. By contrast, compounds **29–42** carrying the arylidene moiety on the 1,3-thiazolidine-4-one scaffold exhibited ≥70% inhibition of HCV RNA replication but displayed ≤45% cellular viability. It may be noted though that this inhibition may partly be attributed to the toxicity of the compounds in cell-based assay.

With the objective of gaining some insight into the chemical properties of this group of compounds, we derived their log *P* values (Table 1) using ALOGPS 2.102 log *P*/log *S* calculation software (http://www.vcclab.org). The derived log *P* values were least for compound **22** and highest for compound **42**. It was apparent from this data that compounds with log *P* values between 3.5 and 4.5 exhibited better inhibition of NS5B RdRp activity, whereas a decrease in hydrophobicity of the compounds to log *P* values below 2.8 negatively impacted both their ability to inhibit *in vitro* NS5B polymerase activity as well as the anti-HCV activity in cell-based assay. This observation may assist in efforts to design better 1,3-thiazolidine-4-one based inhibitors.

Molecular modeling studies

Docking model of the most potent compound 33 at thumb pocket-II of NS5B

The docking studies for the compounds reported here were performed on thumb pocket-II (TP-II) of NS5B using X-ray co-crystal structure of HCV NS5B-PF868554 (PDB ID: 3FRZ) [54] following a similar protocol as mentioned in our previous report [36]. Extra precision (XP) docking results show a correlation between the glide gscore representing the binding energy to the observed IC_{50} values viz. **33** (−5.901 kcal/mol) < **31** (−5.626 kcal/mol) < **35** (−4.537 kcal/mol). A ribbon representation of the docked complex of compound **33** with that of allosteric site on the thumb domain is displayed in Fig. 3. The guanidine-side chain of Arg501 forms a cation–pi interaction with the 3-fluorophenyl ring. In addition, the 3-flurophenyl ring is in vicinity to the side-chains of non-polar residues Leu419, Met423, Tyr477, Ile482, and Leu497. The backbone "NH" of the dipeptide fragment formed by Ser476 and Tyr477 may interact electrostatically with the thiazolidinone ring and the bridging imine nitrogen. The protonated amine nitrogen in the side-chain of Lys533 forms a cation–pi interaction with the thiadiazole ring. Moreover, the N-3 atom of the thiadiazole ring interacts with the guanidine side-chain of Arg422 through electrostatic bonding. The 2-chloro group in the 2-chloro-6-fluorophenyl ring forms electrostatic interactions with the polar side-chains of Asn527 and Arg422. These docking studies will facilitate further lead optimization efforts.

Conclusions

As a part of our continuing efforts to identify potent inhibitors of HCV NS5B polymerase, a novel series of 5-arylidene-4-thiazolidinones were synthesized and evaluated for their NS5B RdRp inhibitory activity. Compounds **22–28** displayed 6–31% inhibition whereas their corresponding arylidene derivatives **29–42** exhibited 71–91% NS5B RdRp inhibitory activity. Compounds **29–42** were further screened for their NS5B inhibition potency and yielded IC_{50} values between 25.3 and 54.1 μM. By performing a high throughput cell-based anti-HCV screening for compounds **22–42**; compounds **22–28** were observed to exhibit ≥68% cellular viability at 100 μM concentration in contrast to compounds **29–42** with ≤45% cellular viability. Docking model of compound **33** with TP-II of NS5B will assist future lead optimization efforts. Outcomes of all these efforts will be

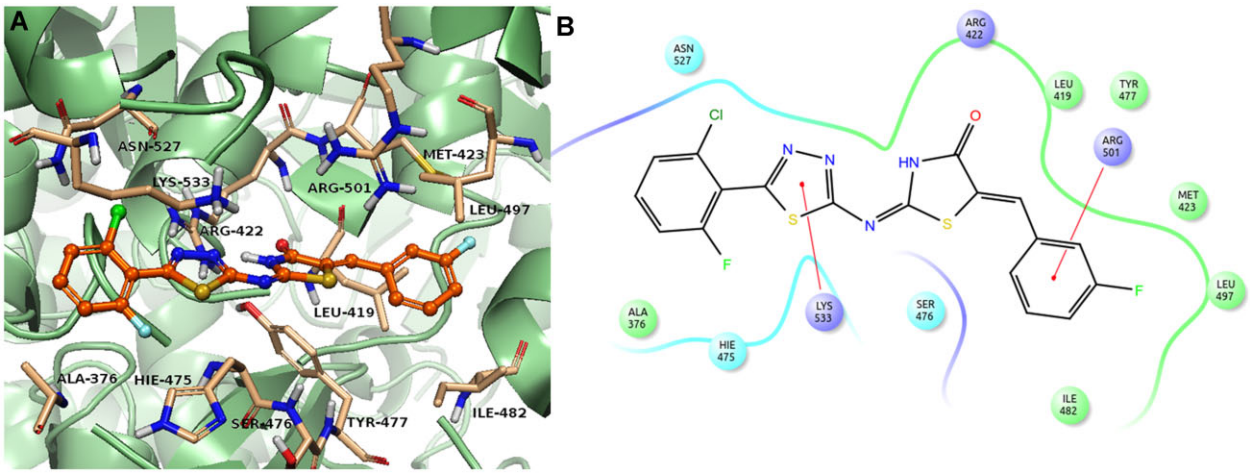


Figure 3. XP docking model of compound **33** at thumb pocket-II of NS5B. (A) NS5B protein is shown in ribbon (pale green) presentation. Selected amino acids are depicted as sticks with the atoms colored as carbon – wheat, hydrogen – white, nitrogen – blue, oxygen – red, sulfur – yellow) whereas the inhibitor is shown as ball and stick model with the same color scheme as above except carbon atoms are represented in orange. The image was generated using pymol v1.6. (B) A 2D representation of the docked model in panel A with the residues within 5 Å of compound **33** of NS5B is shown. The amino acids are shown as colored bubbles; purple indicates charged, cyan indicates polar, and green indicates hydrophobic residues. Red line shows cation–pi interaction.

taken into consideration for designing new analogs with lower cellular toxicity.

Experimental

Chemistry

All solvents and reagents were obtained from commercial sources and used without further purification. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany), using developing systems: S1: chloroform/methanol/acetic acid (93:5:2 v/v/v) and S2: acetone/DMF/ethanol (10:5:1 v/v/v). Spots were detected under UV light at $\lambda = 254$ and 366 nm.

All melting points were determined using Kleinfeld SMP-II basic model point apparatus and are uncorrected. Elemental analyses were obtained using Leco CHNS-932 and are consistent with the assigned structures. High-resolution electron impact mass spectra of compounds **32** and **39** were recorded on Jeol JMS-700 High Resolution instrument. ESI positive and negative ionization (low resolution) mass spectra of the synthesized compounds were obtained using AB SCIEX API 2000 LC–MS/MS instrument.

Infrared spectra were recorded on a Shimadzu FT-IR Affinity-1 and data are expressed in wavenumber (cm^{-1}). NMR spectra were recorded on Bruker AVANCE DPX 300–600 MHz for ^1H NMR and ^{13}C NMR. The chemical shifts were expressed in ppm downfield from tetramethylsilane (TMS) using DMSO-d_6 as solvent. The high-pressure liquid chromatographic system consists of an Agilent 1100 series instrument equipped with quaternary solvent delivery system and a model Agilent series G1315 B photodiode array detector. A Rheodyne syringe loading sample injector with 50 μL sample loop was used for the injection of the analytes. Chromatographic data were collected and processed using Agilent ChemStation plus software. The separation was performed at ambient temperature by using reversed phase ACE C18 (4 mm × 100 mm × 5 μm) column. All experiments were performed in gradient mode. The mobile phase was prepared by mixing acetonitrile and TEA-pH 4.50 phosphate buffer (80:20 v/v during 0–2 min, 70:30 v/v during 2–4 min, 60:40 v/v during 4–6 min, 50:50 v/v during 6–8 min, 25:75 v/v during 8–10 min, 0:100 v/v during 10–18 min, 40:60 v/v during 18–20 min, 80:20 v/v during 20–22 min) and filtered through a 0.45 μm pore filter and subsequently degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 0.8 mL/min. Detection of the analytes was carried out at 210, 230, 254, and 280 nm.

SMILES were generated from the structures using the ACD/ChemSketch version 12.0 molecular editor (http://www.acdlabs.com) and then log *P* values were calculated using ALOGPS 2.102 log *P*/log *S* calculation software [55, 56]. The calculated log *P* values for the compounds are given in Table 1.

*General procedure for the synthesis of 2-(arylmethylidene)hydrazinecarbothioamides (**1–7**)*

The ethanolic solution of thiosemicarbazide (30 mmol) was heated under reflux with various aromatic aldehydes (30 mmol) in the presence of a few drops of acetic acid. The crude products **1–7** were filtered and cyrstallized from appropriate solvent.

*2-(Pyridin-3-ylmethylidene)hydrazinecarbothioamide (**1**)*

Yield 46%. m.p. 218–219°C (MeOH–CH_3COOH) (lit. 216°C [57]). TLC R_f: 0.70 (S1). HPLC t_R (min): 1.28. IR, ν (cm^{-1}): 3391, 3263 (N–H str), 1627–1591 (C=N str), 1360–1264 (C=S str).

*2-(2-Fluorobenzylidene)hydrazinecarbothioamide (**2**)*

Yield 97.4%. m.p. 184–185°C (EtOH) (lit. 183–184°C [58]). TLC R_f: 0.70 (S1). HPLC t_R (min): 7.6. IR, ν (cm^{-1}): 3432, 3370 (N–H str), 1593 (C=N str), 1244 (C=S str).

*2-(3-Fluorobenzylidene)hydrazinecarbothioamide (**3**)*

Yield 84.6%. m.p. 190–192°C (EtOH) (lit. 190°C [59]). TLC R_f: 0.80 (S1). HPLC t_R (min): 5.22. IR, ν (cm^{-1}): 3391, 3235 (N–H str), 1576 (C=N str), 1361–1294 (C=S str).

*2-(2-Chlorobenzylidene)hydrazinecarbothioamide (**4**)*

Yield 78.2%. m.p. 220°C (EtOH) (lit. 220–221°C [60]). TLC R_f: 0.68 (S1). HPLC t_R (min): 8.22. IR, ν (cm^{-1}): 3414, 3245 (N–H str), 1591 (C=N str), 1374–1278 (C=S str).

*2-(2-Chloro-6-fluorobenzylidene)hydrazinecarbothioamide (**5**)*

Yield 57.7%. m.p. 231°C (EtOH) (lit. 228°C [61]). TLC R_f: 0.66 (S1). HPLC t_R (min): 8.53. IR, ν (cm^{-1}): 3406, 3229 (N–H str), 1595 (C=N str), 1285 (C=S str).

*2-(2,4-Dichlorobenzylidene)hydrazinecarbothioamide (**6**)*

Yield 59.3%. m.p. 240°C (EtOH) (lit. 238°C [59]). TLC R_f: 0.62 (S1). HPLC t_R (min): 10.22. IR, ν (cm^{-1}): 3444, 3323 (N–H str), 1587 (C=N str), 1386–1275 (C=S str).

*2-(2,6-Dichlorobenzylidene)hydrazinecarbothioamide (**7**)*

Yield 27%. m.p. 238°C (EtOH) (lit. 236–237°C [62]). TLC R_f: 0.68 (S1). HPLC t_R (min): 8.00. IR, ν (cm^{-1}): 3406, 3235 (N–H str), 1590 (C=N str), 1290 (C=S str).

*General procedure for the synthesis of 5-(aryl)-1,3,4-thiadiazol-2-amines (**8–14**)*

Compounds **1–7** (1 mmol) were dissolved in ethanol and ethanolic ferric chloride solution (4 mmol) was added. The reaction mixtures were heated under reflux for 16–20 h. The mixtures were neutralized using ammonia solution, filtered, and washed with water, dried, and cyrstallized from appropriate solvent to obtain products **8–14**.

*5-(Pyridin-3-yl)-1,3,4-thiadiazol-2-amine (**8**)*

Yield 88%. m.p. 236–239°C (EtOH) (lit. 236–238°C [63]). TLC R_f: 0.71 (S1). HPLC t_R (min): 1.18. IR, ν (cm^{-1}): 3259, 3222 (N–H str), 1643 (N–H bending), 1575 (C=N str).

*5-(2-Fluorophenyl)-1,3,4-thiadiazol-2-amine (**9**)*
Yield 51%. m.p. 226–227°C (EtOH) (lit. 223–225°C [64]). TLC R_f: 0.59 (S1). HPLC t_R (min): 6.29. IR, ν (cm^{-1}): 3231 (N–H str), 1634–1613 (N–H bending), 1581 (C=N str).

*5-(3-Fluorophenyl)-1,3,4-thiadiazol-2-amine (**10**)*
Yield 86%. m.p. 229–230°C (EtOH) (lit. 234–236°C [64]). TLC R_f: 0.55 (S1). HPLC t_R (min): 4.43. IR, ν (cm^{-1}): 3256 (N–H str), 1634–1612 (N–H bending), 1587 (C=N str).

*5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-amine (**11**)*
Yield 54%. m.p. 190–192°C (EtOH/DMF) (lit. 192–195°C [65]). TLC R_f: 0.56 (S1). HPLC t_R (min): 6.98. IR, ν (cm^{-1}): 3273 (N–H str), 1638 (N–H bending), 1566 (C=N str).

*5-(2-Chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-amine (**12**)*
Yield 72%. m.p. 182–184°C (ACN). TLC R_f: 0.53 (S1). HPLC t_R (min): 6.68. IR, ν (cm^{-1}): 3378, 3262 (N–H str), 1600 (N–H bending), 1568 (C=N str). ^{1}H NMR: δ (ppm) 7.38–7.64 (m, 5H, ArH, NH_2). Anal. calcd. for $C_8H_5ClFN_3S$ (229.66): C, 41.84; H, 2.19; N, 18.30; S, 13.96%. Found C, 41.59; H, 2.48; N, 17.93; S, 13.94%.

*5-(2,4-Dichlorophenyl)-1,3,4-thiadiazol-2-amine (**13**)*
Yield 70.7%. m.p. 229°C (EtOH/DMF) (lit. 240–242°C [39]). TLC R_f: 0.48 (S1). HPLC t_R (min): 9.28. IR, ν (cm^{-1}): 3288, 3279 (N–H str), 1613 (N–H bending), 1587 (C=N str).

*5-(2,6-Dichlorophenyl)-1,3,4-thiadiazol-2-amine (**14**)*
Yield 72.2%. m.p. 254–255°C (EtOH/DMF) (lit. 257–259°C [66]). TLC R_f: 0.48 (S1). HPLC t_R (min): 8.42. IR, ν (cm^{-1}): 3288, 3279 (N–H str), 1613 (N–H bending), 1558 (C=N str).

*General procedure for the synthesis of 2-chloro-N-[5-(aryl)-1,3,4-thiadiazol-2-yl]acetamides (**15–21**)*
Compounds **8–14** (5 mmol) were dissolved in DCM and TEA (6 mmol) was added to the reaction mixtures. Chloroacetyl chloride (10 mmol) was slowly added to the reaction mixtures. The reaction mixtures were heated for 2 h under reflux. The crude products were filtered, dried, and cyrstallized from appropriate solvent to obtain products **15–21**.

*2-Chloro-N-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-acetamide (**15**)*
Yield 49%. m.p. 270–272°C (MeOH) (lit. 282–283°C [67]). TLC R_f: 0.83 (S1). HPLC t_R (min): 8.50. IR, ν (cm^{-1}): 3102 (N–H str), 1704 (amide C=O), 1591 (C=N str).

*2-Chloro-N-[5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide (**16**)*
Yield 83%. m.p. 271°C (MeOH). TLC R_f: 0.72. HPLC t_R (min): 9.80 (S1). IR, ν (cm^{-1}): 3190 (N–H str), 1705 (amide C=O), 1583 (C=N str). ^{1}H NMR: δ (ppm) 4.50 (s, 2H, CH_2), 7.36–7.82 (m, 3H, ArH), 8.23–8.28 (m, 1H, ArH), 13.13 (s, 1H, NH). Anal. calcd. for $C_{10}H_7ClFN_3OS$ (271.70): C, 44.21; H, 2.60; N, 15.47; S, 11.80. Found C, 43.78; H, 2.59; N, 15.17; S, 11.65.

*2-Chloro-N-[5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide (**17**)*
Yield 64%. m.p. 239°C (MeOH). TLC R_f: 0.69 (S1). HPLC t_R (min): 7.78. IR, ν (cm^{-1}): 3180 (N–H str), 1705 (amide C=O), 1565 (C=N str). ^{1}H NMR: δ (ppm) 4.50 (s, 2H, CH_2), 7.31–7.43 (m, 1H, ArH), 7.56–7.63 (m, 1H, ArH), 7.75–7.83 (d, 2H, $J = 7.5$ Hz, ArH), 13.13 (s, 1H, NH). Anal. calcd. for $C_{10}H_7ClFN_3OS$ (271.70): C, 44.21; H, 2.60; N, 15.47; S, 11.80%. Found C, 43.96; H, 2.59; N, 15.31; S, 11.75%.

*2-Chloro-N-[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide (**18**)*
Yield 97.2%. m.p. 215–217°C (MeOH) (lit. 210–211°C [39]). TLC R_f: 0.73 (S1). HPLC t_R (min): 9.71. IR, ν (cm^{-1}): 3192 (N–H str), 1709 (amide C=O), 1575 (C=N str).

*2-Chloro-N-[5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]acetamide (**19**)*
Yield 82%. m.p. 231–233°C (MeOH). TLC R_f: 0.66 (S1). HPLC t_R (min): 9.77. IR, ν (cm^{-1}): 3180 (N–H str), 1715 (amide C=O), 1570 (C=N str). ^{1}H NMR: δ (ppm) 4.51 (s, 2H, CH_2), 7.44–7.71 (m, 3H, ArH), 13.22 (s, 1H, NH). Anal. calcd. for $C_{10}H_6Cl_2FN_3OS$ (306.14): C, 39.23; H, 1.98; N, 13.73; S, 10.47%. Found C, 39.48; H, 2.28; N, 13.66; S, 10.38%.

*2-Chloro-N-[5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide (**20**)*
Yield 63.6%. m.p. 248–250°C (MeOH) (lit. 246–247°C [39]). TLC R_f: 0.70 (S1). HPLC t_R (min): 10.41. IR, ν (cm^{-1}): 3174 (N–H str), 1708 (amide C=O), 1581 (C=N str).

*2-Chloro-N-[5-(2,6-dichlorophenyl)-1,3,4-thiadiazol-2-yl]-acetamide (**21**)*
Yield 40.3%. m.p. 218–220°C (EtOH). TLC R_f: 0.74 (S1). HPLC t_R (min): 8.09. IR, ν (cm^{-1}): 3175 (N–H str), 1722 (amide C=O), 1578 (C=N str). ^{1}H NMR: δ (ppm) 4.52 (s, 2H, CH_2), 7.60–7.73 (m, 3H, ArH), 13.20 (s, 1H, NH). Anal. calcd. for $C_{10}H_6Cl_3N_3OS$ (322.60): C, 37.23; H, 1.87; N, 13.03; S, 9.94%. Found C, 37.56; H, 2.02; N, 12.95; S, 9.83%.

*General procedure for the synthesis of 2-{[5-(aryl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-ones (**22–28**)*
Compounds **15–21** (5 mmol) were dissolved in 1,4-dioxane. Ten millimoles of ammonium thiocyanate was dissolved in ethanol and added to the reaction mixtures and heated for 6 h under reflux. The solvent was evaporated under vacuum. The crude products were filtered, dried, and crystallized from appropriate solvent.

*2-{[5-(Pyridin-3-yl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**22**)*
Yield 37%. m.p. 250–254°C (EtOH). TLC R_f: 0.76 (S1). HPLC t_R (min): 9.90. IR, ν (cm^{-1}): 3117 (N–H str), 1730 (C=O str), 1553 (C=N str). ^{1}H NMR: δ (ppm) 4.35 (s, 2H, SCH_2), 7.43–9.32 (m, 4H, ArH), 12.66 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.51 (thiazolidinone C_5), 125.07, 127.06, 135.26, 148.27, 152.21

(Ar–C), 167.50 (thiazolidinone C_2), 174.85 (thiazolidinone C_4). LC–MS-(ESI): Calculated: M_{mi}: 277.0092, $(M+H)^+$: 278.0165, $(M-H)^-$: 276.0019. Found: $(M+H)^+$: 277.9468, $(M-H)^-$: 275.9249.

*2-{[5-(2-Fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**23**)*
Yield 69%. m.p. 269–271°C (EtOH). TLC R_f: 0.81 (S1). HPLC t_R (min): 8.55. IR, ν (cm^{-1}): 3109 (N–H str), 1738 (C=O str), 1558 (C=N str). ^1H NMR: δ (ppm) 4.35 (s, 2H, SCH_2), 7.61–7.99 (m, 3H, ArH), 8.42–8.47 (m, 1H, ArH), 12.63 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.46 (thiazolidinone C_5), 117.07 and 117.36, 118.54 and 118.69, 126.11 and 126.15, 128.71, 133.56 and 133.68, 141.77 and 141.97, 156.92, 157.02 and 157.60, 160.925 (Ar–C), 167.16 (thiadiazole C_2), 171.94 (thiazolidinone C_2), 172.01 (thiadiazole C_5), 174.79 (thiazolidinone C4). Anal. calcd. for $C_{11}H_7FN_4OS_2$ (294.33): C, 44.89; H, 2.40; N, 19.04; S, 21.79%. Found C, 45.44; H, 2.55; N, 18.73; S, 21.60%. LC–MS-(ESI): Calculated: M_{mi}: 294.0045, $(M+H)^+$: 295.0118, $(M-H)^-$: 292.9972. Found: $(M+H)^+$: 294.9925, $(M-H)^-$: 292.9662.

*2-{[5-(3-Fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**24**)*
Yield 37%. m.p. 258–259°C (EtOH). TLC R_f: 0.85 (S1). HPLC t_R (min): 7.53. IR, ν (cm^{-1}): 3109 (N–H str), 1736 (C=O str), 1557 (C=N str). ^1H NMR: δ (ppm) 4.31 (s, 2H, SCH_2), 7.55–7.95 (m, 4H, ArH), 12.58 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.44 (thiazolidinone C_5), 124.11, 124.15, 132.19, 132.31, 132.77, 136.89 (Ar–C), 164.61 (thiadiazole C_2), 167.23 (thiazolidinone C_2), 171.26 (thiadiazole C_5), 174.78 (thiazolidinone C4). Anal. calcd. for $C_{11}H_7FN_4OS_2$ (294.33): C, 44.89; H, 2.40; N, 19.04; S, 21.79%. Found C, 44.92; H, 2.68; N, 18.78; S, 21.61%. LC–MS-(ESI): Calculated: M_{mi}: 294.0045, $(M+H)^+$: 295.0118, $(M-H)^-$: 292.9972. Found: $(M+H)^+$: 295.0114, $(M-H)^-$: 292.9281.

*2-{[5-(2-Chlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**25**)*
Yield 32%. m.p. 228–229°C (EtOH–water) (lit. 222–224°C [43]). TLC R_f: 0.82 (S1). HPLC t_R (min): 9.16. IR, ν (cm^{-1}): 3179 (N–H str), 1709 (C=O str), 1553 (C=N str). ^1H NMR: δ (ppm) 4.15 (s, 2H, SCH_2), 7.52–8.16 (m, 4H, ArH), 12.43 (s, 1H, lactam NH). Anal. calcd. for $C_{11}H_7ClN_4OS_2$ (310.78): C, 42.51; H, 2.27; N, 18.03; S, 20.64%. Found C, 42.73; H, 2.45; N, 17.96; S, 20.20%. LC–MS-(ESI): Calculated: M_{mi}: 309.9750, $(M+H)^+$: 310.9826, $(M-H)^-$: 308.9677. Found: $(M+H)^+$: 310.9221, $(M-H)^-$: 308.9561.

*2-{[5-(2-Chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]-imino}-1,3-thiazolidin-4-one (**26**)*
Yield 86%. m.p. 183–185°C (EtOH). TLC R_f: 0.70 (S1). HPLC t_R (min): 8.45. IR, ν (cm^{-1}): 3164 (N–H str), 1715 (C=O str), 1580 (C=N str). ^1H NMR: δ (ppm) 4.40 (s, 2H, SCH_2), 7.70–7.96 (m, 3H, ArH), 12.69 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.57 (thiazolidinone C_5), 126.92, 126.97, 134.13, 134.26, 134.58, 134.61 (Ar–C), 162.57 (thiadiazole C_2), 167.77 (thiazolidinone C_2), 173.22 (thiadiazole C_5), 174.88 (thiazolidinone C4). Anal. calcd. for $C_{11}H_6ClFN_4OS_2$ (328.77): C, 40.19; H, 1.84; N, 17.04; S, 19.51%. Found C, 40.62; H, 2.15; N, 16.89; S, 19.56%. LC–MS-(ESI): Calculated: M_{mi}: 327.9655, $(M+H)^+$: 328.9728, $(M-H)^-$: 326.9582. Found: $(M+H)^+$: 328.8571, $(M-H)^-$: 326.8817.

*2-{[5-(2,4-Dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**27**)*
Yield 11%. m.p. 190°C (EtOH) (lit. 186–188°C [39]). TLC R_f: 0.86 (S1). HPLC t_R (min): 9.29. IR, ν (cm^{-1}): 3120 (N–H str), 1736–1722 (C=O str), 1591 (C=N str). ^1H NMR: δ (ppm) 4.40 (s, 2H, SCH_2), 7.20, 7.37, 7.54 (3s, 1H, sec amine NH), 7.88 (d, 1H, $J = 8.7$ Hz, ArH), 8.14 (s, 1H, ArH), 8.41 (d, 1H, $J = 8.4$ Hz, ArH), 12.68 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.55 (thiazolidinone C_5), 128.53, 129.97, 130.86, 132.41, 132.81, 136.66 (Ar–C), 159.30 (thiadiazole C_2), 167.49 (thiazolidinone C_2), 172.38 (thiadiazole C_5), 174.83 (thiazolidinone C4). LC–MS-(ESI): Calculated: M_{mi}: 343.9360, $(M+H)^+$: 344.9432, $(M-H)^-$: 342.9287. Found: $(M+H)^+$: 344.7782, $(M-H)^-$: 342.8303.

*2-{[5-(2,6-Dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**28**)*
Yield 80%. m.p. 229–230°C (EtOH). TLC R_f: 0.91 (S1). HPLC t_R (min): 8.05. IR, ν (cm^{-1}): 3174 (N–H str), 1724 (C=O str), 1556 (C=N str). ^1H NMR: δ (ppm) 4.29 (s, 2H, SCH_2), 7.28–7.85 (m, 3H, ArH), 12.60 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 36.47 (thiazolidinone C_5), 128.68, 129.38, 133.79, 135.55 (Ar–C), 158.12 (thiadiazole C_2), 167.67 (thiazolidinone C_2), 173.06 (thiadiazole C_5), 174.77 (thiazolidinone C4). Anal. calcd. for $C_{11}H_6Cl_2N_4OS_2 \cdot \frac{1}{2}H_2O$ (354.23): C, 37.30; H, 1.99; N, 15.82; S, 18.10%. Found C, 36.99; H, 2.41; N, 16.11; S, 17.62%. LC–MS-(ESI): Calculated: M_{mi}: 343.9360, $(M+H)^+$: 344.9433, $(M-H)^-$: 342.9287. Found: $(M+H)^+$: 344.7846, $(M-H)^-$: 342.8371.

*General procedure for the synthesis of 5-(substituted benzylidene)-2-{[5-(aryl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-ones (**29–42**)*
The solution of compounds **22–28** (1.5 mmol) was dissolved in sodium methoxyde (3 mmol) by heating. An excess amount of appropriate aldehyde (1.8 mmol) was dissolved in methanol and added to this mixture and refluxed for 8–10 h. The reaction mixture was cooled at room temperature and 50 mL ice-cold water was poured into it and this mixture was neutralized with glacial acetic acid. The precipitated solid was filtered, washed with water, and then recrystallized from ethanol.

*5-(3-Fluorobenzylidene)-2-{[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**29**)*
Yield 83%. m.p. 305–306°C (EtOH). TLC R_f: 0.90 (S2). HPLC t_R (min): 9.33. IR, ν (cm^{-1}): 3077 (N–H str), 1721 (C=O str), 1599 (C=N str). ^1H NMR: δ (ppm) 7.36–7.72 (m, 9H, ArH, =CH), 13.16 (s, 1H, lactam NH). Anal. calcd. for $C_{17}H_{10}FN_5OS_2 \cdot H_2O$ (401.44): C, 50.86; H, 3.01; N, 17.45; S, 15.98%. Found C, 51.46; H, 3.00; N, 17.72; S, 15.93%. LC–MS-(ESI): Calculated: M_{mi}: 383.0311, $(M+H)^+$: 384.0383, $(M-H)^-$: 382.0238. Found: $(M+H)^+$: 383.8690, $(M-H)^-$: 381.9213.

*5-(3-Fluorobenzylidene)-2-{[5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**30**)*
Yield 70%. m.p. 277–279°C (EtOH). TLC R_f: 0.94 (S2). HPLC t_R (min): 11.48–11.73. IR, ν (cm^{-1}): 3149 (N–H str), 1702 (C=O str), 1568 (C=N str). ^1H NMR: δ (ppm) 7.33–7.75 (m, 7H, ArH), 7.78 (s, 1H, =CH), 8.22–8.26 (s, 1H, ArH), 13.18 (s, 1H, lactam NH). Anal. calcd. for $C_{18}H_{10}F_2N_4OS_2$ (400.42): C, 53.99; H, 2.52; N, 13.99; S, 16.02%. Found C, 53.32; H, 2.56; N, 13.72; S, 15.73%. LC–MS-(ESI): Calculated: M_{mi}: 400.0264, $(M+H)^+$: 401.0337, $(M-H)^-$: 399.0191. Found: $(M+H)^+$: 400.8339, $(M-H)^-$: 398.8876.

*5-(3-Fluorobenzylidene)-2-{[5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**31**)*
Yield 83%. m.p. 293°C (EtOH). TLC R_f: 0.96 (S2). HPLC t_R (min): 10.97. IR, ν (cm^{-1}): 3116 (N–H str), 1717 (C=O str), 1596 (C=N str). ^1H NMR: δ (ppm) 7.36–7.76 (m, 9H, ArH, =CH), NH proton exchanged with DMSO.^{13}C NMR: δ (ppm) 114.10, 114.42, 117.60, 117.91, 118.58, 118.85, 124.23, 126.33, 132.18, 132.75, 136.09, 136.21, 159.50 (ArC, thiazolidinone C_5, =CH–Ar), 164.67 (thiazolidinone C_2, thiadiazole C_5), 167.53 (thiazolidinone C_4), 170.84 (thiadiazole C_2). Anal. calcd. for $C_{18}H_{10}F_2N_4OS_2$ (400.42): C, 53.99; H, 2.52; N, 13.99; S, 16.02%. Found C, 53.72; H, 2.84; N, 13.94; S, 15.99%. LC–MS-(ESI): Calculated: M_{mi}: 400.0264, $(M+H)^+$: 401.0337, $(M-H)^-$: 399.0191. Found: $(M+H)^+$: 400.8158, $(M-H)^-$: 398.8927.

*5-(3-Fluorobenzylidene)-2-{[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**32**)*
Yield 22%. m.p. 310–312°C (EtOH). TLC R_f: 0.58 (S2). HPLC t_R (min): 11.25. IR, ν (cm^{-1}): 3130 (N–H str), 1726 (C=O str), 1597 (C=N str). ^1H NMR: δ (ppm) 7.19–8.11 (m, 9H, ArH, =CH), NH proton exchanged with DMSO. Anal. calcd. for $C_{18}H_{10}ClFN_4OS_2$ (416.88): C, 51.86; H, 2.42; N, 13.44; S, 15.38%. Found C, 51.82; H, 2.54; N, 13.34; S, 14.65%. HR-MS (ESI), *m/z* 417.0038 (MH^+, $C_{18}H_{10}ClFN_4OS_2$), required: 417.0041. LC–MS-(ESI): Calculated: M_{mi}: 415.9968, $(M+H)^+$: 417.0041, $(M-H)^-$: 414.9896. Found: $(M+H)^+$: 416.7717, $(M-H)^-$: 414.8577.

*5-(3-Fluorobenzylidene)-2-{[5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**33**)*
Yield 38%. m.p. 264–266°C (EtOH). TLC R_f: 0.72 (S2). HPLC t_R (min): 9.72. IR, ν (cm^{-1}): 3081 (N–H str), 1725 (C=O str), 1597 (C=N str). ^1H NMR: δ (ppm) 7.34–7.84 (m, 8H, ArH, =CH), 13.16 (s, 1H, lactam NH). Anal. calcd. for $C_{18}H_9ClF_2N_4OS_2$ (434.87): C, 49.71; H, 2.09; N, 12.88; S, 14.75%. Found C, 49.56; H, 2.47; N, 12.29; S, 14.31%. LC–MS-(ESI): Calculated: M_{mi}: 433.9874, $(M+H)^+$: 434.9947, $(M-H)^-$: 432.9802. Found: $(M+H)^+$: 434.7592, $(M-H)^-$: 432.8572.

*5-(3-Fluorobenzylidene)-2-{[5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**34**)*
Yield 48%. m.p. 297–298°C (EtOH). TLC R_f: 0.70 (S2). HPLC t_R (min): 10.26. IR, ν (cm^{-1}): 3156 (N–H str), 1717 (C=O str), 1594 (C=N str). ^1H NMR: δ (ppm) 7.33–7.66 (m, 5H, ArH), 7.78 (s, 1H, =C–H), 7.88 (s, 1H, ArH), 8.18–8.21 (d, 1H, ArH, $J = 8.4$ Hz), NH proton exchanged with DMSO. Anal. calcd. for $C_{18}H_9Cl_2FN_4OS_2 \cdot ½H_2O$ (460.33): C, 46.96; H, 2.19; N, 12.17; S, 13.93%. Found C, 47.44; H, 2.41; N, 11.99; S, 13.68%. LC–MS-(ESI): Calculated: M_{mi}: 449.9578, $(M+H)^+$: 450.9652, $(M-H)^-$: 448.9506. Found: $(M+H)^+$: 450.9312, $(M-H)^-$: 448.7850.

*5-(3-Fluorobenzylidene)-2-{[5-(2,6-dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**35**)*
Yield 81%. m.p. 266–267°C (EtOH). TLC R_f: 0.96 (S2). HPLC t_R (min): 10.94. IR, ν (cm^{-1}): 3142 (N–H str), 1729 (C=O str), 1597 (C=N str). ^1H NMR: δ (ppm) 7.33–7.82 (m, 8H, ArH, =C–H), NH proton exchanged with DMSO. Anal. calcd. for $C_{18}H_9Cl_2FN_4OS_2$ (451.32): C, 47.90; H, 2.01; N, 12.41; S, 14.21%. Found C, 47.94; H, 2.35; N, 11.89; S, 13.26%. LC–MS-(ESI): Calculated: M_{mi}: 449.9578, $(M+H)^+$: 450.9652, $(M-H)^-$: 448.9506. Found: $(M+H)^+$: 450.6951, $(M-H)^-$: 448.7866.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**36**)*
Yield 79%. m.p. 292–294°C (EtOH). TLC R_f: 0.90 (S2). HPLC t_R (min): 9.88. IR, ν (cm^{-1}): 3066 (N–H str), 1717 (C=O str), 1583 (C=N str). ^1H NMR: δ (ppm) 7.52–7.64 (m, 7H, ArH), 7.75 (s, 1H, =CH), NH proton exchanged with DMSO. Anal. calcd. for $C_{17}H_9Cl_2N_5OS_2$ (434.32): C, 47.01; H, 2.09; N, 16.12; S, 14.77%. Found C, 47.14; H, 2.35; N, 16.06; S, 14.73%. LC–MS-(ESI): Calculated: M_{mi}: 432.9625, $(M+H)^+$: 433.9698, $(M-H)^-$: 431.9558. Found: $(M+H)^+$: 433.7563, $(M-H)^-$: 431.8460.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(2-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**37**)*
Yield 40%. m.p. 260–262°C (EtOH). TLC R_f: 0.90 (S2). HPLC t_R (min): 11.77, 12.11. IR, ν (cm^{-1}): 3136 (N–H str), 1723 (C=O str), 1578 (C=N str). ^1H NMR: δ (ppm) 7.39–7.70 (m, 6H, ArH), 7.75 (s, 1H, =CH), 8.16–8.22 (m, 1H, ArH), 13.18 (s, 1H, lactam NH). ^{13}C NMR: δ (ppm) 117.54, 117.81, 118.10, 126.30, 128.62, 129.16, 131.36, 131.86, 132.20, 132,91, 136.15, 159.41, 160.78 (ArC, thiazolidinone C_5, =CH–Ar), 164.54 (thiazolidinone C_2), 161.30 (thiadizole C_5), 167.48 (thiazolidinone C_4), 171.61 (thiadiazole C_2). Anal. calcd. for $C_{18}H_9Cl_2FN_4OS_2$ (451.32): C, 47.90; H, 2.01; N, 12.41; S, 14.21%. Found C, 47.83; H, 2.07; N, 12.29; S, 14.11%. LC–MS-(ESI): Calculated: M_{mi}: 449.9578, $(M+H)^+$: 450.9652, $(M-H)^-$: 448.9506. Found: $(M+H)^+$: 450.6488, $(M-H)^-$: 448.7859.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(3-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**38**)*
Yield 79%. m.p. 260–262°C (EtOH). TLC R_f: 0.93 (S2). HPLC t_R (min): 11.12, 11.42. IR, ν (cm^{-1}): 3123 (N–H str), 1717 (C=O str), 1575 (C=N str). ^1H NMR: δ (ppm) 7.38–7.44 (m, 1H, ArH), 7.51–7.66 (m, 5H, ArH, =CH), 7.75 (d, 2H, ArH), 13.18 (s, 1H, lactam NH). Anal. calcd. for $C_{18}H_9Cl_2FN_4OS_2$ (451.32): C, 47.90; H, 2.01; N, 12.41; S, 14.21%. Found C, 47.61; H, 2.38; N, 12.11; S, 13.97%. LC–MS-(ESI): Calculated: M_{mi}: 449.9578, $(M+H)^+$: 450.9652, $(M-H)^-$: 448.9506. Found: $(M+H)^+$: 450.6702, $(M-H)^-$: 448.7804.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(2-chlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**39**)*
Yield 97%. m.p. 251–252°C (EtOH). TLC R_f: 0.92 (S2). HPLC t_R (min): 12.06. IR, ν (cm^{-1}): 3056 (N–H str), 1709 (C=O str), 1592 (C=N str). ^1H NMR: δ (ppm) 7.15–7.71 (m, 7H, ArH), 7.76 (s, 1H, =CH), 13.19 (s, 1H, lactam NH). Anal. calcd. for $C_{18}H_9Cl_3N_4OS_2 \cdot ½H_2O$ (476.79): C, 45.34; H, 2.11; N, 11.75; S, 13.45%. Found C, 45.28; H, 2.24; N, 11.71; S, 13.40%. HR-MS (ESI), *m/z* 466.9357 (MH^+, $C_{18}H_9Cl_3FN_4OS_2$), required: 466.9356. LC–MS-(ESI): Calculated: M_{mi}: 465.9283, $(M+H)^+$: 466.9356, $(M-H)^-$: 464.9210. Found: $(M+H)^+$: 466.8894, $(M-H)^-$: 464.9371.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(2-chloro-6-fluorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**40**)*
Yield 20%. m.p. 226–227°C (EtOH). TLC R_f: 0.73 (S2). HPLC t_R (min): 9.28. IR, ν (cm^{-1}): 3089 (N–H str), 1710 (C=O str), 1596 (C=N str). ^1H NMR: δ (ppm) 7.44–7.80 (m, 7H, ArH, =CH), 13.23 (s, 1H, lactam NH). Anal. calcd. for $C_{18}H_8Cl_3FN_4OS_2$ (485.77): C, 44.51; H, 1.66; N, 11.53; S, 13.20%. Found C, 44.32; H, 1.97; N, 11.29; S, 13.17%.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(2,4-dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**41**)*
Yield 39%. m.p. 282–283°C (EtOH). TLC R_f: 0.80 (S2). HPLC t_R (min): 10.28. IR, ν (cm^{-1}): 3089 (N–H str), 1706 (C=O str), 1583 (C=N str). ^1H NMR: δ (ppm) 7.51–7.65 (m, 3H, ArH), 7.75 (s, 1H, =CH), 7.88–8.15 (m, 3H, ArH), 13.20 (S, 1H, lactam NH). ^{13}C NMR: δ (ppm) 128.52, 129.96, 130.85, 132.40, 132.81, 136.66 (ArC, thiazolidinone C_5, =CH–Ar), 167.48 (thiazolidinone C_2), 159.28 (thiadizole C_5), 172.37 (thiazolidinone C_4), 174.83 (thiadiazole C_2). Anal. calcd. for $C_{18}H_8Cl_4N_4OS_2$ (502.22): C, 43.05; H, 1.61; N, 11.16; S, 12.77%. Found C, 43.03; H, 1.87; N, 10.83; S, 12.35%.

*5-(2,6-Dichlorobenzylidene)-2-{[5-(2,6-dichlorophenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**42**)*
Yield 17%. m.p. 238–240°C (EtOH). TLC R_f: 0.94 (S2). HPLC t_R (min): 10.59. IR, ν (cm^{-1}): 3128 (N–H str), 1733 (C=O str), 1586 (C=N str). ^1H NMR: δ (ppm) 7.49–7.75 (m, 7H, ArH, =CH), NH proton exchanged with DMSO. Anal. calcd. for $C_{18}H_8Cl_4N_4OS_2$ (502.22): C, 43.05; H, 1.61; N, 11.16; S, 12.77%. Found C, 43.14; H, 1.80; N, 10.86; S, 12.17%.

Biological studies

NS5B inhibition assay

The effect of the compounds on HCV NS5B RdRp activity was evaluated on poly rA-U_{12} template primer in the presence of [α-^{32}P]UTP and $MnCl_2$ as described previously [51, 68]. Reactions in the presence of the compound or DMSO were incubated at 30°C for 1 h and terminated by the addition of 5% TCA. The nascent radiolabeled RNA was precipitated on GF-B filters and counted in a liquid scintillation counter. NS5B activity in the presence of DMSO was set at 100% and that in the presence of the compounds was determined relative to this control. Compounds exhibiting $\geq$50% inhibition at 50 μM concentration were investigated further for their IC_{50} values employing 8–10 concentrations of the serially diluted compounds. The IC_{50} values were analyzed from dose–response curves utilizing Graphpad prism 3.03 software.

Cell-based anti-HCV screening

The effect of the compounds on cell viability and anti-HCV activity was investigated employing Huh7 parental and Huh7/Rep-Feo1b replicon reporter cells, respectively, as previously described [52, 53]. Briefly, 1×10^4 cells were screened at compound concentrations of 100 and 50 μM for 48 h for evaluation of cell viability and antiviral effect, respectively. The concentration of DMSO in cell culture was kept constant at 0.5%. Cell viability was measured by the colorimetric MTS assay employing the CellTiter 96AQueous One Solution assay reagent (Promega, USA). Inhibitory effect of the compounds on HCV RNA replication was measured as the relative levels of firefly luciferase signals in compound-treated cells versus DMSO controls.

Molecular modeling

The compounds were built and prepared for docking using Ligprep v2.7 and Macromodel v10.1 implemented in Maestro v9.5 (Schrödinger, LLC, New York, NY, 2013) following the steps mentioned previously [69]. The X-ray crystal structure of PF868554 bound HCV NS5B (PDB ID:3FRZ) [54] was used for docking into TP-II. The protocols for protein preparation, grid generation, and docking simulations were essentially the same as those mentioned in our earlier report except the modeling tools used were those implemented in Maestro v9.5 (Schrödinger, LLC) [36]. The highest scoring pose of compound **33** within TP-II was used for graphical analysis. All computations were carried out on a Dell Precision 490 dual processor with Linux OS (Ubuntu 12.04 LTS).

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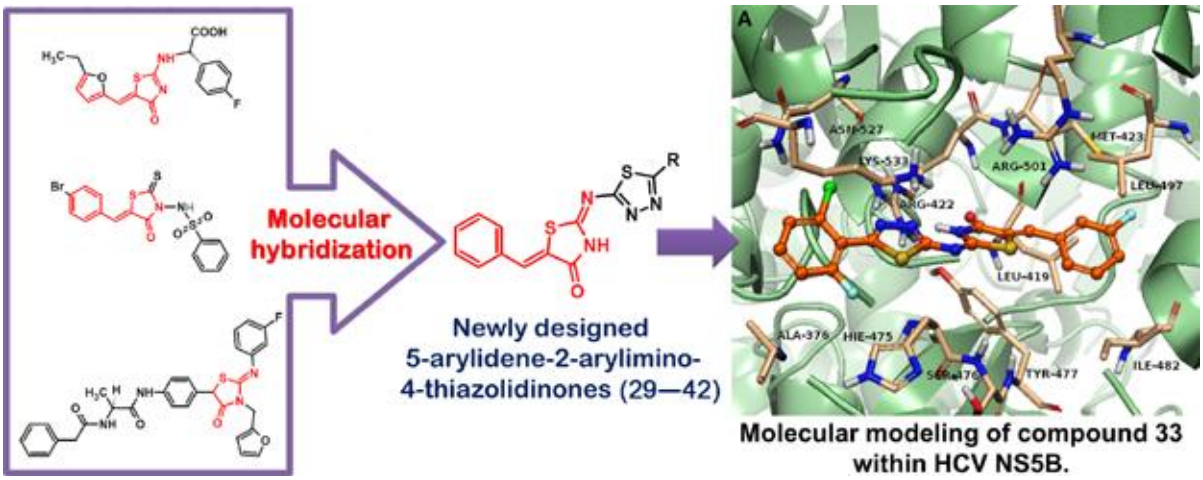

Molecular modeling of compound 33 within HCV NS5B.