Journal of Medicinal Chemistry

Article

Design, Synthesis, and Biological Evaluation of Substituted 4,6-Dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3H)-dione Analogues as Potent NS4B Inhibitors for the Treatment of Dengue Virus Infection

Jimin Xu, Xuping Xie, Na Ye, Jing Zou, Haiying Chen, Mark Andrew White, Pei-Yong Shi, and Jia Zhou

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00698 • Publication Date (Web): 12 Aug 2019

Design, Synthesis, and Biological Evaluation of Substituted 4,6-Dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione Analogues as Potent NS4B Inhibitors for the Treatment of Dengue Virus Infection

Jimin Xu,[†,∥] Xuping Xie,[‡,∥] Na Ye,[†] Jing Zou,[‡] Haiying Chen,[†] Mark A. White,[‡,§] Pei-Yong Shi,[‡,§,*] and Jia Zhou[†,§,*]

[†]Chemical Biology Program, Department of Pharmacology and Toxicology, [‡]Department of Biochemistry and Molecular Biology, and [§]Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas 77555, United States

ABSTRACT:

A series of substituted 4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione analogues were synthesized and evaluated as potent dengue virus inhibitors. Throughout a structure-activity relationship exploration on the amide of the indolone moiety, a wide range of substitutions were found to be well tolerated for chemical optimization at this position. Among these compounds, **15** (**JMX0254**) displayed the most potent and broad inhibitory activities, effective against DENV-1 to -3 with EC_{50} values of 0.78 μM, 0.16 μM and 0.035 μM, respectively, while compounds **16**, **21**, **27-29**, **47** and **70** exhibited relatively moderate to high activities with low micromolar to nanomolar potency against all four serotypes. The biotinylated compound **73** enriched NS4B protein from cell lysates in pull-down studies and the findings together with the mutation investigations further validated dengue NS4B protein as the target of this class of compounds. More importantly, compound **15** exhibited good *in vivo* pharmacokinetic properties and efficacy in the A129 mouse model, indicating its therapeutic potential against the dengue virus infection as a drug candidate for further preclinical development.

INTRODUCTION

Dengue is a viral disease vectored mainly by female *Aedes* mosquitoes that causes flu-like symptoms with high fever, headache, vomiting, rash and joint/bone/muscle pain, and sometimes it can develop into a potentially life-threatening complication called severe dengue, dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[1] Before 1970, only nine countries had experienced severe dengue epidemics. Today, the disease is widespread globally in more than 100 tropical and subtropical countries, putting almost half of the world's population at risk, and the Americas, South-East Asia and Western Pacific regions are the most seriously affected.[2-4] The incidence of dengue has grown dramatically with a 30-fold increase around the world over the past 50 years.[5] As a vast majority of cases are asymptomatic, the actual numbers of dengue cases are underreported and many cases are misclassified. It is estimated that 390 million dengue infections occur annually, of which 96 million manifest clinically (with any severity of disease), including approximately 500,000 cases of severe dengue and 22,000 deaths worldwide.[6, 7] The burden of dengue is considerable, causing human suffering, strained health services and massive economic losses. However, there remains no effective antiviral therapy for dengue fever, and vector control is the main method to prevent dengue outbreaks.[8-17] Recently, the first dengue vaccine CYD-TDV, developed by Sanofi Pasteur, has been licensed in a number of countries but has limited efficacy and safety issues.[18, 19] Because of this, it is limited to individuals ranging from 9-45 years of age and given as 3-dose series with 6 months between each dose. Moreover, in September 2018, World Health Organization (WHO) updated its recommendations regarding the use of CYD-TDV based on the evidence that seronegative vaccine recipients have an excess risk of hospitalized and severe dengue compared to seronegative non-vaccinated individuals.[20]

Therefore, there is an urgent need to develop safe and effective therapeutics for the treatment of dengue virus infection.

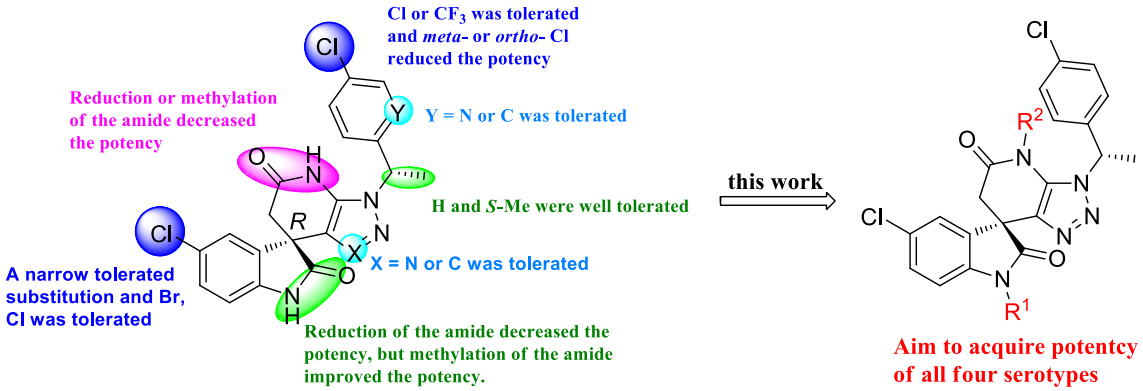


Figure 1. Previous work (SAR summary on DENV-2) and drug design for the current work.

Dengue viruses (DENV) belong to the genus *Flavivirus* within the *Flaviviridae* family, and there are 4 distinct, but closely related, serotypes of the virus (DENV-1, DENV-2, DENV-3, and DENV-4).[21, 22] A geographic region may be affected by one or more DENV serotypes simultaneously.[23-25] Recovery from infection by one serotype provides lifelong immunity against that particular serotype, while cross-immunity against the other serotypes is only partial and temporary.[26, 27] A second infection with a different dengue serotype is more likely to develop severe dengue.[28-32] Hence an ideal dengue antiviral should be effective against all four serotypes.[33] The dengue viral genome is a positive single-strand RNA of approximately 11 kb in length. This RNA encodes three structural proteins (capsid, envelope, and membrane) that form the virus particle and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that are essential for replication of the virus.[34] Dengue NS4B is a small integral membrane, consisting of 248 amino acid residues. It is moderately conserved, with about 83-89% amino acid similarity among the four dengue serotypes. Due to its high hydrophobicity, neither the crystal nor NMR structure of flavivirus NS4B is currently available.[35, 36] In the recent years, some

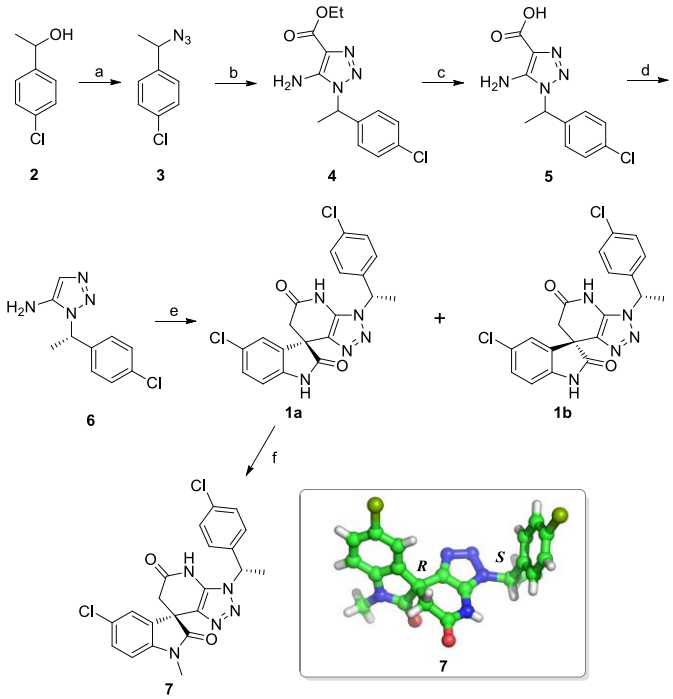
progresses have been made to figure out the biological functions of NS4B, including its engagement in host innate immunity and interactions with other viral proteins.[37-45] However, the exact mechanisms of flavivirus NS4B involved in the viral replication cycle remain elusive. Despite many gaps in our knowledge of its structure and function, NS4B protein has become an attractive therapeutic target and a relatively large number of NS4B inhibitors were discovered by phenotypic screening.[46-51] Our team discovered a series of spiropyrazolopyridone analogues as potent inhibitors against DENV-2 and DENV-3, while these compounds have a lack of potency against DENV-1 and DENV-4.[51-53] Based on the previous structure-activity relationship (SAR) studies as depicted in **Figure 1**, a narrow range of substitutions are tolerated on the core spiro scaffold and only methylation on the amide of the indolone moiety improved the potency against DENV-2. Herein, we reported our continued SAR optimization efforts mainly focused on two amide moieties, aiming to elucidate the chemical space for the potential to acquire enhanced potency of all four serotypes.

RESULTS AND DISCUSSION

Chemistry. According to the previous work, the three component condensation of substituted aminotriazoles, isatin derivatives, and Meldrum's acid can provide the access to a series of spirotrizolopyridone analogues for investigating the substitution on the two amide groups.[51, 52] However, this condensation yields the final product as two enantiomers or diastereoisomers with great discrepancy in potency, and it will bring a large amount of separation work to get each pure and potent stereoisomer. Surprisingly, when 1.2 equivalent of methyl iodide was added, direct methylation of pure diastereoisomer **1a** afforded mono-methylated product **7** with a high yield of 76% (**Scheme 1**). The structure of methylated derivative **7** was subsequently determined by X-

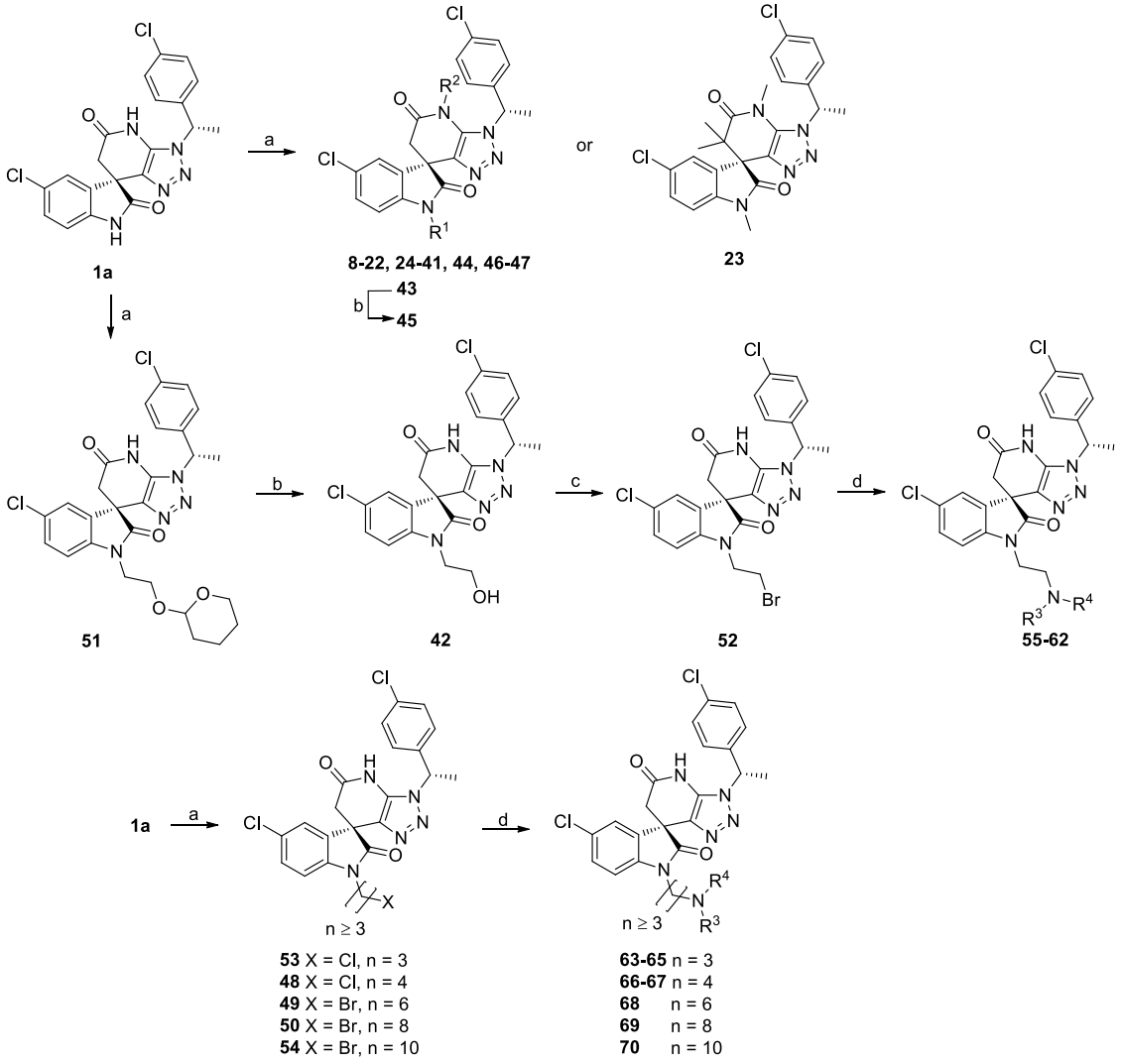
ray crystallography to confirm the methylation position and the absolute configurations of the chiral centers. These results demonstrated that the amide of the indolone moiety has a higher nucleophilic reactivity in comparison with the one of the pyridone moiety, and it provides a good chance for a quick SAR investigation on this part using pure stereoisomer **1a** as a starting material. The synthesis of diastereoisomer **1a** commenced with commercially available 1-(4-chlorophenyl)ethanol (**2**), which was brominated followed by azidation to generate azide **3**.[52] This intermediate was treated with ethyl 2-cyanoacetate and sodium in ethanol to provide aminocarboxylate **4**, which was then converted to (*S*)-aminotriazole **6** *via* hydrolysis, decarboxylation, and then chiral HPLC separation. Aminotriazole **6** was condensed with 5-chloroisatin and Meldrum's acid in acetic acid to give the final products as two diastereoisomers with a ratio of approximately 1:1, which were successfully separated by column chromatography to afford pure stereoisomer **1a** and **1b**.

Scheme 1. Synthesis of Compounds 1a and 7[a]



[a]Reagents and conditions: (a) i. PBr_3, CH_2Cl_2, 0 °C to r.t, 4 h; ii. NaN_3, DMSO, r.t, 5 h. (b) ethyl 2-cyanoacetate, Na, EtOH, 85 °C, 5 h. (c) NaOH, EtOH/H_2O, 85 °C, 4 h. (d) i. DMF, 200 °C, 20 min; ii. chiral HPLC separation. (e) 5-chloroisatin, Meldrum's acid, AcOH, 100 °C, 4 h. (f) CH_3I, NaH, DMF, 0 °C to r.t, 2 h.

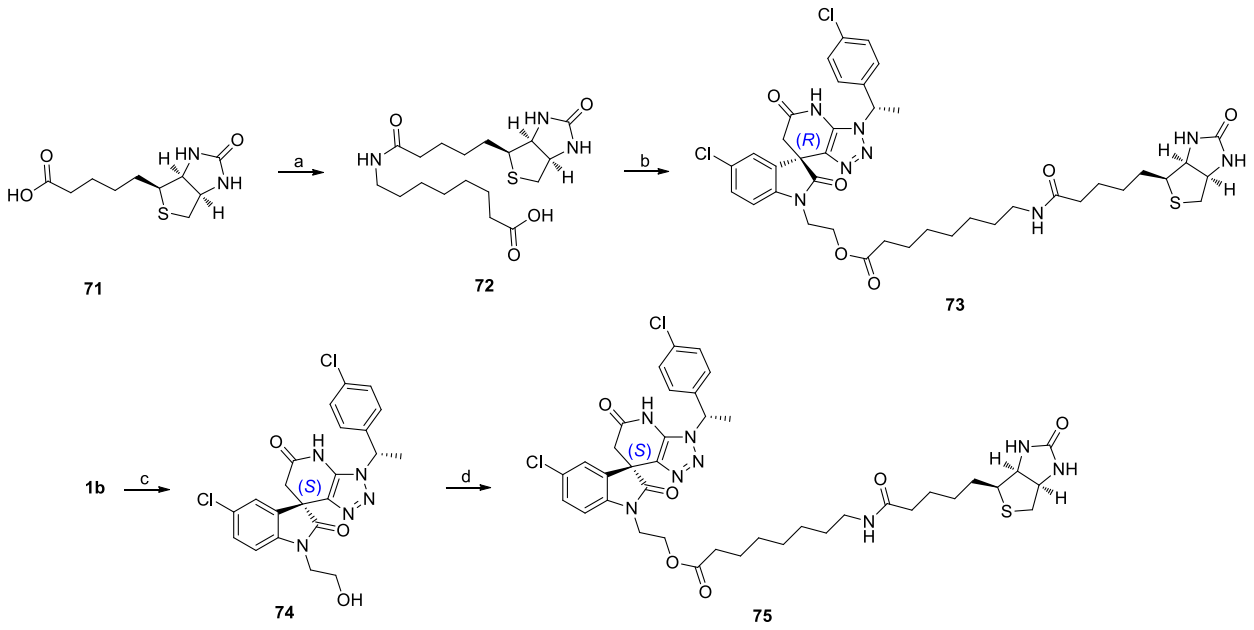
Scheme 2. Synthesis of Substituted 4,6-Dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione Analogues 8-50 and 55-70[a]



[a]Reagents and conditions: (a) RI or RBr, NaH, DMF, 0 °C to r.t or 50 °C. (b) TsOH, MeOH, r.t. (c) PPh_3, CBr_4, DCM, 0 °C to r.t. (d) R^3R^4NH, K_2CO_3, CH_3CN, 60 °C ~ 80 °C.

With pure stereoisomer **1a** in hand, the effect of the substitution on the two amide moieties was successfully explored. As outlined in **Scheme 2**, direct substitution of **1a** with corresponding iodide or bromide afforded a series of substituted 4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione analogues **8-41**, **43-44, 46-51** and **53-54**. Acid analogue **45** was accessed from ester **43** *via* hydrolysis. Depyranylation of THP ether **51** gave alcohol **42**, which was then brominated to give bromide **52**. Substitution of bromide or chloride **48-50** and **52-54** with different amines afforded the corresponding amine analogues **55-70**. To assist the target validation and mechanistic studies of this series of molecules, two biotinylated compounds **73** and **75** were designed and synthesized as the chemical probe tools (**Scheme 3**). Condensation of D-(+)-Biotin (**71**) with methyl 8-aminooctanoate was followed by hydrolysis to provide acid **72**, which was then condensed with alcohol **42** to give the biotinylated analogue **73**. The biotinylated analogue **75** was prepared using the same route as that for its diastereoisomer **73** starting from compound **1b** for comparison.

Scheme 3. Synthesis of Biotinylated Analogues 73 and 75[a]

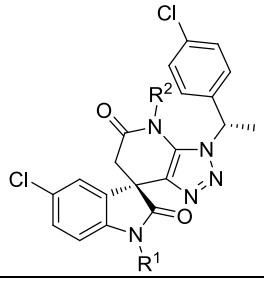


[a]Reagents and conditions: (a) i. methyl 8-aminooctanoate hydrochloride, EDCI, HOBt, DIEA, DMF, r.t; ii. NaOH, MeOH/H_2O, r.t. (b) **42**, EDCI, DMAP, DMF, r.t. (c) i. 2-(2-bromoethoxy)tetrahydro-2*H*-pyran, NaH, DMF, 0 °C to r.t.; ii. TsOH, MeOH, r.t. (d) **72**, EDCI, DMAP, DMF, r.t.

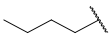
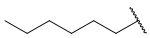
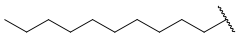
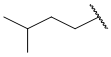
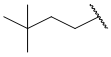
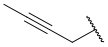
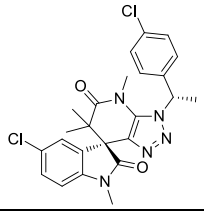
***In Vitro* Evaluation of DENV Inhibition.** All new compounds were first screened against DENV-2, and the active compounds screened out (DENV-2 EC_{50} < 1 μM) were further evaluated against other three DENV serotypes. Considering the high hydrophobicity of NS4B protein, we first introduced different alkyl groups on the amide of indolone or pyridone moiety (**Table 1**). As expected, compound **7** with methyl on the amide of indolone showed increased inhibitory activities against DENV-2 and DENV-3 (EC_{50} = 0.019 μM and 0.004 μM, respectively) compared to the parent compound **1a**. However, neither of these two compounds showed potency against DENV-1 or DENV-4 (EC_{50} > 5 μM). Compound **8** with ethyl began to show inhibitory activity against DENV-1 with an EC_{50} of 2.5 μM, while it maintained the same level of potency against DENV-2 and DENV-3 (EC_{50} = 0.024 μM and 0.006 μM, respectively). When we increased the length of alkyl, the corresponding compounds (**9**, **10** and **11**) showed improved potency against DENV-1 with EC_{50} values of 1.9 μM, 1.3 μM and 0.83 μM, respectively. Nevertheless, *n*-hexyl group (**11)** resulted in a significant loss of potency against DENV-2 and DENV-3 (EC_{50} = 0.81 μM and 0.14 μM, respectively); *n*-decyl group (**12**) led to a complete loss of potency against DENV-2 (EC_{50} > 10 μM), while it showed potency against DENV-1, -3 and -4 with EC_{50} values of 3.4 μM, 1.2 μM and 7.3 μM, respectively. These results indicated that the long length of alkyl was unfavorable for DENV-2 potency. Compounds with the branched alkyl groups (**13**-**15**) showed potency against DENV-1 to -3 with the same trend. Among these compounds, **15** with isopentyl showed the most potent inhibitory activity against DENV-1 with an EC_{50} of 0.78 μM, meanwhile maintaining a similar level of potency against

DENV-2 and DENV-3 (EC_{50} = 0.16 μM and 0.035 μM, respectively). Unfortunately, all these compounds showed no obvious potency against DENV-4 (EC_{50} > 5 μM). Interestingly, compound **16** with 3,3-dimethylbutyl maintained potency against DENV-1 to -3 (EC_{50} = 1.1 μM, 0.87 μM and 0.082 μM, respectively), while also showing inhibitory activity against DENV-4 with EC_{50} of 3.9 μM. Compounds **17** with cyclopropylmethyl and **18**-**20** with unsaturated alkyls all exhibited good potency against DENV-2 and DENV-3 and weak inhibitory activities against DENV-1 and DENV-4. Compound **21** with isopropyl on the amide of pyridone also showed broad inhibitory activities against DENV-1 to -4 with EC_{50} of 2.1 μM, 0.86 μM, 0.31 μM and 4.4 μM, respectively. However, considering the significant loss of potency against DENV-2 when introducing the alkyl group on the amide of pyridone (**21**), the subsequent investigation was mainly focused on the indolone moiety. Two isopropyl substitutions (**22**) further decreased the antiviral activity against DENV-2 (EC_{50} > 10 μM) and DENV-3 (EC_{50} = 1.5 μM), while it maintained potency against DENV-1 and DENV-4 (EC_{50} = 3.8 μM and 3.0 μM, respectively). Compound **23** with four methyl groups (α-carbonyl carbon was di-methylated) resulted in significant loss of potency against all four DENV serotypes (EC_{50} > 5 μM).

Table 1. **Antiviral Activity and Cytotoxicity of Compounds 1a and 7-23**



Compd	R^1	R^2	EC_{50} (μM) [a]				CC_{50} (μM) [b]
			DENV-1	DENV-2	DENV-3	DENV-4	
1a	H	H	>5.0	0.039	0.010	>5.0	>10

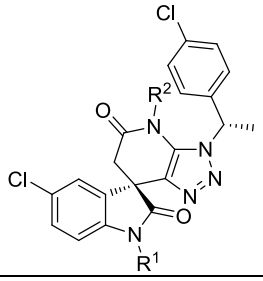
7		H	>5.0	0.019	0.004	>5.0	>10
8		H	2.5	0.024	0.006	>5.0	>10
9		H	1.9	0.071	0.010	>5.0	>10
10		H	1.3	0.075	0.018	>5.0	>10
11		H	0.83	0.81	0.14	>5.0	>10
12		H	3.4	>10	1.2	7.3	>10
13		H	5.7	0.023	0.009	>5.0	>10
14		H	1.3	0.15	0.020	>5.0	>10
15		H	0.78	0.16	0.035	>5.0	>10
16		H	1.1	0.87	0.082	3.9	>10
17		H	3.1	0.11	0.017	>5.0	>10
18		H	2.1	0.038	0.005	>5.0	>10
19		H	>5.0	0.069	0.003	>5.0	>10
20		H	>5.0	0.063	0.020	>5.0	>10
21	H		2.1	0.86	0.31	4.4	>10
22			3.8	>10	1.5	3.0	>10
23			>5.0	>5.0	>5.0	>5.0	>10

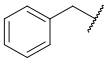
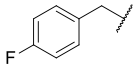
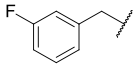
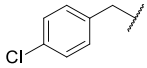
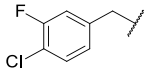
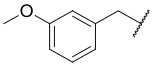
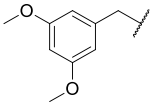
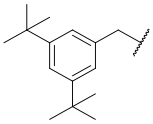
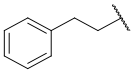
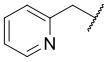
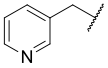
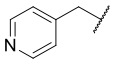
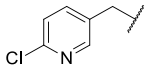
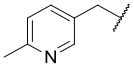
[a]EC_{50} values were determined on Huh7 cells stably expressing DENV-1 to -4 replicon. [b]CC_{50} values were measured using Huh7 cells stably expressing DENV-2 replicon.

To explore the effect of introducing aryl groups on the amide of indolone moiety, compounds **24-37** were prepared and evaluated as shown in **Table 2**. Compounds **24** with benzyl

and **25-26** with fluorobenzyl showed moderate potency against DENV-2 (EC_{50} = 0.14 ~ 0.29 μM) and DENV-3 (EC_{50} = 0.020 ~ 0.051 μM), weak potency against DENV-1 (EC_{50} = 2.0 μM, 5.0 μM and 10.0 μM, respectively), and no obvious potency against DENV-4. Compounds **27** with 4-chlorobenzyl and **28** with 4-chloro-3-fluorobenzyl resulted in a significant loss of potency against DENV-2 (EC_{50} = 3.3 μM and 4.0 μM, respectively) and DENV-3 (EC_{50} = 0.20 μM and 0.29 μM, respectively). However, these two compounds showed potency against both DENV-1 (EC_{50} = 2.2 μM and 1.5 μM, respectively) and DENV-4 (EC_{50} = 6.5 μM and 5.1 μM, respectively). 3-Methoxybenzyl substitution (**29**) showed moderate potency against DENV-2 and DENV-3 (EC_{50} = 0.83 μM and 0.087 μM, respectively) and weak potency against DENV-1 and DENV-4 (EC_{50} = 2.6 μM and 6.2 μM, respectively). 3,5-Dimethoxybenzyl substitution (**30**) displayed decreased potency against DENV-2 and DENV-3 (EC_{50} = 3.1 μM and 0.79 μM, respectively) and maintained the same level of potency against DENV-1 and DENV-4 (EC_{50} = 3.4 μM and 7.4 μM, respectively). Interestingly, 3,5-di-*tert*-butylbenzyl substitution (**31**) showed no obvious potency against DENV-2 (EC_{50} > 10 μM), while it maintained potency against DENV-1 and DENV-4 (EC_{50} = 4.6 μM and 4.9 μM, respectively). Compound **32** with homobenzyl exhibited improved potency against DENV-1 (EC_{50} = 1.1 μM) and decreased potency against DENV-2 and DENV-3 (EC_{50} = 0.60 μM and 0.19 μM, respectively) in comparison with **24**. Excitingly, compounds **33-37** with pyridine moieties displayed highly potent inhibitory activities against DENV-2 (EC_{50} = 0.020 ~ 0.15 μM) and DENV-3 (EC_{50} = 0.003 ~ 0.025 μM). However, no significant improvement of potency against DENV-4 was observed for these compounds.

Table 2. **Antiviral Activity and Cytotoxicity of Compounds 24-37**

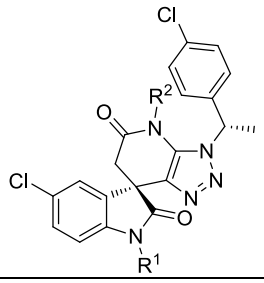


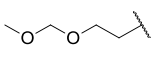
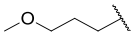
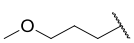
Compd	R_1	R_2	EC_{50} (μM) [a]				CC_{50} (μM) [b]
			DENV-1	DENV-2	DENV-3	DENV-4	
24		H	2.0	0.15	0.020	>5.0	>10
25		H	5.0	0.16	0.048	10	>10
26		H	10.0	0.29	0.051	>5.0	>10
27		H	2.2	3.3	0.20	6.5	>10
28		H	1.5	4.0	0.29	5.1	>10
29		H	2.6	0.83	0.087	6.2	>10
30		H	3.4	3.1	0.79	7.4	>10
31		H	4.6	>10	5.9	4.9	>10
32		H	1.1	0.60	0.19	>5.0	>10
33		H	>5.0	0.020	0.003	>5.0	>10
34		H	3.2	0.029	0.021	>5.0	>10
35		H	>5.0	0.072	0.025	>5.0	>10
36		H	>5.0	0.078	0.006	>5.0	>10
37		H	>5.0	0.15	0.011	>5.0	>10

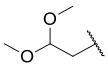
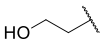
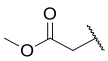
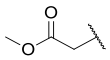
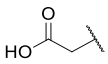
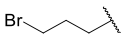
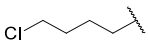
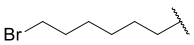
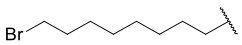
[a]EC_{50} values were determined on Huh7 cells stably expressing DENV-1 to -4 replicon. [b]CC_{50} values were measured using Huh7 cells stably expressing DENV-2 replicon.

Next, a wide range of functional groups was investigated. As listed in **Table 3**, ether (**38** and **39**), acetal (**41**), alcohol (**42**), ester (**43**), 2-fluoroethyl (**46**) and 4-chlorobutyl (**48**) groups were all well tolerated on the amide of indolone moiety, presenting potent inhibitory activities against DENV-2 (EC_{50} = 0.037 ~ 0.29 μM) and DENV-3 (EC_{50} = 0.009 ~ 0.076 μM). As expected, ether (**40**) and ester (**44**) on the amide of pyridone moiety resulted in a significant loss of potency against DENV-2 (EC_{50} = 8.7 μM and > 10 μM, respectively), compared to the corresponding substitutions on the amide of indolone moiety (**39** and **43**). Neither of these two compounds showed potency against DENV-1 and DENV-4 (EC_{50} > 10 μM). The carboxyl group (**45**) diminished the potency against DENV-2 (EC_{50} = 2.4 μM) in comparison of the corresponding ester (**43**). Surprisingly, compound **47** with 3-bromopropyl showed potency against all four serotypes DENV-1 to -4 (EC_{50} = 2.4 μM, 0.25 μM, 0.076 μM and 3.2 μM, respectively). Substitutions with long carbon chain (**49**-**50**) showed decreased potency against DENV-2, consistent with the trend observed before.

Table 3. **Antiviral Activity and Cytotoxicity of Compounds 38-50**



Compd	R_1	R_2	EC_{50} (μM) [a]				CC_{50} (μM)[b]
			DENV-1	DENV-2	DENV-3	DENV-4	
38		H	>5.0	0.092	0.019	>5.0	>10
39		H	>5.0	0.040	0.009	>5.0	>10
40	H		>10	8.7	0.91	>10	>10

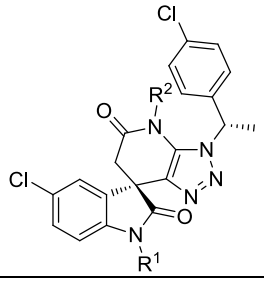
41		H	>5.0	0.29	0.076	>5.0	>10
42		H	>5.0	0.037	0.010	>5.0	>10
43		H	>5.0	0.077	0.011	>5.0	>10
44	H		>10	>10	2.7	>10	>10
45		H	ND[c]	2.4	ND	ND	>10
46		H	>5.0	0.038	0.009	>5.0	>10
47		H	2.4	0.25	0.076	3.2	>10
48		H	>5.0	0.073	0.019	>5.0	>10
49		H	ND	3.0	ND	ND	>10
50		H	ND	>5.0	ND	ND	>10

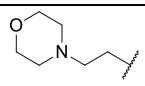
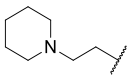
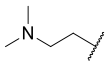
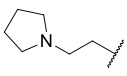
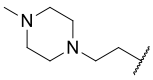
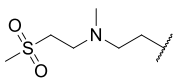
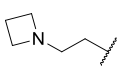
[a]EC_{50} values were determined on Huh7 cells stably expressing DENV-1 to -4 replicon. [b]CC_{50} values were measured using Huh7 cells stably expressing DENV-2 replicon. [c]ND: not detected.

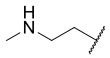
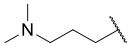
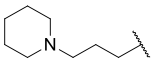
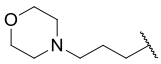
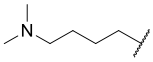
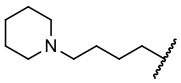
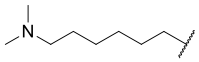
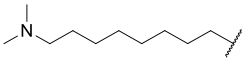
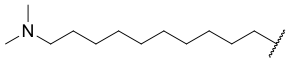
To improve the aqueous solubility, we attempted to introduce various alkylamino groups and compounds **55**-**70** were designed, synthesized and evaluated (**Table 4**). Intriguingly, amino analogs **55**-**62** with two carbon chain and **63**-**65** with three carbon chain all showed great potency against DENV-2 (EC_{50} = 0.025 ~ 0.13 μM) and DENV-3 (EC_{50} = 0.001 ~ 0.030 μM). When the length of carbon chain was increased from 4 to 10, the potency of the compounds (**66**-**70**) against DENV-2 exhibited a declining trend. Among these compounds, **68** with 6-(dimethylamino)hexyl group was effective against three serotypes DENV-1 to -3 (EC_{50} = 1.5 μM, 0.90 μM and 0.066 μM, respectively); **70** with 10-(dimethylamino)decyl group showed moderate to high potency against all four serotypes DENV-1 to -4 (EC_{50} = 2.7 μM, 3.5 μM, 0.098 μM and 3.5 μM, respectively). However, all these amino compounds except **70** lack potency against DENV-4. Taken together, the substitution on the amide of indolone moiety was widely tolerated against

DENV-2 and DENV-3, and various functional groups such as amino, hydroxyl, and ester with proper carbon chain maintained the same level of potency against these two serotypes. The alkyl groups with proper length are favorable for DENV-1 potency, while the long and bulky alkyl groups are beneficial for DENV-4 potency. The substitutions on the amide of pyridone moiety or on both amide moieties diminished the potency against DENV-2 and DENV-3. However, compounds with the proper substitutions at these positions maintained the micromolar level of potency against both DENV-1 and DENV-4. Unfortunately, during this SAR investigation, no highly potent compounds were discovered against all four serotypes DENV-1 to -4 except a few compounds such as **16**, **21**, **27-29, 47** and **70** with a relatively moderate potency.

Table 4. **Antiviral Activity and Cytotoxicity of Compounds 55-70**



Compd	R_1	R_2	EC_{50} (μM) [a]				CC_{50} (μM)[b]
			DENV-1	DENV-2	DENV-3	DENV-4	
55		H	>5.0	0.075	0.017	>5.0	>10
56		H	>5.0	0.074	0.005	>5.0	>10
57		H	>5.0	0.025	0.002	>5.0	>10
58		H	>5.0	0.038	0.006	>5.0	>10
59		H	>5.0	0.30	0.030	>5.0	>10
60		H	>5.0	0.077	0.018	>5.0	>10
61		H	>5.0	0.067	0.007	>5.0	>10

62		H	>5.0	0.12	0.022	>5.0	>10
63		H	>5.0	0.025	0.001	>5.0	>10
64		H	>5.0	0.13	0.012	>5.0	>10
65		H	>5.0	0.076	0.005	>5.0	>10
66		H	>5.0	0.19	0.038	>5.0	>10
67		H	>5.0	0.35	0.078	>5.0	>10
68		H	1.5	0.90	0.066	>5.0	>10
69		H	>5.0	0.49	0.042	>5.0	>10
70		H	2.7	3.5	0.098	3.5	>10

[a]EC_{50} values were determined on Huh7 cells stably expressing DENV-1 to -4 replicon. [b]CC_{50} values were measured using Huh7 cells stably expressing DENV-2 replicon.

Biotinylated Compound 73 Can Pull Down NS4B Protein from Cell Lysates. Previous studies revealed that the spiropyrazolopyridone derivatives inhibited DENV replication by targeting NS4B protein.[53] To facilitate the validation of NS4B as the target of this class of compounds, a biotin group with a proper linker was added to compound **1a** and its diastereoisomer **1b**, resulting in compounds **73** and **75** (**Figure 2A**). Consistent with previous SAR results, the (*R*,*S*)-diastereoisomer **73** but not the (*S*,*S*)-diastereoisomer **75** remains active against DENV-2 and DENV-3 with EC_{50} values of 0.041 μM and 0.017 μM, respectively (**Figure 2B**).[51, 52] In addition, compound **73** has no significant activity against DENV-1 and DENV-4 at the concentration up to 10 μM (data not shown). To further prove the association of compound **73** with viral NS4B protein, a pull-down assay was performed by mixing compounds with the lysates of DENV-2 infected cells. As shown in **Figure 2C**, only compound **73** but not

75 can pull down viral NS4B protein, suggesting that this class of compounds likely target viral NS4B protein.

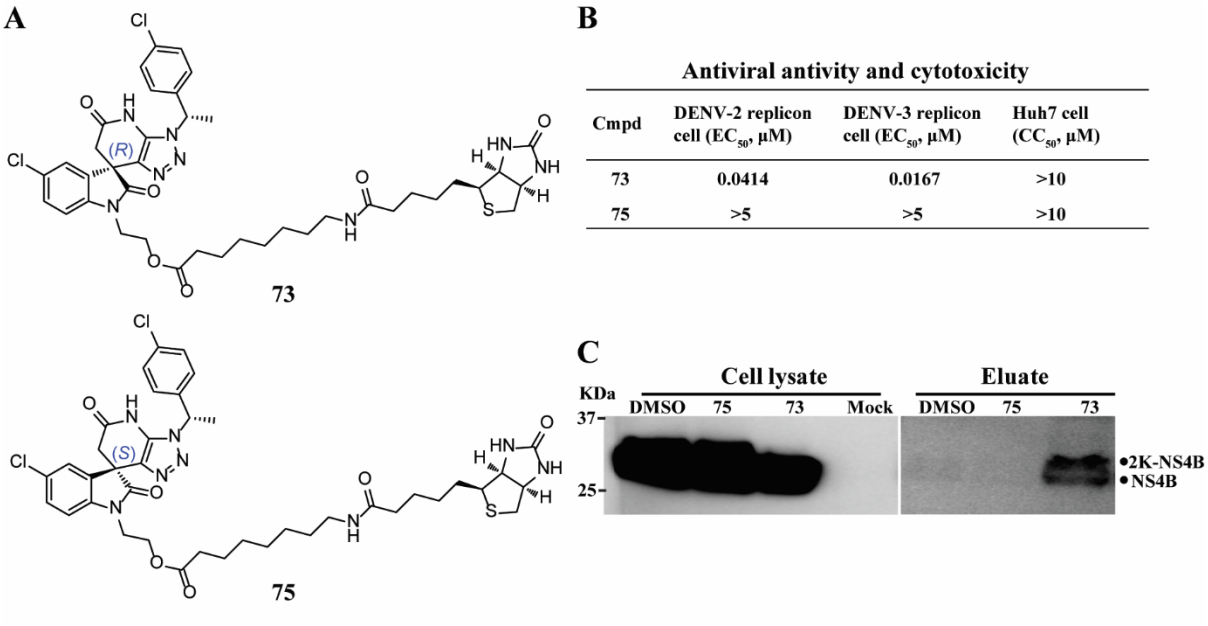
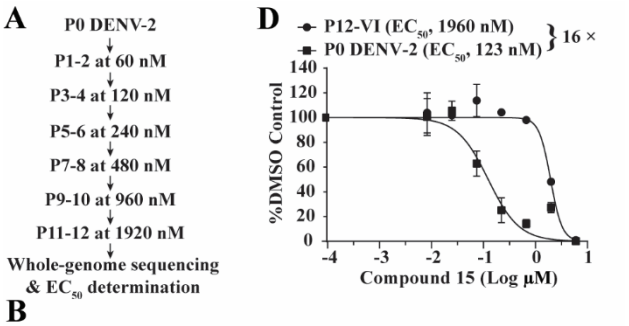


Figure 2. Antiviral activity, cytotoxicity and the association with NS4B of the biotinylated compounds. (A) Chemical structures of biotinylated compound (*R*,*S*)-**73** and its (*S*,*S*)-diastereoisomer **75**. (B) Antiviral activity and cytotoxicity of compound **73** and **75** in cell culture. (C) Compound **73** is associated with DENV-2 viral NS4B protein. NS4B in the cell lysates and eluates were detected by Western blot using a mouse monoclonal antibody against DENV-2 NS4B protein.

Compound 63 Resistance Maps to the Viral NS4B Protein. To further validate whether the series of compounds generated in this study still target DENV NS4B protein, we raised resistant virus by consecutively culturing DENV-2 with increasing concentrations of compound **63** (**Figure 3A**), a compound with excellent potency against DENV-2 and DENV-3. After twelve rounds of selection, all four independent selections in the presence of compound **63** (selections III to VI) showed increased EC_{50} values by >100 folds (**Figure 3B**). Sequencing of the entire genome of the resistant viruses revealed that the amino acid change at position 63 (V63M or V63L) in the NS4B protein occurred in those compound **63**-treated isolates but not those DMSO-

treated isolates (**Figures 3B** and **3C**). The same mutation has been previously determined responsible for the viral resistance to the spiropyrazolopyridone inhibitors.[53] The data demonstrated that DENV NS4B protein is still the target of this series of compounds. Moreover, we evaluated the sensitivity of the P12 viruses (using P12-VI as an example) to compound **15**, a compound with excellent efficacy against three serotypes DENV-1 to -3 (**Figure 3D**). The mutation NS4B V63L increased the viral resistance to compound **15** by 16 folds. Notably, compound **15** remained effective against resistant viruses P12-VI with an EC_{50} of 2.0 μM, suggesting that other sequence variations beyond V63 in NS4B protein may influence its sensitivity to compound treatment.



B

Isolates	Selection	Mutations	EC_{50}	Resistance fold
P12-I	DMSO	NS3 T606A	48 nM	0.80
P12-II		NS3 T606A	56 nM	0.93
P12-III	Compound 63	NS3 T606A; NS4B V63M	> 6000 nM	>100
P12-IV		NS4B V63M;	> 6000 nM	>100
P12-V		NS4B V63M; NS4B P104S	> 6000 nM	>100
P12-VI		NS3 T606A; NS4B V63L	> 6000 nM	>100
P0 DENV-2	--	--	60 nM	--

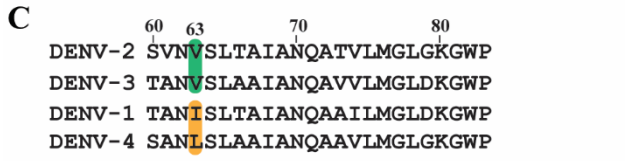


Figure 3. Mutations in NS4B confer to viral resistance to the inhibition by this series of compounds. (A) Scheme of selection and validation of resistant viruses. See details in the Method. DENV-2 strain NGC was used for the selection on Huh7 cells. (B) Resistant profile of P12 mutant viruses. Mutations at position V63 of DENV-2 NS4B were consistently recovered from four independent selections. DMSO (0.45%) was used as a negative control during resistance selection. The resistance fold was calculated by comparing the sensitivities (indicated

by EC_{50}) of the resistant virus to the that of the P0 DENV-2. (C) NS4B protein sequence alignment among four DENV serotypes. The sequences between positions 60 to 83 are shown. V63 residue is highlighted. (D) Sensitivity of P12-VI to the treatment of compound **15**. EC_{50} values were determined by viral reduction assay on Huh7 cells.

***In Vivo* PK and Efficacy of Compound 15**. Considering its excellent inhibitory activities against three serotypes DENV-1 to -3, Compound **15** (**JMX0254**) was selected for further *in vivo* PK and efficacy studies. First, plasma pharmacokinetics after 10 mg/kg intravenous and 20 mg/kg oral administration of compound **15** to male SD rats were characterized. As listed in **Table 5**, compound **15** showed good oral exposure (6,240 ng·h/mL) and oral bioavailability (30%). Following an oral dose of 20 mg/kg, the rat plasma concentration of **15** reached a maximum (1,517 ng/mL) at 1.0 h postdose administration.

Table 5. Pharmacokinetic Parameters of Compound 15 Following 20 mg/kg Oral and 10 mg/kg Intravenous Dosing in Rats[a]

	$AUC_{0-\infty}$ (ng·h/mL)	MRT (h)	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	CL (L/h/kg)	V_{ss} (L/kg)	F (%)
po	6,240	3.46	2.24	1.00	1,517	3.57	11.54	30
iv	10,334	2.28	2.29		10,334	0.82	2.67	

[a]C_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; AUC, area under the curve (t = 0 to 24 h); MRT, mean residence time; V_{ss}, volume of distribution at steady state; CL, plasma clearance; $t_{1/2}$, terminal half-life; F, absolute oral bioavailability. Formulation for po and iv: DMSO: 20% HP-β-CD in saline = 1:9.

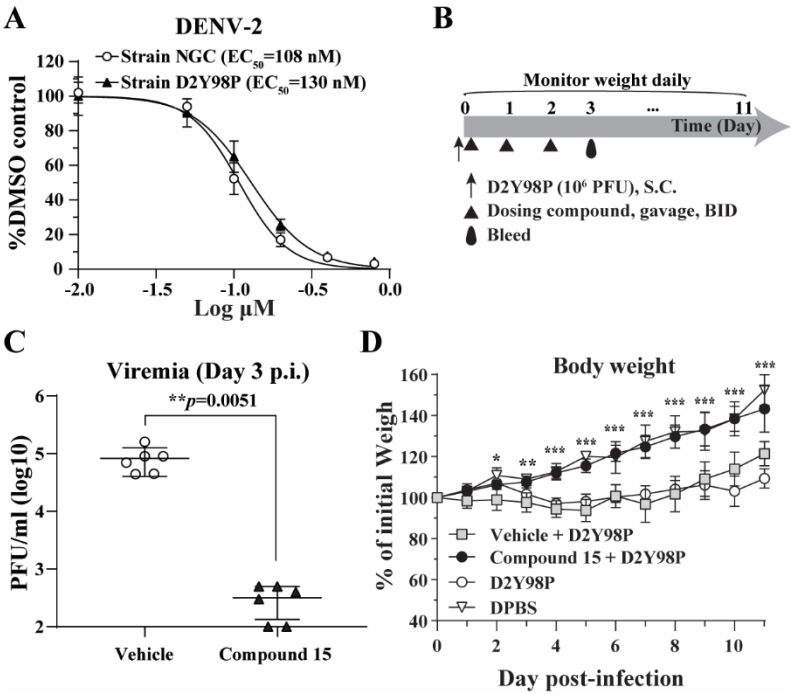


Figure 4. *In vitro* activity and *in vivo* efficacy profile of compound **15** (**JMX0254**). (A) Antiviral activity of compound **15** against DENV-2 strains NGC and D2Y98P. (B) Schematic diagram of evaluating the efficacy of compound **15** against DENV-2 strain D2Y98P *in vivo*. 3-week old A129 mice were subcutaneously (S.C.) inoculated with 10^6 PFU D2Y98P virus. Immediately, the mice were administrated orally at 100 mg/kg twice daily (BID) with compound **15** or vehicle for 3 days. On day 3 post-infection (p.i.), mice were retro-orbitally bled. Viremia were determined by plaque assay. (C) Viremia on day 3 p.i. (D) Mouse body weight after infection. The statistical significances between the group "vehicle + D2Y98P" and the group "compound **15** + D2Y98P" are shown. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

We first confirmed the *in vitro* activity of **15** on DENV-2 strain D2Y98P, using a viral titer reduction assay. As shown in **Figure 4A**, consistent with the screening results using the replicon cell line, compound **15** inhibited both DENV-2 strains NGC (for *in vitro* study) and D2Y98P (for *in vivo* study) in a dose-dependent manner on Huh7 cells, with EC_{50} values of about 100 nM. The *in vivo* efficacy of compound **15** was then tested in a DENV-2 (strain D2Y98P) viremia A129 mouse model (**Figure 4B**). Compared to vehicle control, the compound **15** treatment (when administrated orally at 100 mg/kg twice daily for 3 days) significantly reduced viremia (2.4 log, $p = 0.0051$) (**Figure 4C**). In addition, this compound eliminated the

morbidity caused by DENV-2 infection (**Figure 4D**). In summary, compound **15** clearly demonstrated a promising efficacy profile in both cell culture and mouse model.

CONCLUSION

In conclusion, we optimized a series of substituted 4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione analogues as potent dengue NS4B inhibitors. Throughout a SAR exploration on the amide of the indolone moiety, a wide range of substitutions were found to be well tolerated at this position. Among these compounds, **15** (**JMX0254**) showed the most potent and broad inhibitory activities, effective against DENV-1 to -3 with nanomolar to submicromolar EC_{50} values. Compounds **16**, **21**, **27-29**, **47** and **70** displayed relatively moderate to high activities with low micromolar to nanomolar potency against all four serotypes. The biotinylated compound **73** can enrich NS4B protein from cell lysates in pull-down studies and the findings together with the mutation investigations further validated dengue NS4B protein as the target of this class of compounds. More importantly, compound **15** exhibited good *in vivo* pharmacokinetic properties and promising efficacy in the A129 mouse model, indicating its therapeutic potential against the dengue virus infection as a drug candidate for further preclinical development. The SAR studies also revealed that a new chemical space where biotin was conjugated (e.g. **73** with maintained excellent potency) could be utilized for further design and synthesis of novel protein degraders. Proteolysis-targeting chimaera (PROTAC) technology utilizes bifunctional molecules, whereby one end binds to the protein of interest while the other end hijacks cellular E3 ligase-mediated ubiquitination to induce the degradation of the target protein.[54, 55] Since only a transient binding event is sufficient for protein degradation activity, the strong binding to an active site of NS4B protein based on the

traditionally occupancy-driven pharmacology is likely not required. Such PROTAC strategy may open the new avenue to tackle the challenges with respect to the enhanced activities towards all four serotypes, and our effort along this line is currently ongoing.

EXPERIMENTAL SECTION

General Chemistry Information. All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063-0.200 mm (70-230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Brucker-600 and Brucker-300 (^{1}H, 600 & 300 MHz; ^{13}C, 150 & 75 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts were expressed in ppm, and *J* values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: Nano ESI spray voltage was 1.8 kV; Capillary temperature was 275 °C and the resolution was 60,000; Ionization was achieved by positive mode. Melting points were measured on a Thermo Scientific Electrothermal Digital Melting Point Apparatus and uncorrected. Purities of final compounds were established by analytical HPLC, which was carried out on a Shimadzu HPLC system (model: CBM-20A LC-20AD SPD-20A UV/VIS). HPLC analysis conditions: Waters μBondapak C18 (300 × 3.9 mm); flow rate 0.5 mL/min; UV detection at 270 and 254 nm; linear gradient from 10% acetonitrile in water to

100% acetonitrile in water in 20 min followed by 30 min of the last-named solvent (0.1% TFA was added into both acetonitrile and water). All biologically evaluated compounds are > 95% pure.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (1a). Compound **1a** was prepared by a reported route decribed in **Scheme 1**. The title compound was obtained as an off-white solid. HPLC purity 95.9% (t_R = 17.21 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.72 (s, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.41 – 7.29 (m, 4H), 6.93 (d, *J* = 8.4 Hz, 1H), 5.85 (q, *J* = 6.9 Hz, 1H), 3.34 (d, *J* = 16.2 Hz, 1H), 2.61 (d, *J* = 16.2 Hz, 1H), 1.88 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.5, 168.5, 141.3, 139.3, 136.4, 132.7, 132.1, 128.8, 128.8 (2C), 128.5 (2C), 126.8, 126.1, 124.7, 111.4, 55.6, 46.7, 21.2. HRMS (ESI) calcd for $C_{20}H_{16}Cl_2N_5O_2$ 428.0681 $(M + H)^+$, found 428.0679.

(*S*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (1b). Compound **1b** was prepared by a reported route decribed in **Scheme 1**. The title compound was obtained as an off-white solid. HPLC purity 99.8% (t_R = 16.92 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.23 (s, 1H), 10.72 (s, 1H), 7.47 (d, *J* = 8.7 Hz, 2H), 7.41 – 7.30 (m, 4H), 6.93 (d, *J* = 8.4 Hz, 1H), 5.85 (q, *J* = 6.9 Hz, 1H), 3.34 (d, *J* = 16.2 Hz, 1H), 2.61 (d, *J* = 16.2 Hz, 1H), 1.87 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 177.5, 168.5, 141.3, 139.3, 136.4, 132.7, 132.1, 128.8, 128.8 (2C), 128.5 (2C), 126.8, 126.1, 124.7, 111.4, 55.6, 46.7, 21.2. HRMS (ESI) calcd for $C_{20}H_{16}Cl_2N_5O_2$ 428.0681 $(M + H)^+$, found 428.0677.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-methyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (**7**)**.** To a solution of (*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (**1**) (33 mg, 0.077 mmol) in anhydrous DMF (3 mL) was added NaH (6 mg, 0.15 mmol, 60% dispersion in mineral oil) at 0 °C. The resulting mixture was stirred at r.t. for 30 min. Then CH_3I (6 μL, 0.093 mmol) was added and the resulting mixture was stirred at r.t. for 2 h till the reaction was completed monitored by TLC. The reaction mixture was poured into NH_4Cl (aq., 15 mL) and extracted with EtOAc/MeOH (3:1, 2×45 mL). The organic phase was washed with water (2 × 15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated. The residue was purified by preparative TLC developed by 60% EtOAc in hexane to afford compound **7** as a white solid (26 mg, 76%). HPLC purity 98.5% (t_R = 18.50 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.45 (s, 1H), 7.36 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.33 – 7.27 (m, 4H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.54 (q, *J* = 6.9 Hz, 1H), 3.26 (s, 3H), 3.00 (s, 2H), 1.94 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 169.9, 142.2, 138.2, 135.5, 134.6, 130.9, 129.8, 129.3 (2C), 129.2, 128.2 (2C), 127.1, 124.4, 110.2, 57.7, 46.8, 40.8, 27.0, 21.8. HRMS (ESI) calcd for $C_{21}H_{18}Cl_2N_5O_2$ 442.0838 $(M + H)^+$, found 442.0831.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-ethyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (8). Compound **8** (20 mg, 93%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.4% (t_R = 18.58 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.31 (s, 1H), 7.38 – 7.27 (m, 5H), 7.14 (d, *J* = 1.8 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.80 (q, *J* = 7.2 Hz, 2H), 3.00 (s, 2H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 170.0, 141.4, 138.2, 135.4, 134.7,

131.1, 129.7, 129.4 (2C), 129.0, 128.2 (2C), 127.3, 124.7, 110.3, 57.8, 46.7, 40.9, 35.6, 21.8, 12.7. HRMS (ESI) calcd for $C_{22}H_{20}Cl_2N_5O_2$ 456.0994 $(M + H)^+$, found 456.0986.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-propyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (9). Compound **9** (20 mg, 90%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.6% (t_R = 19.10 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1H), 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.84 – 3.53 (m, 2H), 3.04 (d, *J* = 16.5 Hz, 1H), 2.97 (d, *J* = 16.8 Hz, 1H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.83 – 1.71 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.1, 141.7, 138.2, 135.4, 134.7, 131.2, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.4, 124.6, 110.5, 57.74, 46.7, 42.3, 40.9, 21.8, 20.8, 11.4. HRMS (ESI) calcd for $C_{23}H_{22}Cl_2N_5O_2$ 470.1151 $(M + H)^+$, found 470.1146.

(*R*)-1'-Butyl-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3H)-dione (10). Compound **10** (20 mg, 88%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 97.7% (t_R = 19.65 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, 1H), 7.36 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.85 – 3.64 (m, 2H), 3.03 (d, *J* = 16.5 Hz, 1H), 2.97 (d, *J* = 16.5 Hz, 1H), 1.96 (d, *J* = 7.2 Hz, 3H), 1.76 – 1.61 (m, 2H), 1.48 – 1.33 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.1, 141.7, 138.2, 135.4, 134.7, 131.2, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.4, 124.6, 110.4, 57.8, 46.7, 40.9, 40.6, 29.5, 21.8, 20.2, 13.8. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_2$ 484.1307 $(M + H)^+$, found 484.1303.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-hexyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (11). Compound **11** (21 mg, 87%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. HPLC purity 99.5 % (t_R = 20.81 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.98 (s, 1H), 7.38 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.81 – 3.65 (m, 2H), 3.00 (s, 2H), 1.96 (d, *J* = 7.2 Hz, 3H), 1.76 – 1.63 (m, 2H), 1.42 – 1.25 (m, 6H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.1, 141.7, 138.2, 135.4, 134.7, 131.1, 129.7, 129.4 (2C), 129.0, 128.2 (2C), 127.4, 124.6, 110.4, 57.8, 46.7, 40.9, 40.8, 31.5, 27.4, 26.6, 22.6, 21.8, 14.1. HRMS (ESI) calcd for $C_{26}H_{28}Cl_2N_5O_2$ 512.1620 $(M + H)^+$, found 512.1614.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-decyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3H)-dione (12). Compound **12** (22 mg, 83%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.5% (t_R = 23.61 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.81 – 3.63 (m, 2H), 3.04 – 2.93 (m, 2H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.76 – 1.63 (m, 2H), 1.42 – 1.20 (m, 14H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.1, 141.7, 138.2, 135.4, 134.7, 131.1, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.3, 124.6, 110.4, 57.7, 46.7, 40.9, 40.9, 32.0, 29.6, 29.6, 29.4, 29.4, 27.4, 27.0, 22.8, 21.8, 14.2. HRMS (ESI) calcd for $C_{30}H_{36}Cl_2N_5O_2$ 568.2246 $(M + H)^+$, found 568.2241.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-isopropyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3*H*)-dione (13). To a solution of (*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-

7,3'-indoline]-2',5(3*H*)-dione (**7**) (42 mg, 0.099 mmol) in anhydrous DMF (3 mL) was added NaH (16 mg, 0.40 mmol, 60% dispersion in mineral oil) at 0 °C. The resulting mixture was stirred at r.t. for 30 min. Then 2-bromopropane (32 mg, 0.24 mmol) was added and the resulting mixture was stirred at 50 °C for 24 h. The reaction mixture was cooled to r.t. and poured into NH_4Cl (aq., 15 mL) and extracted with EtOAc/MeOH (3:1, 2 × 45 mL). The organic phase was washed with water (2 × 15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC developed by 40% EtOAc in hexane to afford compound **13** as an off-white solid (12 mg, 26%). HPLC purity 95.3% (t_R = 19.08 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (s, 1H), 7.35 – 7.28 (m, 5H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.03 (d, *J* = 8.7 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 4.65 – 4.50 (m, 1H), 3.03 – 2.91 (m, 2H), 1.97 (d, *J* = 7.2 Hz, 3H), 1.52 (d, *J* = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 170.1, 141.1, 138.1, 135.4, 134.7, 131.3, 129.5, 129.4 (2C), 128.7, 128.2 (2C), 127.6, 124.7, 111.7, 57.8, 46.7, 45.0, 41.0, 21.8, 19.5, 19.5. HRMS (ESI) calcd for $C_{23}H_{22}Cl_2N_5O_2$ 470.1151 $(M + H)^+$, found 470.1146.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-isobutyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (14). Compound **14** (13 mg, 57%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.4% (t_R = 19.57 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.36 (s, 1H), 7.36 – 7.27 (m, 5H), 7.12 (d, *J* = 1.8 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.61 (dd, *J* = 13.8, 7.8 Hz, 1H), 3.50 (dd, *J* = 13.8, 7.2 Hz, 1H), 3.06 (d, *J* = 16.5 Hz, 1H), 2.98 (d, *J* = 16.5 Hz, 1H), 2.25 – 2.10 (m, 1H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.03 – 0.94 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.2, 142.0, 138.2, 135.3, 134.7, 131.2, 129.6, 129.4 (2C), 128.9, 128.2 (2C), 127.5, 124.5, 110.7, 57.8, 48.1, 46.7, 40.9,

27.1, 21.8, 20.3, 20.2. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_2$ 484.1307 $(M + H)^+$, found 484.1299.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-isopentyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (15). Compound **15** (18 mg, 77%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.42 (s, 1H), 7.38 – 7.26 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.87 – 3.60 (m, 2H), 2.99 (s, 2H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.73 – 1.54 (m, 3H), 0.98 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 170.2, 141.6, 138.2, 135.4, 134.7, 131.1, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.3, 124.6, 110.4, 57.7, 46.7, 40.9, 39.2, 36.0, 26.1, 22.6, 22.5, 21.8. HRMS (ESI) calcd for $C_{25}H_{26}Cl_2N_5O_2$ 498.1464 $(M + H)^+$, found 498.1457.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3,3-dimethylbutyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (16). Compound **16** (29 mg, 65%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 99.6% (t_R = 19.71 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.39 – 7.26 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.56 (d, *J* = 6.9 Hz, 1H), 3.84 – 3.64 (m, 2H), 2.99 (s, 2H), 1.95 (d, *J* = 6.9 Hz, 3H), 1.67 – 1.50 (m, 2H), 1.02 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 170.1, 141.6, 138.3, 135.4, 134.6, 131.2, 129.7, 129.3 (2C), 128.9, 128.2 (2C), 127.2, 124.6, 110.2, 57.7, 46.7, 40.8, 40.4, 37.4, 30.1, 29.3 (3C), 21.8. HRMS (ESI) calcd for $C_{26}H_{28}Cl_2N_5O_2$ 512.1620 $(M + H)^+$, found 512.1617.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(cyclopropylmethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (17). Compound **17** (43 mg, 84%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. HPLC purity 98.4% (t_R = 19.58 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.56 (s, 1H), 7.37 – 7.26 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 5.67 – 5.42 (q, *J* = 6.9 Hz, 1H), 3.64 (d, *J* = 6.9 Hz, 2H), 3.05 (d, *J* = 16.5 Hz, 1H), 2.98 (d, *J* = 16.5 Hz, 1H), 1.95 (d, *J* = 6.9 Hz, 3H), 1.22 – 1.10 (m, 1H), 0.61 – 0.53 (m, 2H), 0.44 – 0.35 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.2, 141.9, 138.3, 135.4, 134.6, 131.1, 129.7, 129.3 (2C), 128.9, 128.2 (2C), 127.2, 124.5, 110.6, 57.7, 46.8, 45.1, 41.0, 21.8, 9.6, 4.2, 4.1. HRMS (ESI) calcd for $C_{24}H_{22}Cl_2N_5O_2$ 482.1151 $(M + H)^+$, found 482.1146.

(*R*)-1'-Allyl-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (18). Compound **18** (22 mg, 70%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light-yellow solid. HPLC purity 98.1% (t_R = 19.54 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.08 (s, 1H), 7.37 – 7.28 (m, 5H), 7.15 (d, *J* = 2.1 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 5.93 – 5.78 (m, 1H), 5.53 (q, *J* = 6.9 Hz, 1H), 5.34 – 5.23 (m, 2H), 4.48 – 4.29 (m, 2H), 3.09 – 2.95 (m, 2H), 1.95 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 169.8, 141.4, 138.2, 135.4, 134.7, 130.9, 130.4, 129.7, 129.4 (2C), 129.2, 128.2 (2C), 127.3, 124.5, 118.3, 111.1, 57.8, 46.8, 42.9, 40.8, 21.8. HRMS (ESI) calcd for $C_{23}H_{20}Cl_2N_5O_2$ 468.0994 $(M + H)^+$, found 468.0988.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(prop-2-yn-1-yl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3*H*)-dione (19). To a solution of (*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-

indoline]-2',5(3*H*)-dione (**1**) (41 mg, 0.096 mmol) in anhydrous DMF (4 mL) was added NaH (8 mg, 0.19 mmol, 60% dispersion in mineral oil) at 0 °C. The resulting mixture was stirred at r.t. for 30 min. Then propargyl bromide (14 mg, 0.12 mmol) was added at 0 °C, and the resulting mixture was stirred at 0 °C for 30 min. The reaction mixture was then poured into NH_4Cl (aq., 15mL) and extracted with EtOAc/MeOH (3:1, 2×50 mL). The organic phase was washed with water (2×15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by preparative TLC developed by 50% EtOAc in hexane to afford compound **19** as a yellow solid (41 mg, 85%). HPLC purity 97.4% (t_R = 18.96 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.52 (s, 1H), 7.39 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.17 (d, *J* = 1.8 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 5.54 (q, *J* = 6.9 Hz, 1H), 4.63 (dd, *J* = 17.8, 2.4 Hz, 1H), 4.46 (dd, *J* = 17.8, 2.4 Hz, 1H), 3.02 (s, 2H), 2.32 (t, *J* = 2.4 Hz, 1H), 1.93 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 169.8, 140.3, 138.3, 135.6, 134.6, 130.6, 129.8, 129.7, 129.3 (2C), 128.2 (2C), 126.8, 124.6, 111.3, 76.1, 73.5, 57.8, 46.8, 40.8, 30.1, 21.9. HRMS (ESI) calcd for $C_{23}H_{18}Cl_2N_5O_2$ 466.0838 $(M + H)^+$, found 466.0833.

(*R*)-1'-(But-2-yn-1-yl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (20). Compound **20** (40 mg, 79%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. HPLC purity 98.8% (t_R = 19.08 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.45 (s, 1H), 7.38 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.16 (d, *J* = 2.1 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 5.49 (q, *J* = 6.9 Hz, 1H), 4.59 – 4.37 (m, 2H), 3.00 (s, 2H), 1.90 (d, *J* = 6.9 Hz, 3H), 1.78 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 169.5, 140.7, 138.4, 135.8, 134.5, 130.7, 129.7, 129.5, 129.3 (2C), 128.2 (2C), 126.9, 124.5, 111.4, 81.3, 71.5,

57.7, 46.8, 40.9, 30.5, 21.8, 3.6. HRMS (ESI) calcd for $C_{24}H_{20}Cl_2N_5O_2$ 480.0994 $(M + H)^+$, found 480.0991.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4-isopropyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3*H*)-dione (21). Compound **21** (9 mg, 19%) was prepared by a procedure same to that used to prepare compound **13**. The title compound was obtained as a white solid. HPLC purity 97.0% (t_R = 20.85 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (br s, 1H), 7.40 – 7.30 (m, 4H), 7.10 – 7.02 (m, 1H), 6.91 (d, *J* = 1.8 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 5.80 (q, *J* = 7.2 Hz, 1H), 5.39 – 5.20 (m, 1H), 3.06 (d, *J* = 17.4 Hz, 1H), 2.73 (d, *J* = 17.4 Hz, 1H), 2.02 (d, *J* = 7.2 Hz, 3H), 1.36 – 1.30 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 168.0, 142.4, 139.3, 139.1, 134.1, 133.4, 129.2, 129.1 (2C), 128.9, 128.6 (2C), 128.1, 124.0, 111.7, 71.6, 57.1, 47.1, 36.8, 21.7, 21.6, 20.8. HRMS (ESI) calcd for $C_{23}H_{22}Cl_2N_5O_2$ 470.1151 $(M + H)^+$, found 470.1143.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1',4-diisopropyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-b]pyridine-7,3'-indoline]-2',5(3*H*)-dione (22). compound **22** (7.4 mg, 14%) was prepared by a procedure same to that used to prepare compound **13**. The title compound was obtained as a light-yellow solid. HPLC purity 99.3% (t_R = 22.62 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 4H), 7.26 – 7.21 (m, 1H), 7.01 (d, *J* = 2.1 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 5.77 (q, *J* = 7.2 Hz, 1H), 5.37 – 5.23 (m, 1H), 4.64 – 4.49 (m, 1H), 2.93 (d, *J* = 17.4 Hz, 1H), 2.74 (d, *J* = 17.1 Hz, 1H), 2.00 (d, *J* = 7.5 Hz, 3H), 1.51 – 1.45 (m, 6H), 1.38 – 1.28 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 167.8, 142.3, 140.7, 139.4, 134.0, 133.4, 129.0 (3C), 128.9, 128.5 (2C), 128.3, 124.4, 111.4, 71.4, 56.9, 46.6, 44.7, 37.0, 21.7, 21.6, 21.0, 19.5, 19.5. HRMS (ESI) calcd for $C_{26}H_{28}Cl_2N_5O_2$ 512.1620 $(M + H)^+$, found 512.1622.

(*S*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1',4,6,6-tetramethyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (23). To a solution of (*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (**1**) (42 mg, 0.098 mmol) in anhydrous DMF (4 mL) was added NaH (24 mg, 0.59 mmol, 60% dispersion in mineral oil) at 0 °C. The resulting mixture was stirred at r.t. for 30 min. Then CH_3I (37 μL, 0.59 mmol) was added and the resulting mixture was stirred at r.t. for 2 h till the reaction was completed monitored by TLC. The reaction mixture was poured into NH_4Cl (aq., 15 mL) and extracted with EtOAc/MeOH (3:1, 2 × 45 mL). The organic phase was washed with water (2 × 15 mL) and brine (15 mL), dried over Na_2SO_4 and concentrated. The residue was purified by preparative TLC developed by 40% EtOAc in hexane to afford compound **23** as a white solid (41 mg, 86%). HPLC purity 98.0% (t_R = 20.73 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.35 – 7.28 (m, 3H), 7.07 – 7.01 (m, 2H), 6.85 (d, *J* = 8.1 Hz, 1H), 5.78 (q, *J* = 6.9 Hz, 1H), 3.36 (s, 3H), 3.14 (s, 3H), 2.06 (d, *J* = 6.9 Hz, 3H), 1.09 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 174.2, 143.6, 139.6, 138.0, 134.5, 130.8, 129.7, 129.5 (2C), 128.4, 127.0 (2C), 126.9, 126.5, 109.8, 59.5, 54.4, 46.8, 31.3, 26.4, 22.4, 21.5, 18.9. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_2$ 484.1307 $(M + H)^+$, found 484.1304.

(*R*)-1'-Benzyl-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (24). Compound **24** (47 mg, 85%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.9% (t_R = 20.11 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, 1H), 7.38 – 7.28 (m, 5H), 7.27 – 7.18 (m, 5H), 7.14 (d, *J* = 1.8 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 5.53 (q, *J* = 6.9 Hz, 1H), 5.06 (d, *J* = 15.9 Hz, 1H), 4.91 (d, *J* = 15.9 Hz,

1H), 3.15 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.8 Hz, 1H), 1.90 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 169.8, 141.2, 138.3, 135.6, 134.6, 134.4, 131.1, 129.6, 129.3, 129.2 (2C), 129.2 (2C), 128.1 (2C), 127.1 (2C), 127.0, 124.4, 111.2, 57.7, 46.9, 44.3, 40.8, 21.8. HRMS (ESI) calcd for $C_{27}H_{22}Cl_2N_5O_2$ 518.1151 $(M + H)^+$, found 518.1159.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(4-fluorobenzyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (25). Compound **25** (29 mg, 92%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.5% (t_R = 20.17 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.33 – 7.27 (m, 6H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.09 – 6.97 (m, 2H), 6.72 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.84 (d, *J* = 15.9 Hz, 1H), 3.13 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.5 Hz, 1H), 1.96 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 169.9, 162.4 (d, *J* = 245.4 Hz), 140.9, 138.0, 135.3, 134.5, 131.0, 130.3 (d, *J* = 3.3 Hz), 129.6, 129.3 (3C), 128.8 (d, *J* = 8.2 Hz, 2C), 128.0 (2C), 127.1, 124.4, 116.1 (d, *J* = 21.6 Hz, 2C), 111.0, 57.7, 46.7, 43.6, 40.6, 21.7. HRMS (ESI) calcd for $C_{27}H_{21}Cl_2FN_5O_2$ 536.1056 $(M + H)^+$, found 536.1052.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-fluorobenzyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (26). Compound **26** (30 mg, 95%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.3% (t_R = 20.18 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.54 (s, 1H), 7.36 – 7.26 (m, 5H), 7.26 – 7.21 (m, 1H), 7.17 – 7.06 (m, 2H), 7.05 – 6.93 (m, 2H), 6.72 (d, *J* = 8.4 Hz, 1H), 5.55 (q, *J* = 6.9 Hz, 1H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.90 (d, *J* = 15.9 Hz, 1H), 3.14 (d, *J* = 16.5 Hz, 1H), 3.04 (d, *J* = 16.5 Hz, 1H), 1.94 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 170.0, 163.2 (d, *J* = 246.0 Hz), 140.9, 138.2,

137.3 (d, *J* = 7.0 Hz), 135.4, 134.6, 131.1, 130.9 (d, *J* = 8.3 Hz), 129.8, 129.5, 129.3 (2C), 128.1 (2C), 127.1, 124.6, 122.6 (d, *J* = 2.9 Hz), 115.2 (d, *J* = 20.9 Hz), 114.2 (d, *J* = 22.0 Hz), 111.1, 57.8, 46.8, 43.9, 40.7, 21.8. HRMS (ESI) calcd for $C_{27}H_{21}Cl_2FN_5O_2$ 536.1056 $(M + H)^+$, found 536.1052.

(*R*)-5'-Chloro-1'-(4-chlorobenzyl)-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (27). Compound **27** (57 mg, 94%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. HPLC purity 99.8% (t_R = 20.11min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.62 (s, 1H), 7.34 – 7.20 (m, 9H), 7.13 (d, *J* = 1.8 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 5.00 (d, *J* = 15.9 Hz, 1H), 4.83 (d, *J* = 15.9 Hz, 1H), 3.12 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.5 Hz, 1H), 1.95 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.1, 140.9, 138.2, 135.4, 134.6, 134.1, 133.2, 131.1, 129.7, 129.4, 129.4 (2C), 129.3 (2C), 128.5 (2C), 128.1 (2C), 127.1, 124.5, 111.0, 57.8, 46.8, 43.7, 40.6, 21.8. HRMS (ESI) calcd for $C_{27}H_{21}Cl_3N_5O_2$ 552.0761 $(M + H)^+$, found 552.0761.

(*R*)-5'-Chloro-1'-(4-chloro-3-fluorobenzyl)-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (28). Compound **28** (15 mg, 30%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 95.8% (t_R = 19.66 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.05 (s, 1H), 7.43 – 7.27 (m, 5H), 7.26 – 7.22 (m, 1H), 7.18 – 7.02 (m, 3H), 6.69 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 4.98 (d, *J* = 16.2 Hz, 1H), 4.84 (d, *J* = 16.2 Hz, 1H), 3.11 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.5 Hz, 1H), 2.00 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 169.7, 158.5 (d, *J* = 249.0 Hz), 140.7, 137.9, 135.7 (d, *J* = 6.3 Hz), 135.1 (d, *J* = 29.6 Hz), 131.5 (2C), 131.0, 129.8, 129.6, 129.5 (2C), 128.1 (2C), 127.3, 124.7, 123.5 (d,

J = 3.7 Hz), 121.0 (d, *J* = 17.6 Hz), 115.6 (d, *J* = 21.8 Hz), 110.9, 58.0, 46.8, 43.5, 40.6, 21.8. HRMS (ESI) calcd for $C_{27}H_{20}Cl_3FN_5O_2$ 570.0667 $(M + H)^+$, found 570.0668.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-methoxybenzyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (29). Compound **29** (49 mg, 93%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 99.6% (t_R = 18.97 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.32 – 7.22 (m, 6H), 7.16 (d, *J* = 2.1 Hz, 1H), 6.93 – 6.80 (m, 3H), 6.76 (d, *J* = 8.4 Hz, 1H), 5.53 (q, *J* = 6.9 Hz, 1H), 5.05 (d, *J* = 15.9 Hz, 1H), 4.88 (d, *J* = 15.9 Hz, 1H), 3.79 (s, 3H), 3.15 (d, *J* = 16.5 Hz, 1H), 3.05 (d, *J* = 16.5 Hz, 1H), 1.94 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 169.7, 160.3, 141.2, 138.3, 136.2, 135.5, 134.4, 131.1, 130.3, 129.7, 129.3, 129.3 (2C), 128.1 (2C), 127.1, 124.4, 119.1, 113.4, 112.7, 111.2, 57.7, 55.4, 46.9, 44.2, 40.7, 21.8. HRMS (ESI) calcd for $C_{28}H_{24}Cl_2N_5O_3$ 548.1256 $(M + H)^+$, found 548.1256.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3,5-dimethoxybenzyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (30). Compound **30** (30 mg, 53%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 99.8% (t_R = 19.11 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.48 (s, 1H), 7.29 – 7.18 (m, 5H), 7.13 (d, *J* = 1.8 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 2H), 6.33 (t, *J* = 2.1 Hz, 1H), 5.47 (q, *J* = 6.9 Hz, 1H), 5.02 (d, *J* = 15.9 Hz, 1H), 4.79 (d, *J* = 15.9 Hz, 1H), 3.74 (s, 6H), 3.14 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.5 Hz, 1H), 1.88 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 169.7, 161.5 (2C), 141.2, 138.3, 137.0, 135.5, 134.4, 131.1, 129.7, 129.3, 129.2 (2C), 128.1 (2C), 127.1, 124.4, 111.2,

104.8 (2C), 99.6, 57.7, 55.5 (2C), 46.9, 44.2, 40.6, 21.7. HRMS (ESI) calcd for $C_{29}H_{26}Cl_2N_5O_4$ 578.1362 $(M + H)^+$, found 578.1364.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3,5-di-tert-butylbenzyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (31). Compound **31** (45 mg, 87%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 99.5% (t_R = 21.93 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.55 (s, 1H), 7.36 – 7.22 (m, 6H), 7.17 – 7.11 (m, 3H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 4.94 (s, 2H), 3.05 (s, 2H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.30 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.1, 151.7 (2C), 141.6, 138.3, 135.5, 134.5, 134.0, 130.9, 129.6, 129.3 (2C), 129.1, 128.2 (2C), 127.2, 124.5, 122.0, 121.3 (2C), 111.2, 57.7, 46.8, 44.7, 41.0, 35.0 (2C), 31.5 (6C), 21.9. HRMS (ESI) calcd for $C_{35}H_{38}Cl_2N_5O_2$ 630.2403 $(M + H)^+$, found 630.2400.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-phenethyl-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (32). Compound **32** (16 mg, 64%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.4% (t_R = 19.95 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.35 – 7.25 (m, 7H), 7.24 – 7.17 (m, 3H), 7.11 (d, *J* = 1.8 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 4.08 – 3.87 (m, 2H), 3.01 (t, *J* = 7.2 Hz, 2H), 2.92 (d, *J* = 16.5 Hz, 1H), 2.82 (d, *J* = 16.5 Hz, 1H), 1.99 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 170.0, 141.6, 138.1, 137.7, 135.3, 134.7, 130.8, 129.6, 129.4 (2C), 129.1 (2C), 128.9 (3C), 128.2 (2C), 127.3, 127.0, 124.6, 110.3, 57.8, 46.6, 42.1, 40.8, 33.7, 21.8. HRMS (ESI) calcd for $C_{28}H_{24}Cl_2N_5O_2$ 532.1307 $(M + H)^+$, found 532.1305.

(*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(pyridin-2-ylmethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (33). Compound **33** (32 mg, 87%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.8% (t_R = 19.08 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.63 (s, 1H), 8.55 (d, *J* = 4.2 Hz, 1H), 7.64 (td, *J* = 7.8, 1.8 Hz, 1H), 7.31 – 7.26 (m, 5H), 7.25 – 7.16 (m, 2H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 5.19 (d, *J* = 16.2 Hz, 1H), 4.98 (d, *J* = 16.2 Hz, 1H), 3.17 (d, *J* = 16.8 Hz, 1H), 3.04 (d, *J* = 16.5 Hz, 1H), 1.92 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.0, 154.7, 149.7, 141.2, 138.3, 137.4, 135.5, 134.5, 131.0, 129.7, 129.3 (3C), 128.1 (2C), 127.2, 124.4, 123.1, 121.5, 111.4, 57.7, 46.9, 46.2, 40.7, 21.9. HRMS (ESI) calcd for $C_{26}H_{21}Cl_2N_6O_2$ 519.1103 $(M + H)^+$, found 519.1104.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(pyridin-3-ylmethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (34). Compound **34** (45 mg, 81%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.6% (t_R = 19.80 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.56 (s, 1H), 8.65 (s, 1H), 8.54 (d, *J* = 4.2 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.26 (m, 5H), 7.26 – 7.22 (m, 1H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 5.60 (q, *J* = 6.9 Hz, 1H), 5.03 (d, *J* = 15.9 Hz, 1H), 4.90 (d, *J* = 15.9 Hz, 1H), 3.12 (d, *J* = 16.5 Hz, 1H), 3.03 (d, *J* = 16.5 Hz, 1H), 1.97 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 169.9, 149.6, 148.8, 140.7, 138.1, 135.4, 135.1, 134.7, 131.1, 130.7, 129.8, 129.6, 129.4 (2C), 128.1 (2C), 127.1, 124.7, 124.2, 110.8, 57.8, 46.8, 41.9, 40.7, 21.9. HRMS (ESI) calcd for $C_{26}H_{21}Cl_2N_6O_2$ 519.1103 $(M + H)^+$, found 519.1105.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(pyridin-4-ylmethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (35). Compound **35** (13 mg, 43%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.9% (t_R = 19.49 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.42 (s, 1H), 8.59 (d, *J* = 6.0 Hz, 2H), 7.23 – 7.28 (m, 4H), 7.27 – 7.21 (m, 3H), 7.17 (d, *J* = 2.1 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 5.59 (q, *J* = 6.9 Hz, 1H), 5.09 (d, *J* = 16.8 Hz, 1H), 4.83 (d, *J* = 16.8 Hz, 1H), 3.15 (d, *J* = 16.5 Hz, 1H), 3.06 (d, *J* = 16.8 Hz, 1H), 1.99 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 169.8, 150.6 (2C), 143.8, 140.6, 138.0, 135.3, 134.8, 131.0, 129.9, 129.7, 129.5 (2C), 128.1 (2C), 127.2, 124.7, 121.9 (2C), 110.8, 57.9, 46.8, 43.2, 40.6, 21.9. HRMS (ESI) calcd for $C_{26}H_{21}Cl_2N_6O_2$ 519.1103 $(M + H)^+$, found 519.1103.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-((6-chloropyridin-3-yl)methyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (36). Compound **36** (23 mg, 71%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as an off-white solid. HPLC purity 99.5% (t_R = 19.51 min). ^{1}H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 8.37 (d, *J* = 2.4 Hz, 1H), 7.62 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.34 – 7.21 (m, 6H), 7.15 (d, *J* = 2.1 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 5.61 (q, *J* = 6.9 Hz, 1H), 4.98 (d, *J* = 15.9 Hz, 1H), 4.84 (d, *J* = 15.9 Hz, 1H), 3.01 (d, *J* = 16.5 Hz, 1H), 2.87 (d, *J* = 16.5 Hz, 1H), 1.96 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 176.6, 168.7, 151.3, 148.8, 140.7, 138.6, 138.5, 136.8, 134.7, 131.1, 130.5, 129.9, 129.8, 129.4 (2C), 128.3 (2C), 126.9, 125.3, 125.0, 110.9, 57.7, 47.0, 41.1, 40.6, 21.7. HRMS (ESI) calcd for $C_{26}H_{20}Cl_3N_6O_2$ 553.0713 $(M + H)^+$, found 553.0714.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-((6-methylpyridin-3-yl)methyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (37). To a solution of (*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (**1**) (30 mg, 0.070 mmol) in anhydrous THF (10 mL) was added LiHMDS (0.7 mL, 0.70 mmol, 1 M in THF) at 0 °C. The resulting mixture was stirred at 0 °C for 15 min. Then 5-(bromomethyl)-2-methylpyridine hydrobromide (28 mg, 0.105 mmol) was added at 0 °C and the resulting mixture was stirred at r.t. for 5 d. The reaction mixture was diluted with EtOAc/MeOH (3:1, 60 mL), washed with water (10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by preparative TLC developed by 3% MeOH in EtOAc to afford compound **37** (6 mg, 16%) as a light yellow solid. HPLC purity 99.9% (t_R = 20.16 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (br s, 1H), 8.52 (d, *J* = 1.8 Hz, 1H), 7.53 (dd, *J* = 8.1, 2.4 Hz, 1H), 7.33 – 7.27 (m, 4H), 7.26 – 7.22 (m, 1H), 7.17 – 7.10 (m, 2H), 6.74 (d, *J* = 8.4 Hz, 1H), 5.59 (q, *J* = 6.9 Hz, 1H), 4.97 (d, *J* = 15.9 Hz, 1H), 4.87 (d, *J* = 15.9 Hz, 1H), 3.10 (d, *J* = 16.5 Hz, 1H), 3.02 (d, *J* = 16.5 Hz, 1H), 2.53 (s, 3H), 1.99 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 169.9, 158.7, 148.2, 140.8, 138.0, 135.5, 135.3, 134.8, 131.1, 129.8, 129.5 (3C), 128.1 (2C), 127.6, 127.3, 124.6, 123.9, 110.9, 57.9, 46.8, 41.8, 40.7, 24.3, 21.9. HRMS (ESI) calcd for $C_{27}H_{23}Cl_2N_6O_2$ 533.1260 $(M + H)^+$, found 533.1257.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(methoxymethoxy)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (38). Compound **38** (60 mg, 81%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 98.9% (t_R = 18.79 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.53 (s, 1H), 7.36 – 7.27 (m, 5H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 4.57 (s, 2H), 3.96 (t, *J* = 5.4 Hz, 2H), 3.80 (t, *J* = 5.4 Hz, 2H),

3.26 (s, 3H), 3.00 (s, 2H), 1.95 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.0, 142.0, 138.3, 135.5, 134.5, 130.8, 129.6, 129.3 (2C), 129.0, 128.2 (2C), 127.1, 124.3, 111.2, 96.6, 64.9, 57.6, 55.5, 46.6, 41.0, 40.9, 21.8. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_4$ 516.1205 $(M + H)^+$, found 516.1199.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-methoxypropyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (39). Compound **39** (43 mg, 83%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.5% (t_R = 18.92 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.60 (s, 1H), 7.37 – 7.26 (m, 5H), 7.11 (d, *J* = 2.1 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.93 – 3.74 (m, 2H), 3.41 (t, *J* = 5.7 Hz, 2H), 3.30 (s, 3H), 3.08 – 2.92 (m, 2H), 2.03 – 1.89 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.2, 141.8, 138.3, 135.4, 134.5, 131.0, 129.7, 129.3 (2C), 128.9, 128.2 (2C), 127.2, 124.4, 110.5, 69.5, 58.8, 57.6, 46.7, 40.7, 38.0, 27.7, 21.8. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_3$ 500.1256 $(M + H)^+$, found 500.1254.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4-(3-methoxypropyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (40). Compound **40** (5 mg, 10%) was prepared by a procedure same to that used to prepare compound **39**. The title compound was obtained a light-yellow solid. HPLC purity 97.5% (t_R = 18.54 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (s, 1H), 7.38 – 7.26 (m, 5H), 7.14 (d, *J* = 1.8 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.83 (t, *J* = 6.9 Hz, 2H), 3.41 (t, *J* = 5.7 Hz, 2H), 3.29 (s, 3H), 3.03 (d, *J* = 16.5 Hz, 1H), 2.93 (d, *J* = 16.5 Hz, 1H), 2.07 – 1.90 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 169.4, 141.8, 137.4, 135.2, 135.1, 131.2, 129.7 (3C), 128.8, 128.1 (3C), 124.4,

110.4, 69.6, 58.8, 58.1, 46.6, 40.8, 38.1, 27.7, 21.6. HRMS (ESI) calcd for $C_{24}H_{24}Cl_2N_5O_3$ 500.1256 $(M + H)^+$, found 500.1247.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2,2-diethoxyethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (41). Compound **41** (10 mg, 24%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a yellow solid. HPLC purity 99.6% (t_R = 19.81 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (s, 1H), 7.36 – 7.29 (m, 5H), 7.14 – 7.10 (m, 2H), 5.58 (q, *J* = 6.9 Hz, 1H), 4.69 (t, *J* = 5.4 Hz, 1H), 3.88 – 3.82 (m, 2H), 3.81 – 3.69 (m, 2H), 3.59 – 3.46 (m, 2H), 3.07 – 2.93 (m, 2H), 1.99 (d, *J* = 6.9 Hz, 3H), 1.21 – 1.10 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.0, 142.1, 138.0, 135.3, 134.8, 130.6, 129.5 (3C), 128.9, 128.2 (2C), 127.4, 124.1, 112.0, 100.7, 64.3, 64.0, 57.9, 46.6, 44.3, 41.0, 21.79, 15.5, 15.4. HRMS (ESI) calcd for $C_{26}H_{28}Cl_2N_5O_4$ 544.1518 $(M + H)^+$, found 544.1515.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-hydroxyethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (42). The intermediate (3'*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-((tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione **51** (46 mg, 74%) was prepared by a procedure similar to that used to prepare compound **7.**

To a solution of compound **51** (125 mg, 0.23 mmol) in 10 mL of EtOH was added TsOH (8 mg, 0.045 mmol). The resulting mixture was stirred at r.t. for 24 h. Then the mixture was concentrated and purified by preparative TLC developed by 4% MeOH in DCM to give compound **42** (90 mg, 84%) as a white solid. HPLC purity 99.3% (t_R = 17.56 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, 1H), 7.36 – 7.23 (m, 5H), 7.04 (d, *J* = 1.8 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 4.05 – 3.54 (m, 5H), 3.03 (d, *J* = 16.5 Hz, 1H), 2.93 (d, *J* =

16.5 Hz, 1H), 1.89 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 169.8, 142.0, 138.2, 135.7, 134.7, 131.0, 129.7, 129.4 (2C), 129.0, 128.1 (2C), 127.2, 124.2, 111.2, 59.7, 57.8, 46.7, 43.7, 40.5, 21.8. HRMS (ESI) calcd for $C_{22}H_{20}Cl_2N_5O_3$ 472.0943 $(M + H)^+$, found 472.0942.

Methyl 2-((*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-2',5-dioxo-3,4,5,6-tetrahydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indolin]-1'-yl)acetate (43). Compound **43** (47 mg, 79%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.6% (t_R = 18.61 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.41 – 7.30 (m, 5H), 7.19 (d, *J* = 2.1 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 5.60 (q, *J* = 6.9 Hz, 1H), 4.73 (d, *J* = 17.7 Hz, 1H), 4.33 (d, *J* = 17.4 Hz, 1H), 3.80 (s, 3H), 3.16 – 3.01 (m, 2H), 2.00 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 170.0, 167.6, 141.0, 138.3, 135.6, 134.6, 130.6, 129.8, 129.5, 129.3 (2C), 128.2 (2C), 126.8, 124.7, 110.2, 57.7, 53.0, 46.7, 41.7, 41.0, 21.8. HRMS (ESI) calcd for $C_{23}H_{20}Cl_2N_5O_4$ 500.0892 $(M + H)^+$, found 500.0886.

Methyl 2-((*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-2',5-dioxo-5,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indolin]-4(3*H*)-yl)acetate (44). Compound **44** (3 mg, 5%) was prepared by a procedure same to that used to prepare compound **43**. The title compound was obtained as a white solid. HPLC purity 95.0% (t_R = 18.20 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.38 – 7.28 (m, 5H), 7.19 (d, *J* = 1.8 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 5.59 (q, *J* = 6.9 Hz, 1H), 4.71 (d, *J* = 17.7 Hz, 1H), 4.27 (d, *J* = 17.4 Hz, 1H), 3.78 (s, 3H), 3.09 (d, *J* = 16.5 Hz, 1H), 3.00 (d, *J* = 16.5 Hz, 1H), 2.01 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 169.4, 167.6, 140.9, 137.4, 135.3, 135.0, 130.8, 129.8, 129.7 (2C), 129.5, 128.1 (2C), 127.5, 124.7, 110.2, 58.2, 53.0, 46.6, 41.7, 40.9, 21.6. HRMS (ESI) calcd for $C_{23}H_{20}Cl_2N_5O_4$ 500.0892 $(M + H)^+$, found 500.0881.

2-((*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-2',5-dioxo-3,4,5,6-tetrahydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indolin]-1'-yl)acetic acid (45). To a solution of compound **43** (124 mg, 0.25 mmol) in THF (6 mL) was added NaOH (50 mg, 1.24 mmol, in 2 mL of H_2O). The resulting mixture was stirred at r.t for 1 h. Then the pH value of the mixture was adjusted to 3~4 with 1 M HCl (aq.). The resulting mixture was extracted with EtOAc/MeOH (3:1, 2×60 mL), washed with water (2×15 mL) and concentrated *in vacuo* to give compound **45** (100 mg, 83%) as a white solid. HPLC purity 98.4% (t_R = 11.91 min). ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 – 7.30 (m, 6H), 7.03 (d, *J* = 8.4 Hz, 1H), 5.74 (q, *J* = 6.9 Hz, 1H), 4.57 (d, *J* = 18.0 Hz, 1H), 4.46 (d, *J* = 18.0 Hz, 1H), 3.31 (d, *J* = 16.2 Hz, 1H), 2.77 (d, *J* = 16.5 Hz, 1H), 1.97 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 177.9, 170.4, 170.1, 143.1, 140.0, 138.5, 135.3, 131.9, 130.5, 130.1, 130.1 (2C), 129.3 (2C), 128.1, 125.8, 111.8, 58.5, 47.9, 42.2, 41.3, 21.8. HRMS (ESI) calcd for $C_{22}H_{18}Cl_2N_5O_4$ 486.0736 $(M + H)^+$, found 486.0728.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-fluoroethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (46). Compound **46** (20 mg, 41%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.6% (t_R = 18.70 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.37 – 7.28 (m, 5H), 7.14 (d, *J* = 2.1 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 4.69 (dt, *J* = 46.8, 4.8 Hz, 2H), 4.19 – 3.93 (m, 2H), 3.03 (s, 2H), 1.96 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1, 170.1, 141.6, 138.2, 135.4, 134.7, 130.7, 129.8, 129.4 (2C), 129.3, 128.2 (2C), 127.1, 124.5, 111.0, 110.9, 81.7 (d, *J* = 170.9 Hz), 57.8, 46.7, 41.5 (d, *J* = 21.0 Hz), 41.0, 21.9. HRMS (ESI) calcd for $C_{22}H_{19}Cl_2FN_5O_2$ 474.0900 $(M + H)^+$, found 474.0907.

(*R*)-1'-(3-Bromopropyl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (47). Compound **47** (32 mg, 50%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 96.2% (t_R = 19.78 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.38 (s, 1H), 7.41 – 7.29 (m, 5H), 7.14 (t, *J* = 2.1 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 4.03 – 3.78 (m, 2H), 3.50 – 3.40 (m, 2H), 3.10 – 2.95 (m, 2H), 2.35 – 2.22 (m, 2H), 1.98 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.1, 141.4, 138.1, 135.3, 134.7, 131.0, 129.9, 129.4 (2C), 129.2, 128.2 (2C), 127.3, 124.6, 110.4, 57.8, 46.6, 40.6, 39.3, 30.5, 30.2, 21.9. HRMS (ESI) calcd for $C_{23}H_{21}BrCl_2N_5O_2$ 548.0256 $(M + H)^+$, found 548.0256.

(*R*)-5'-Chloro-1'-(4-chlorobutyl)-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (48). Compound **48** (64 mg, 87%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a white solid. HPLC purity 99.4% (t_R = 19.88 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.64 (s, 1H), 7.38 – 7.27 (m, 5H), 7.12 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.92 – 3.68 (m, 2H), 3.66 – 3.50 (m, 2H), 3.09 – 2.95 (m, 2H), 2.01 – 1.78 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.3, 141.3, 138.2, 135.3, 134.6, 131.1, 129.8, 129.3 (2C), 129.1, 128.2 (2C), 127.2, 124.6, 110.4, 57.7, 46.7, 44.4, 40.8, 39.8, 29.4, 24.6, 21.9. HRMS (ESI) calcd for $C_{24}H_{23}Cl_3N_5O_2$ 518.0917 $(M + H)^+$, found 518.0921.

(*R*)-1'-(6-Bromohexyl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (49). Compound **49** (17 mg, 47%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 99.6% (t_R = 20.68 min). ^{1}H NMR

(300 MHz, $CDCl_3$) δ 10.17 (s, 1H), 7.38 – 7.27 (m, 5H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 3.87 – 3.62 (m, 2H), 3.39 (t, *J* = 6.9 Hz, 2H), 3.00 (s, 2H), 1.98 (d, *J* = 6.9 Hz, 3H), 1.91 – 1.67 (m, 4H), 1.54 – 1.33 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.0, 141.6, 138.1, 135.3, 134.8, 131.1, 129.7, 129.5 (2C), 129.0, 128.2 (2C), 127.4, 124.6, 110.4, 57.8, 46.7, 40.8, 40.5, 33.8, 32.6, 27.8, 27.2, 26.0, 21.8. HRMS (ESI) calcd for $C_{26}H_{27}BrCl_2N_5O_2$ 590.0725 $(M + H)^+$, found 590.0723.

(*R*)-1'-(8-bromooctyl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (50). Compound **50** (17 mg, 47%) was prepared by a procedure similar to that used to prepare compound **7**. The title compound was obtained as a light yellow solid. HPLC purity 97.2 % (t_R = 21.82 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.18 (s, 1H), 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.85 – 3.63 (m, 2H), 3.39 (t, *J* = 6.9 Hz, 2H), 3.00 (s, 2H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.88 – 1.77 (m, 2H), 1.75 – 1.67 (m, 2H), 1.45 – 1.26 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.0, 141.7, 138.1, 135.3, 134.7, 131.1, 129.7, 129.5 (2C), 129.0, 128.2 (2C), 127.4, 124.6, 110.4, 57.8, 46.7, 40.9, 40.7, 34.1, 32.8, 29.1, 28.7, 28.1, 27.3, 26.7, 21.8. HRMS (ESI) calcd for $C_{28}H_{31}BrCl_2N_5O_2$ 618.1038 $(M + H)^+$, found 618.1036.

(*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-morpholinoethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (55). To a solution of compound **42** (90 mg, 0.19 mmol) and CBr_4 (190 mg, 0.57 mmol) in 8 mL of DCM was added PPh_3 (150 mg, 0.57mmol) at 0 °C. After addition, the resulting mixture was stirred at r.t for 24 h. The mixture was concentrated and purified by column chromatography (Hex/EtOAc=1/1 to 1/2) to give (*R*)-1'-(2-bromoethyl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo [4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione **52** (90 mg, 97%)

as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.63 (s, 1H), 7.39 – 7.27 (m, 5H), 7.13 (d, *J* = 1.8 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 5.61 (q, *J* = 6.9 Hz, 1H), 4.27 – 4.03 (m, 2H), 3.70 – 3.56 (m, 2H), 3.07 (d, *J* = 16.8 Hz, 1H), 3.00 (d, *J* = 16.8 Hz, 1H), 1.97 (d, *J* = 6.9 Hz, 3H).

To a solution of compound **52** (21 mg, 0.039 mmol) and K_2CO_3 (6 mg, 0.039 mmol) in 4 mL of MeCN was added morpholine (17 mg, 0.19 mmol). The resulting mixture was stirred at 80 °C for 24 h. Then the solids were filtered out. The resulting mixture was concentrated and purified by preparative TLC developed by 5% MeOH in DCM to give compound **55** (19 mg, 90%) as an off-white solid. HPLC purity 95.6% (t_R =17.15 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 5H), 7.16 (d, *J* = 2.1 Hz, 1H), 6.89 (t, *J* = 8.4 Hz, 1H), 5.60 (q, *J* = 6.9 Hz, 1H), 4.03 – 3.91 (m, 1H), 3.80 – 3.59 (m, 5H), 3.08 – 2.97 (m, 2H), 2.76 – 2.50 (m, 4H), 2.47 – 2.36 (m, 2H), 1.98 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 170.4, 141.5, 138.3, 135.5, 134.6, 130.9, 129.6, 129.3 (2C), 128.9, 128.2 (2C), 127.1, 124.7, 110.4, 67.1 (2C), 57.7, 55.0, 53.8 (2C), 46.7, 41.5, 37.7, 21.8. HRMS (ESI) calcd for $C_{26}H_{27}Cl_2N_6O_3$ 541.1522 $(M + H)^+$, found 541.1519.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(piperidin-1-yl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (56). Compound **56** (13 mg, 86%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 99.0% (t_R = 17.78 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 1.8 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 5.60 (q, *J* = 6.9 Hz, 1H), 4.05 – 3.90 (m, 1H), 3.89 – 3.74 (m, 1H), 3.07 – 2.93 (m, 2H), 2.76 – 2.35 (m, 6H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.65 – 1.51 (m, 4H), 1.48 – 1.37 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.1, 141.7, 138.2, 135.5, 134.7, 131.0, 129.7, 129.4 (2C), 128.9, 128.2 (2C),

127.3, 124.6, 110.7, 57.7, 55.4, 54.9 (2C), 46.7 (2C), 41.2, 38.3, 25.9, 24.2, 21.8. HRMS (ESI) calcd for $C_{27}H_{29}Cl_2N_6O_2$ 539.1729 $(M + H)^+$, found 539.1729.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(dimethylamino)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (57). Compound **57** (16 mg, 85%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a white solid. HPLC purity 98.2% (t_R = 17.07 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.94 – 3.77 (m, 2H), 3.04 – 2.96 (m, 2H), 2.71 – 2.51 (m, 2H), 2.32 (s, 6H), 1.96 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.1, 141.6, 138.2, 135.5, 134.6, 131.0, 129.7, 129.4 (2C), 129.0, 128.2 (2C), 127.2, 124.6, 110.5, 57.8, 56.0, 46.7, 45.7 (2C), 41.0, 39.1, 21.8. HRMS (ESI) calcd for $C_{24}H_{25}Cl_2N_6O_2$ 499.1416 $(M + H)^+$, found 499.1416.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(pyrrolidin-1-yl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (58). Compound **58** (12 mg, 87%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 99.5% (t_R = 17.50 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 7.14 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 4.00 – 3.78 (m, 2H), 2.99 (s, 2H), 2.90 – 2.79 (m, 1H), 2.74 – 2.48 (m, 5H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.84 – 1.72 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.1, 141.7, 138.2, 135.5, 134.7, 131.0, 129.7, 129.4 (2C), 129.0, 128.2 (2C), 127.3, 124.6, 110.5, 57.8, 54.4 (2C), 52.6, 46.7, 41.1, 40.0, 23.7 (2C), 21.8. HRMS (ESI) calcd for $C_{26}H_{27}Cl_2N_6O_2$ 525.1573 $(M + H)^+$, found 5251574.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(4-methylpiperazin-1-yl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (59). Compound **59** (12 mg, 81%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 98.9% (t_R = 17.33 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 – 7.27 (m, 5H), 7.15 (d, *J* = 2.1 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 5.90 (br s, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.98 – 3.72 (m, 2H), 3.00 (s, 2H), 2.81 – 2.30 (m, 10H), 2.27 (s, 3H), 1.99 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 170.0, 141.6, 138.1, 135.5, 134.7, 130.9, 129.7, 129.5 (2C), 128.9, 128.2 (2C), 127.3, 124.7, 110.5, 57.8, 55.2 (2C), 54.6, 53.3 (2C), 46.7, 46.1, 41.2, 38.2, 21.8. HRMS (ESI) calcd for $C_{27}H_{30}Cl_2N_7O_2$ 554.1838 $(M + H)^+$, found 554.1859.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(methyl(2-(methylsulfonyl)ethyl) amino)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (60). Compound **60** (5 mg, 30%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a white solid. HPLC purity 95.0% (t_R = 17.02 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.40 – 7.26 (m, 5H), 7.15 (d, *J* = 2.1 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 3.91 – 3.74 (m, 2H), 3.08 (t, *J* = 6.6 Hz, 2H), 3.00 – 2.85 (m, 7H), 2.81 – 2.68 (m, 2H), 2.33 (s, 3H), 1.99 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.0, 169.4, 141.5, 137.8, 135.5, 134.9, 130.9, 129.8, 129.6 (2C), 129.2, 128.1 (2C), 127.5, 124.8, 110.3, 58.0, 54.4, 52.9, 51.0, 46.7, 42.2, 42.2, 40.9, 38.7, 21.6. HRMS (ESI) calcd for $C_{26}H_{29}Cl_2N_6O_4S$ 591.1348 $(M + H)^+$, found 591.1345.

(*R*)-1'-(2-(Azetidin-1-yl)ethyl)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (61). Compound **61** (12 mg, 78%) was prepared by a procedure similar to that used to prepare compound **55**. The

title compound was obtained as a white solid. HPLC purity 95.1% (t_R = 17.28 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.71 (t, *J* = 6.9 Hz, 2H), 3.30 – 3.18 (m, 4H), 3.06 – 2.92 (m, 2H), 2.80 – 2.62 (m, 2H), 2.06 (p, *J* = 7.2 Hz, 2H), 1.96 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 169.9, 141.7, 138.1, 135.6, 134.7, 131.0, 129.7, 129.5 (2C), 129.0, 128.2 (2C), 127.3, 124.6, 110.5, 57.8, 56.2, 55.8 (2C), 46.7, 41.0, 39.5, 21.8, 18.0. HRMS (ESI) calcd for $C_{25}H_{25}Cl_2N_6O_2$ 511.1416 $(M + H)^+$, found 511.1418.

(*R*)-5'-chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(2-(methylamino)ethyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (62). Compound **62** (14 mg, 77%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 96.5% (t_R = 16.79 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 7.10 (d, *J* = 1.8 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 5.66 (q, *J* = 6.9 Hz, 1H), 4.92 (br s, 2H), 4.04 – 3.79 (m, 2H), 3.09 – 2.86 (m, 4H), 2.45 (s, 3H), 1.94 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 169.9, 141.4, 138.3, 135.8, 134.6, 131.2, 129.8, 129.4 (2C), 129.2, 128.1 (2C), 127.3, 124.5, 110.6, 57.8, 48.6, 46.7, 40.6, 40.3, 35.9, 21.9. HRMS (ESI) calcd for $C_{23}H_{23}Cl_2N_6O_2$ 485.1260 $(M + H)^+$, found 485.1259.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-(dimethylamino)propyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (63). Compound **63** (20 mg, 51%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as an off-white solid. HPLC purity 98.7% (t_R = 17.10 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 5H), 7.12 (d, *J* = 2.1 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.15 (br s, 1H), 5.60 (q, *J* = 6.9 Hz, 1H), 3.88 – 3.73 (m, 2H), 3.05 – 2.91 (m, 2H), 2.37 (t, *J* = 6.9 Hz, 2H), 2.23 (s, 6H), 1.96 (d, *J* = 7.0 Hz, 3H), 1.93 – 1.82 (m, 2H). ^{13}C NMR (75 MHz,

$CDCl_3$) δ 175.9, 170.1, 141.8, 138.2, 135.5, 134.6, 131.1, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.4, 124.5, 110.5, 57.7, 56.6, 46.7, 45.4 (2C), 40.8, 38.9, 25.5, 21.8. HRMS (ESI) calcd for $C_{25}H_{27}Cl_2N_6O_2$ 513.1573 $(M + H)^+$, found 513.1557.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-(piperidin-1-yl)propyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (64). Compound **64** (22 mg, 57%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as an off-white solid. HPLC purity 99.8% (t_R = 17.79 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.11 (d, *J* = 2.1 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.08 (br s, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.90 – 3.66 (m, 2H), 2.99 (s, 2H), 2.46 – 2.21 (m, 6H), 2.07 – 1.79 (m, 5H), 1.67 – 1.51 (m, 4H), 1.49 – 1.37 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.2, 141.9, 138.2, 135.5, 134.6, 131.0, 129.6, 129.4 (2C), 128.9, 128.2 (2C), 127.3, 124.4, 110.8, 57.7, 55.8, 54.6 (2C), 46.7, 40.9, 38.9, 26.1 (2C), 24.9, 24.5, 21.8. HRMS (ESI) calcd for $C_{28}H_{31}Cl_2N_6O_2$ 553.1886 $(M + H)^+$, found 553.1884.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(3-morpholinopropyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (65). Compound **65** (25 mg, 65%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 99.7% (t_R = 17.09 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 5H), 7.12 (d, *J* = 1.8 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 3.93 – 3.63 (m, 6H), 3.00 (s, 2H), 2.48 – 2.32 (m, 6H), 2.03 – 1.81 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.2, 141.8, 138.2, 135.3, 134.7, 131.0, 129.6, 129.4 (2C), 129.0, 128.2 (2C), 127.3, 124.5, 110.5, 67.1 (2C), 57.8, 55.5, 53.7 (2C), 46.7, 40.8, 38.7, 24.4, 21.9. HRMS (ESI) calcd for $C_{27}H_{29}Cl_2N_6O_3$ 555.1678 $(M + H)^+$, found 555.1676.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(4-(dimethylamino)butyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (66). Compound **66** (20 mg, 98%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as an off-white solid. HPLC purity 99.5% (t_R = 17.32 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.12 (d, *J* = 2.1 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.76 (br s, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.87 – 3.64 (m, 2H), 2.98 (s, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.21 (s, 6H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.82 – 1.67 (m, 2H), 1.61 – 1.47 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.2, 141.6, 138.2, 135.6, 134.6, 131.1, 129.7, 129.4 (2C), 129.0, 128.2 (2C), 127.3, 124.6, 110.5, 59.0, 57.7, 46.7, 45.4 (2C), 40.9, 40.5, 25.2, 24.7, 21.8. HRMS (ESI) calcd for $C_{26}H_{29}Cl_2N_6O_2$ 527.1729 $(M + H)^+$, found 527.1729.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(4-(piperidin-1-yl)butyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (67). Compound **67** (19 mg, 96%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 99.4% (t_R = 18.13 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 5H), 7.12 (d, *J* = 2.1 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.81 (br s, 1H), 5.58 (q, *J* = 6.9 Hz, 1H), 3.82 – 3.65 (m, 2H), 3.05 – 2.91 (m, 2H), 2.47 – 2.24 (m, 6H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.80 – 1.66 (m, 2H), 1.64 – 1.50 (m, 6H), 1.43 (d, *J* = 4.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.2, 141.6, 138.3, 135.8, 134.6, 131.2, 129.6, 129.4 (2C), 128.9, 128.2 (2C), 127.3, 124.5, 110.5, 58.5, 57.7, 54.6 (2C), 46.8, 40.8, 40.5, 26.0 (2C), 25.3, 24.5, 23.9, 21.8. HRMS (ESI) calcd for $C_{29}H_{33}Cl_2N_6O_2$ 567.2042 $(M + H)^+$, found 567.2043.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(6-(dimethylamino)hexyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (68). Compound **68** (9 mg, 73%) was prepared by a procedure similar to that used to prepare compound **55**. The title

compound was obtained as a white solid. HPLC purity 99.3% (t_R = 17.81 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 5H), 7.12 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 5.56 (q, *J* = 6.9 Hz, 1H), 3.81 – 3.61 (m, 2H), 3.01 (d, *J* = 16.5 Hz, 1H), 2.95 (d, *J* = 16.5 Hz, 1H), 2.28 – 2.16 (m, 8H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.77 – 1.64 (m, 2H), 1.51 – 1.31 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 170.0, 141.7, 138.2, 135.6, 134.7, 131.2, 129.7, 129.4 (2C), 128.9, 128.2 (2C), 127.4, 124.6, 110.4, 59.8, 57.7, 46.7, 45.6 (2C), 40.9, 40.7, 27.6, 27.4, 27.2, 26.9, 21.8. HRMS (ESI) calcd for $C_{28}H_{33}Cl_2N_6O_2$ 555.2042 $(M + H)^+$, found 555.2039.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(8-(dimethylamino)octyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (69). Compound **69** (13 mg, 92%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 98.9% (t_R = 19.66 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 5.84 (s, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 3.82 – 3.63 (m, 2H), 2.97 (s, 2H), 2.29 – 2.16 (m, 8H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.76 – 1.64 (m, 2H), 1.48 – 1.22 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 169.9, 141.7, 138.1, 135.7, 134.7, 131.2, 129.7, 129.5 (2C), 128.9, 128.1 (2C), 127.4, 124.6, 110.4, 60.0, 57.7, 46.7, 45.6 (2C), 40.9, 40.7, 29.5, 29.2, 27.7, 27.5, 27.4, 26.9, 21.7. HRMS (ESI) calcd for $C_{30}H_{37}Cl_2N_6O_2$ 583.2355 $(M + H)^+$, found 583.2354.

(*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-1'-(10-(dimethylamino)decyl)-4,6-dihydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indoline]-2',5(3*H*)-dione (70). Compound **70** (12 mg, 85%) was prepared by a procedure similar to that used to prepare compound **55**. The title compound was obtained as a yellow solid. HPLC purity 95.0% (t_R = 19.46 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 7.13 (d, *J* = 2.1 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 5.57 (q, *J* = 6.9 Hz, 1H), 5.46 (s, 1H), 3.79 – 3.63 (m, 2H), 2.96 (s, 2H), 2.31 – 2.19 (m, 8H), 1.97 (d,

J = 6.9 Hz, 3H), 1.76 – 1.62 (m, 2H), 1.51 – 1.40 (m, 2H), 1.37 – 1.22 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 169.8, 141.7, 138.1, 135.9, 134.7, 131.1, 129.6, 129.5 (2C), 128.9, 128.1 (2C), 127.5, 124.6, 110.4, 60.0, 57.7, 46.7, 45.6 (2C), 41.0, 40.7, 29.6, 29.5, 29.5, 29.2, 27.8, 27.6, 27.4, 26.8, 21.7. HRMS (ESI) calcd for $C_{32}H_{41}Cl_2N_6O_2$ 611.2668 $(M + H)^+$, found 611.2664.

2-((*R*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-2',5-dioxo-3,4,5,6-tetrahydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indolin]-1'-yl)ethyl 8-(5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)octanoate (73). To a solution of methyl 8-aminooctanoate hydrochloride (129 mg, 0.614 mmol) and *D*-(+)-Biotin (100 mg, 0.409 mmol) in 6 mL of DMF was added EDCI (118 mg, 0.614 mmol), HOBt (83 mg, 0.614 mmol) and DIEA (106 mg, 0.818 mmol) successively. The resulting mixture was stirred at r.t. overnight and then diluted with EtOAc/MeOH (150 mL, 3/1). The mixture was washed with water (3×30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in MeOH/H_2O (6 mL/4 mL), and then NaOH (82 mg, 2.05 mmol) was added. The resulting mixture was stirred at r.t. for 3 h. Then the solvent (MeOH) was evaporated and the pH value was adjusted to 5~6 with 2 M HCl (aq.) at 0 °C. The white precipitate was isolated by filtration, washed with water and dried under vacuum to give 8-(5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)octanoic acid **72** (110 mg, 70% in two steps) as a white solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 11.99 (s, 1H), 7.73 (t, *J* = 5.4 Hz, 1H), 6.50 – 6.20 (m, 2H), 4.30 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.12 (dd, *J* = 7.8, 4.5 Hz, 1H), 3.14 – 3.05 (m, 1H), 3.04 – 2.95 (m, 2H), 2.82 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.57 (d, *J* = 12.3 Hz, 1H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.04 (t, *J* = 7.2 Hz, 2H), 1.67 – 1.19 (m, 16H). ^{13}C NMR (75 MHz, DMSO-*d6*) δ 174.5, 171.8, 162.7, 61.0, 59.2, 55.4, 38.8, 35.2, 33.7, 29.1, 28.5, 28.5, 28.2, 28.0, 26.3, 25.3, 24.4.

To a solution of compound **72** (42 mg, 0.11 mmol) and compound **42** (26 mg, 0.055 mmol) in 3 mL of DMF was added EDCI (21 mg, 0.011 mmol) and DMAP (2 mg, 0.011mmol). The resulting mixture was stirred at 50 °C for 48 h. Then the mixture was diluted with EtOAc/MeOH (80 mL, 3/1), washed with water (2 × 20 mL) and brine (15 mL), and concentrated. The residue was purified by preparative TLC developed by 5% MeOH in DCM to give compound **73** (9 mg, 63%) as a white solid, and 18 mg of compound **42** was recovered. HPLC purity 95.0% (t_R = 19.18 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.76 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.36 – 7.28 (m, 3H), 7.16 (d, *J* = 1.8 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.67 (s, 1H), 6.01 (s, 1H), 5.80 (q, *J* = 6.9 Hz, 1H), 5.16 (s, 1H), 4.62 – 4.48 (m, 2H), 4.42 – 4.32 (m, 1H), 4.25 – 4.06 (m, 2H), 3.83 – 3.71 (m, 1H), 3.29 – 3.08 (m, 3H), 3.02 – 2.68 (m, 4H), 2.31 – 2.08 (m, 4H), 1.97 (d, *J* = 6.9 Hz, 3H), 1.79 – 1.57 (m, 4H), 1.53 – 1.33 (m, 6H), 1.24 – 0.99 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.6, 173.4, 169.6, 164.3, 141.7, 138.8, 136.5, 134.3, 131.1, 129.4, 129.2 (2C), 129.0, 128.6 (2C), 126.6, 124.8, 110.2, 62.2, 60.7, 60.3, 57.0, 55.5, 46.6, 40.9, 40.8, 40.0, 39.4, 36.1, 34.0, 29.3, 28.6, 28.5, 28.2, 28.1, 26.3, 25.7, 24.3, 21.8. HRMS (ESI) calcd for $C_{40}H_{49}Cl_2N_8O_6S$ 839.2873 $(M + H)^+$, found 839.2877.

2-((*S*)-5'-Chloro-3-((*S*)-1-(4-chlorophenyl)ethyl)-2',5-dioxo-3,4,5,6-tetrahydrospiro[[1,2,3]triazolo[4,5-*b*]pyridine-7,3'-indolin]-1'-yl)ethyl 8-(5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)octanoate (75). Compound **75** (19 mg, 60%) was prepared by a route similar to that used to prepare compound **73** starting from compound **1b** and **72**. The title compound was obtained as a white solid. HPLC purity 98.3% (t_R = 19.15 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.69 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.34 – 7.27 (m, 3H), 7.13 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.59 (s, 1H), 6.37 (s, 1H), 5.81 (q, *J* = 6.9 Hz, 1H), 5.49 (s, 1H), 4.60 – 4.38 (m, 2H), 4.37 – 4.17 (m, 2H), 4.17 – 4.05 (m, 1H), 3.88 –

3.75 (m, 1H), 3.29 – 3.09 (m, 3H), 2.99 – 2.81 (m, 3H), 2.73 (d, *J* = 12.9 Hz, 1H), 2.27 – 2.13 (m, 4H), 1.96 (d, *J* = 6.9 Hz, 3H), 1.75 – 1.56 (m, 4H), 1.51 – 1.35 (m, 6H), 1.32 – 1.08 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 173.7, 173.5, 169.5, 164.3, 141.4, 138.8, 136.5, 134.3, 131.2, 129.5, 129.2 (2C), 129.0, 128.6 (2C), 126.7, 124.7, 110.2, 62.1, 60.5, 60.4, 57.1, 55.7, 46.7, 41.1, 40.7, 39.8, 39.6, 36.1, 34.0, 29.3, 28.5 (2C), 28.3, 28.0, 26.4, 25.7, 24.3, 21.9. HRMS (ESI) calcd for $C_{40}H_{49}Cl_2N_8O_6S$ 839.2873 $(M + H)^+$, found 839.2875.

Luciferase Reporter Replicon-Based Screening. Huh7 cells containing a luciferase reporter replicon of DENV-1 (strain WestPac), DENV-2 (New Guinea C strain, NGC), DENV-3 (strain D3MY05-34640) and DENV-4 (strain D4MY01-22713) were used in this study. The replicon cells containing the *Renilla* luciferase and neomycin-resistance genes were generated using a similar strategy as described previously.[56] Briefly, Huh7 DENV-1 to -4 replicon cells were seeded at a density of 10k per well in a 96-well microplate. After incubation at 37 °C with 5% CO_2 overnight, the cells were treated with 2-fold serial dilutions of compounds. Experiments were performed in duplicates. After 48 h of incubation, luciferase activities were measured using the EnduRen live-cell substrate (Promega) by following the manufacturer's instructions. The dose-dependent curve was plotted and EC_{50} values were calculated using four parameter logistic regression in GraphPad software Prism 8.0.

Cytotoxicity Assay. Huh7 cells or Huh7 replicon cells were seeded in a 96-well plate. After incubation at 37 °C with 5% CO_2 overnight, the cells were treated with 2-fold serial dilutions of compounds. Experiments were performed in duplicates. After 48 h of incubation, cell viability was measured by adding the CellTiter-Glo reagent (Promega) to each well. The cell viability in 0.45% DMSO-treatment groups was treated at 100%. The cell viability in compound-treated

wells was normalized to that of DMSO-treated wells. CC_{50} values were calculated using four parameter logistic regression in GraphPad software Prism 8.0.

Viral Titer Reduction Assay. Huh7 cells were seeded in a 96-well plate. The next day, cells were infected with DENV-2 strains NGC or D2Y98P at a MOI of 0.3 in the presence of 2-fold serial dilutions of compounds. Experiments were performed in triplicates. After incubation at 37 °C for 48 h, cell culture fluids were harvested. The infectious viruses in the culture fluids were determined by plaque assay using a protocol as described previously.[48] The EC_{50} values were calculated using four parameter logistic regression in GraphPad software Prism 8.0.

Pull-Down Assay. 4 $\times 10^6$ Huh7 cells were seeded in a T-75 flask. The next day, cells were infected with DENV-2 strain NGC at a MOI of 2.0 in the presence of 10 μM compound **73** or **75**. Cells treated with 0.25% DMSO were used as a control. After 48 h-incubation at 37 °C, cells were harvested and lysed in 500 μL lysis buffer comprising 20 mM Tris-HCL pH 7.5, 100 mM NaCl, 0.5% DDM, and EDTA-free protease inhibitor cocktail (Roche). After removing the cell debris by centrifugation at 15000 rpm for 10 min, the lysates were mixed with corresponding compound at a final concentration of 100 μM or DMSO (0.25%) prior to incubation with 80 μL Streptavidin Magnetic Beads (ThermoFisher Scientific). After incubation at 4 °C overnight with agitation, the beads were washed 5 times with PBST buffer (PBS containing 0.1% Tween 20). The beads were finally mixed with 1 × LDS sample buffer (ThermoFisher Scientific) containing 100 mM dithiothreitol (DTT, Sigma-Aldrich) and heated at 95 °C for 10 minutes to elute the bead-associated proteins. The eluates and cell lysates were loaded onto a 12% SDS-PAGE and viral NS4B proteins were detected by a mouse monoclonal antibody according to the protocol as reported previously.[57]

Selection of Resistant Viruses. Resistant viruses were selected by serial passaging of the DENV-2 strain NGC strain on Huh7 cells in the presence of increasing concentrations of compound **63** using a previously described protocol with some modifications.[53] Four selections in the presence of compound **63** and two selections in the presence of 0.45% DMSO were performed independently. In brief, Huh7 cells in a 12-well plate were infected by DENV-2 Strain NGC or previously passaged viruses. After incubation at 30°C for 1 h, the inoculum was changed to fresh medium with 2% FBS containing corresponding concentration of compound **63** or DMSO. The cells were incubated at 37°C for 3 days. A part of the supernatants from each passage were collected and stored at -80°C. The P12 viruses were tested for compound sensitivity by viral reduction assay. For whole-genome sequencing, viral RNA was extracted from the P12 supernatants. cDNA of the viral entire genome was amplified by RT-PCR and subjected to Sanger sequencing.

***In Vivo* PK Studies**. The PK profiles of compound **15** were determined in male SD rats following intravenous (i.v.) and oral (p.o.) administration of 10 and 20 mg/kg, respectively. The formulations used for i.v. and p.o. were solution in DMSO: 20% HP-β-CD in saline = 1:9. IV bolus injection, animal number N = 3; dose level: 10 mg/kg; sample collection time points: 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h; PO: animal number N = 3; fasting 12 h, dose level: 20 mg/kg; sample collection time points: 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 24 h. Plasma concentrations of compound **15** were measured by API-4000/5500 LC-MS/MS with 8 point standard curve and 6 QCs. Pharmacokinetic parameters were calculated by a non-compartmental approach using WinNonLin software.

***In Vivo* Efficacy Studies.** Animal studies were performed as approved by the University of Texas Medical Branch (UTMB) Institutional Animal Care and Use Committee (IACUC). All efforts were made to minimize animal suffering. The *in vivo* efficacy of compound **15** was evaluated in a dengue viremia model.[58] 3-week-old A129 mice (with knockout of IFN-α/β receptors) and DENV-2 strain D2Y98P were used in this model. The virus stock was propagated in BHK-21 cells grown in RPMI 1640 medium. 100 μL (10^7 PFU/ml) of D2Y98P viruses were injected subcutaneously (S.C.) into the mice. The mice (n = 6 per group) immediately were dosed orally twice daily (BID) with compound **15** (100 mg/kg) or the vehicle for 3 days. The compound was reconstituted as a suspension in the vehicle solution containing 2.5% DMSO, 50% PEG-400 and 47.5% DPBS. On day 3 post-infection (p.i.), the mice were bled via the retro-orbital (R.O.) route. The plasma was collected and used for measuring the viral loads in the sera (Viremia) by plaque assay. The means and standard deviations were presented. Statistical analysis was performed by an unpaired parametric test using Prism software (GraphPad). Mouse weight was carefully monitored for 12 d after infection. A two-way ANOVA with multiple comparison was performed to analyze the statistically significant difference between compound **15** and vehicle-treated groups.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

^{1}H, ^{13}C NMR spectra of all new compounds and X-ray crystal analysis data for compound **7** (PDF)

Molecular formulation strings and some data (CSV)

AUTHOR INFORMATION

Corresponding Author

For P.S.: phone, (409) 772-6370; fax, (409) 772-4298; e-mail, peshi@utmb.edu.

For J.Z.: phone, (409) 772-9748; fax, (409) 772-9818; e-mail, jizhou@utmb.edu.

Author Contributions

‖These two authors contributed equally and should be considered as co-first authors.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the John S. Dunn Foundation, the Amon G. Carter Foundation, the Kleberg Foundation, the Gilson Longenbaugh Foundation, the Summerfield Robert Foundation, CDC grant U01CK0000512, and NIH grants U19AI142759, 1R41AI136126, R01AI127744, John Sealy Memorial Endowment Fund, and Institute for Translational Sciences (ITS) at UTMB.

ABBREVIATIONS USED

DENV, dengue virus; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; SAR, structure-activity relationship; WHO, World Health Organization; TLC, thin layer chromatography; UV, ultraviolet; TMS, tetramethylsilane; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; HOBt, 1-hydroxybenzotriazole;

DIEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; LiHDMS, lithium bis(trimethylsilyl)amide; THF, tetrahydrofuran; TsOH, *p*-toluenesulfonic acid; iv, intravenous injection; po, oral administration; PROTAC, proteolysis targeting chimera.

REFERENCES

1. *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*. World Health Organization: Geneva, 2009.

2. Whitehorn, J.; Simmons, C. P. The Pathogenesis of Dengue. *Vaccine*. **2011,** *29*, 7221-7228.

3. Brady, O. J.; Gething, P. W.; Bhatt, S.; Messina, J. P.; Brownstein, J. S.; Hoen, A. G.; Moyes, C. L.; Farlow, A. W.; Scott, T. W.; Hay, S. I. Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. *PLoS Negl. Trop. Dis.* **2012,** *6*, e1760.

4. Jentes, E. S.; Lash, R. R.; Johansson, M. A.; Sharp, T. M.; Henry, R.; Brady, O. J.; Sotir, M. J.; Hay, S. I.; Margolis, H. S.; Brunette, G. W. Evidence-Based Risk Assessment and Communication: A New Global Dengue-Risk Map for Travellers and Clinicians. *J. Travel Med.* **2016,** *23*, 1-5.

5. *Global Strategy for Dengue Prevention and Control 2012-2020*. World Health Organization: Geneva, 2012.

6. Bhatt, S.; Gething, P. W.; Brady, O. J.; Messina, J. P.; Farlow, A. W.; Moyes, C. L.; Drake, J. M.; Brownstein, J. S.; Hoen, A. G.; Sankoh, O.; Myers, M. F.; George, D. B.; Jaenisch, T.; Wint, G. R.; Simmons, C. P.; Scott, T. W.; Farrar, J. J.; Hay, S. I. The Global Distribution and Burden of Dengue. *Nature*. **2013,** *496*, 504-507.

7. Stanaway, J. D.; Shepard, D. S.; Undurraga, E. A.; Halasa, Y. A.; Coffeng, L. E.; Brady, O. J.; Hay, S. I.; Bedi, N.; Bensenor, I. M.; Castaneda-Orjuela, C. A.; Chuang, T. W.; Gibney, K. B.; Memish, Z. A.; Rafay, A.; Ukwaja, K. N.; Yonemoto, N.; Murray, C. J. L. The Global Burden of Dengue: An Analysis from the Global Burden of Disease Study 2013. *Lancet Infect. Dis.* **2016,** *16*, 712-723.

8. Stevens, A. J.; Gahan, M. E.; Mahalingam, S.; Keller, P. A. The Medicinal Chemistry of Dengue Fever. *J. Med. Chem.* **2009,** *52*, 7911-7926.

9. Chao, B.; Tong, X. K.; Tang, W.; Li, D. W.; He, P. L.; Garcia, J. M.; Zeng, L. M.; Gao, A. H.; Yang, L.; Li, J.; Nan, F. J.; Jacobs, M.; Altmeyer, R.; Zuo, J. P.; Hu, Y. H. Discovery and Optimization of 2,4-Diaminoquinazoline Derivatives as a New Class of Potent Dengue Virus Inhibitors. *J. Med. Chem.* **2012,** *55*, 3135-3143.

10. Lim, S. P.; Wang, Q. Y.; Noble, C. G.; Chen, Y. L.; Dong, H.; Zou, B.; Yokokawa, F.; Nilar, S.; Smith, P.; Beer, D.; Lescar, J.; Shi, P. Y. Ten Years of Dengue Drug Discovery: Progress and Prospects. *Antiviral Res.* **2013,** *100*, 500-519.

11. Nitsche, C.; Holloway, S.; Schirmeister, T.; Klein, C. D. Biochemistry and Medicinal Chemistry of the Dengue Virus Protease. *Chem. Rev.* **2014,** *114*, 11348-11381.

12. Beesetti, H.; Khanna, N.; Swaminathan, S. Drugs for Dengue: A Patent Review (2010-2014). *Expert Opin. Ther. Pat.* **2014,** *24*, 1171-1184.

13. Behnam, M. A.; Nitsche, C.; Boldescu, V.; Klein, C. D. The Medicinal Chemistry of Dengue Virus. *J. Med. Chem.* **2016,** *59*, 5622-5649.

14. *Informing Vaccination Programs: A Guide to the Design and Conduct of Dengue Serosurveys*. World Health Organization: Geneva, 2017.

15. Ye, N.; Chen, H.; Wold, E. A.; Shi, P. Y.; Zhou, J. Therapeutic Potential of Spirooxindoles as Antiviral Agents. *ACS Infect. Dis.* **2016,** *2*, 382-392.

16. Verdonck, S.; Pu, S.-Y.; Sorrell, F. J.; Elkins, J. M.; Froeyen, M.; Gao, L.-J.; Prugar, L. I.; Dorosky, D. E.; Brannan, J. M.; Barouch-Bentov, R.; Knapp, S.; Dye, J. M.; Herdewijn, P.; Einav, S.; De Jonghe, S. Synthesis and Structure–Activity Relationships of 3,5-Disubstituted-Pyrrolo[2,3-*b*]Pyridines as Inhibitors of Adaptor-Associated Kinase 1 with Antiviral Activity. *J. Med. Chem.* **2019,** *62*, 5810-5831.

17. Yang, Y.; Cao, L.; Gao, H.; Wu, Y.; Wang, Y.; Fang, F.; Lan, T.; Lou, Z.; Rao, Y. Discovery, Optimization, and Target Identification of Novel Potent Broad-Spectrum Antiviral Inhibitors. *J. Med. Chem.* **2019,** *62*, 4056-4073.

18. *Global Advisory Committee on Vaccine Safety, 6–7 June 2018*. Weekly Epidemiological Record: World Health Organization: Geneva, 2018; Vol. *93*, p 389-396.

19. Vannice, K. S.; Wilder-Smith, A.; Barrett, A. D. T.; Carrijo, K.; Cavaleri, M.; de Silva, A.; Durbin, A. P.; Endy, T.; Harris, E.; Innis, B. L.; Katzelnick, L. C.; Smith, P. G.; Sun, W.; Thomas, S. J.; Hombach, J. Clinical Development and Regulatory Points for Consideration for Second-Generation Live Attenuated Dengue Vaccines. *Vaccine*. **2018,** *36*, 3411-3417.

20. *Dengue Vaccine: WHO Position Paper–September 2018*. Weekly Epidemiological Record: World Health Organization: Geneva, 2018; Vol. *93*, p 457-476.

21. Messina, J. P.; Brady, O. J.; Scott, T. W.; Zou, C.; Pigott, D. M.; Duda, K. A.; Bhatt, S.; Katzelnick, L.; Howes, R. E.; Battle, K. E.; Simmons, C. P.; Hay, S. I. Global Spread of Dengue Virus Types: Mapping the 70 Year History. *Trends Microbiol.* **2014,** *22*, 138-146.

22. Guzman, M. G.; Harris, E. Dengue. *Lancet*. **2015,** *385*, 453-465.

23. Halsey, E. S.; Marks, M. A.; Gotuzzo, E.; Fiestas, V.; Suarez, L.; Vargas, J.; Aguayo, N.; Madrid, C.; Vimos, C.; Kochel, T. J.; Laguna-Torres, V. A. Correlation of Serotype-Specific Dengue Virus Infection with Clinical Manifestations. *PLoS Negl. Trop. Dis.* **2012,** *6*, e1638.

24. Rocha, B. A. M.; Guilarde, A. O.; Argolo, A. F. L. T.; Tassara, M. P.; da Silveira, L. A.; Junqueira, I. C.; Turchi, M. D.; Féres, V. C. R.; Martelli, C. M. T. Dengue-Specific Serotype Related to Clinical Severity During the 2012/2013 Epidemic in Centre of Brazil. *Infect. Dis. Poverty*. **2017,** *6*, 116.

25. Yung, C. F.; Lee, K. S.; Thein, T. L.; Tan, L. K.; Gan, V. C.; Wong, J. G. X.; Lye, D. C.; Ng, L. C.; Leo, Y. S. Dengue Serotype-Specific Differences in Clinical Manifestation, Laboratory Parameters and Risk of Severe Disease in Adults, Singapore. *Am. J. Trop. Med. Hyg.* **2015,** *92*, 999-1005.

26. Montoya, M.; Gresh, L.; Mercado, J. C.; Williams, K. L.; Vargas, M. J.; Gutierrez, G.; Kuan, G.; Gordon, A.; Balmaseda, A.; Harris, E. Symptomatic Versus Inapparent Outcome in Repeat Dengue Virus Infections Is Influenced by the Time Interval between Infections and Study Year. *PLoS Negl. Trop. Dis.* **2013,** *7*, e2357.

27. Reich, N. G.; Shrestha, S.; King, A. A.; Rohani, P.; Lessler, J.; Kalayanarooj, S.; Yoon, I. K.; Gibbons, R. V.; Burke, D. S.; Cummings, D. A. Interactions between Serotypes of Dengue Highlight Epidemiological Impact of Cross-Immunity. *J. R. Soc. Interface*. **2013,** *10*, 20130414.

28. Sangkawibha, N.; Rojanasuphot, S.; Ahandrik, S.; Viriyapongse, S.; Jatanasen, S.; Salitul, V.; Phanthumachinda, B.; Halstead, S. B. Risk Factors in Dengue Shock Syndrome: A Prospective Epidemiologic Study in Rayong, Thailand. I. The 1980 Outbreak. *Am. J. Epidemiol.* **1984,** *120*, 653-669.

29. Thein, S.; Aung, M. M.; Shwe, T. N.; Aye, M.; Zaw, A.; Aye, K.; Aye, K. M.; Aaskov, J. Risk Factors in Dengue Shock Syndrome. *Am. J. Trop. Med. Hyg.* **1997,** *56*, 566-572.

30. Graham, R. R.; Juffrie, M.; Tan, R.; Hayes, C. G.; Laksono, I.; Ma'roef, C.; Erlin; Sutaryo; Porter, K. R.; Halstead, S. B. A Prospective Seroepidemiologic Study on Dengue in Children Four to Nine Years of Age in Yogyakarta, Indonesia I. Studies in 1995-1996. *Am. J. Trop. Med. Hyg.* **1999,** *61*, 412-419.

31. Endy, T. P.; Yoon, I. K.; Mammen, M. P. Prospective Cohort Studies of Dengue Viral Transmission and Severity of Disease. *Curr. Top. Microbiol. Immunol.* **2010,** *338*, 1-13.

32. Guzman, M. G.; Alvarez, M.; Halstead, S. B. Secondary Infection as a Risk Factor for Dengue Hemorrhagic Fever/Dengue Shock Syndrome: An Historical Perspective and Role of Antibody-Dependent Enhancement of Infection. *Arch. Virol.* **2013,** *158*, 1445-1459.

33. Yokokawa, F.; Nilar, S.; Noble, C. G.; Lim, S. P.; Rao, R.; Tania, S.; Wang, G.; Lee, G.; Hunziker, J.; Karuna, R.; Manjunatha, U.; Shi, P. Y.; Smith, P. W. Discovery of Potent Non-Nucleoside Inhibitors of Dengue Viral RNA-Dependent RNA Polymerase from a Fragment Hit Using Structure-Based Drug Design. *J. Med. Chem.* **2016,** *59*, 3935-3952.

34. Noble, C. G.; Shi, P. Y. Structural Biology of Dengue Virus Enzymes: Towards Rational Design of Therapeutics. *Antiviral Res.* **2012,** *96*, 115-126.

35. Li, Y.; Wong, Y. L.; Lee, M. Y.; Li, Q.; Wang, Q. Y.; Lescar, J.; Shi, P. Y.; Kang, C. Secondary Structure and Membrane Topology of the Full-Length Dengue Virus NS4B in Micelles. *Angew. Chem. Int. Ed. Engl.* **2016,** *55*, 12068-12072.

36. Xie, X.; Zou, J.; Wang, Q. Y.; Shi, P. Y. Targeting Dengue Virus NS4B Protein for Drug Discovery. *Antiviral Res.* **2015,** *118*, 39-45.

37. Zou, J.; Xie, X.; Lee le, T.; Chandrasekaran, R.; Reynaud, A.; Yap, L.; Wang, Q. Y.; Dong, H.; Kang, C.; Yuan, Z.; Lescar, J.; Shi, P. Y. Dimerization of Flavivirus NS4B Protein. *J. Virol.* **2014,** *88*, 3379-3391.

38. Youn, S.; Li, T.; McCune, B. T.; Edeling, M. A.; Fremont, D. H.; Cristea, I. M.; Diamond, M. S. Evidence for a Genetic and Physical Interaction between Nonstructural Proteins NS1 and NS4B That Modulates Replication of West Nile Virus. *J. Virol.* **2012,** *86*, 7360-7371.

39. Umareddy, I.; Chao, A.; Sampath, A.; Gu, F.; Vasudevan, S. G. Dengue Virus NS4B Interacts with NS3 and Dissociates It from Single-Stranded RNA. *J. Gen. Virol.* **2006,** *87*, 2605-2614.

40. Zou, J.; Lee le, T.; Wang, Q. Y.; Xie, X.; Lu, S.; Yau, Y. H.; Yuan, Z.; Geifman Shochat, S.; Kang, C.; Lescar, J.; Shi, P. Y. Mapping the Interactions between the NS4B and NS3 Proteins of Dengue Virus. *J. Virol.* **2015,** *89*, 3471-3483.

41. Tajima, S.; Takasaki, T.; Kurane, I. Restoration of Replication-Defective Dengue Type 1 Virus Bearing Mutations in the N-Terminal Cytoplasmic Portion of NS4A by Additional Mutations in NS4B. *Arch. Virol.* **2011,** *156*, 63-69.

42. Li, X. D.; Ye, H. Q.; Deng, C. L.; Liu, S. Q.; Zhang, H. L.; Shang, B. D.; Shi, P. Y.; Yuan, Z. M.; Zhang, B. Genetic Interaction between NS4A and NS4B for Replication of Japanese Encephalitis Virus. *J. Gen. Virol.* **2015,** *96*, 1264-1275.

43. Zou, J.; Xie, X.; Wang, Q. Y.; Dong, H.; Lee, M. Y.; Kang, C.; Yuan, Z.; Shi, P. Y. Characterization of Dengue Virus NS4A and NS4B Protein Interaction. *J. Virol.* **2015,** *89*, 3455-3470.

44. Munoz-Jordan, J. L.; Laurent-Rolle, M.; Ashour, J.; Martinez-Sobrido, L.; Ashok, M.; Lipkin, W. I.; Garcia-Sastre, A. Inhibition of Alpha/Beta Interferon Signaling by the NS4B Protein of Flaviviruses. *J. Virol.* **2005,** *79*, 8004-8013.

45. Munoz-Jordan, J. L.; Sanchez-Burgos, G. G.; Laurent-Rolle, M.; Garcia-Sastre, A. Inhibition of Interferon Signaling by Dengue Virus. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** *100*, 14333-14338.

46. Kounde, C. S.; Yeo, H. Q.; Wang, Q. Y.; Wan, K. F.; Dong, H.; Karuna, R.; Dix, I.; Wagner, T.; Zou, B.; Simon, O.; Bonamy, G. M. C.; Yeung, B. K. S.; Yokokawa, F. Discovery of 2-Oxopiperazine Dengue Inhibitors by Scaffold Morphing of a Phenotypic High-Throughput Screening Hit. *Bioorg. Med. Chem. Lett.* **2017,** *27*, 1385-1389.

47. Bardiot, D.; Koukni, M.; Smets, W.; Carlens, G.; McNaughton, M.; Kaptein, S.; Dallmeier, K.; Chaltin, P.; Neyts, J.; Marchand, A. Discovery of Indole Derivatives as Novel and Potent Dengue Virus Inhibitors. *J. Med. Chem.* **2018,** *61*, 8390-8401.

48. Xie, X.; Wang, Q. Y.; Xu, H. Y.; Qing, M.; Kramer, L.; Yuan, Z.; Shi, P. Y. Inhibition of Dengue Virus by Targeting Viral NS4B Protein. *J. Virol.* **2011,** *85*, 11183-11195.

49. van Cleef, K. W.; Overheul, G. J.; Thomassen, M. C.; Kaptein, S. J.; Davidson, A. D.; Jacobs, M.; Neyts, J.; van Kuppeveld, F. J.; van Rij, R. P. Identification of a New Dengue Virus Inhibitor That Targets the Viral NS4B Protein and Restricts Genomic RNA Replication. *Antiviral Res.* **2013,** *99*, 165-171.

50. Patkar, C. G.; Larsen, M.; Owston, M.; Smith, J. L.; Kuhn, R. J. Identification of Inhibitors of Yellow Fever Virus Replication Using a Replicon-Based High-Throughput Assay. *Antimicrob. Agents Chemother.* **2009,** *53*, 4103-4114.

51. Zou, B.; Chan, W. L.; Ding, M.; Leong, S. Y.; Nilar, S.; Seah, P. G.; Liu, W.; Karuna, R.; Blasco, F.; Yip, A.; Chao, A.; Susila, A.; Dong, H.; Wang, Q. Y.; Xu, H. Y.; Chan, K.; Wan, K. F.; Gu, F.; Diagana, T. T.; Wagner, T.; Dix, I.; Shi, P. Y.; Smith, P. W. Lead Optimization of Spiropyrazolopyridones: A New and Potent Class of Dengue Virus Inhibitors. *ACS Med. Chem. Lett.* **2015,** *6*, 344-348.

52. Chan, W. L.; Ding, M.; Zou, B. Preparation of Spiropyrazolopyridine Derivatives and Uses Thereof for the Treatment of Viral Infections. WO2014167528A1, 2014.

53. Wang, Q. Y.; Dong, H.; Zou, B.; Karuna, R.; Wan, K. F.; Zou, J.; Susila, A.; Yip, A.; Shan, C.; Yeo, K. L.; Xu, H.; Ding, M.; Chan, W. L.; Gu, F.; Seah, P. G.; Liu, W.; Lakshminarayana, S. B.; Kang, C.; Lescar, J.; Blasco, F.; Smith, P. W.; Shi, P. Y. Discovery of Dengue Virus NS4B Inhibitors. *J. Virol.* **2015,** *89*, 8233-8244.

54. Lai, A. C.; Crews, C. M. Induced Protein Degradation: An Emerging Drug Discovery Paradigm. *Nat. Rev. Drug Discovery*. **2016,** *16*, 101-114.

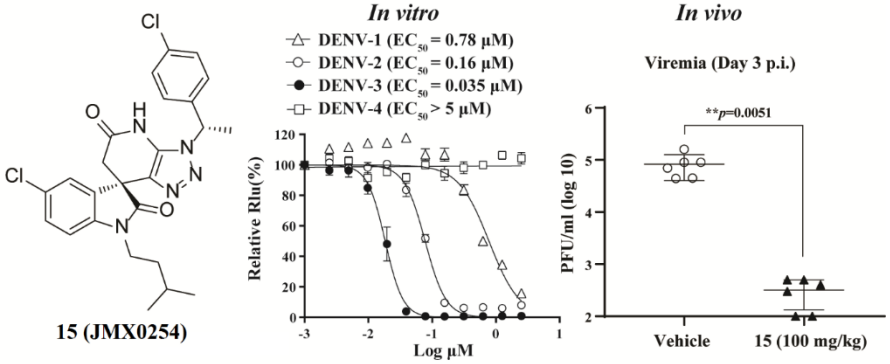
55. Wang, P.; Zhou, J. Proteolysis Targeting Chimera (Protac): A Paradigm-Shifting Approach in Small Molecule Drug Discovery. *Curr. Top. Med. Chem.* **2018,** *18*, 1354-1356.

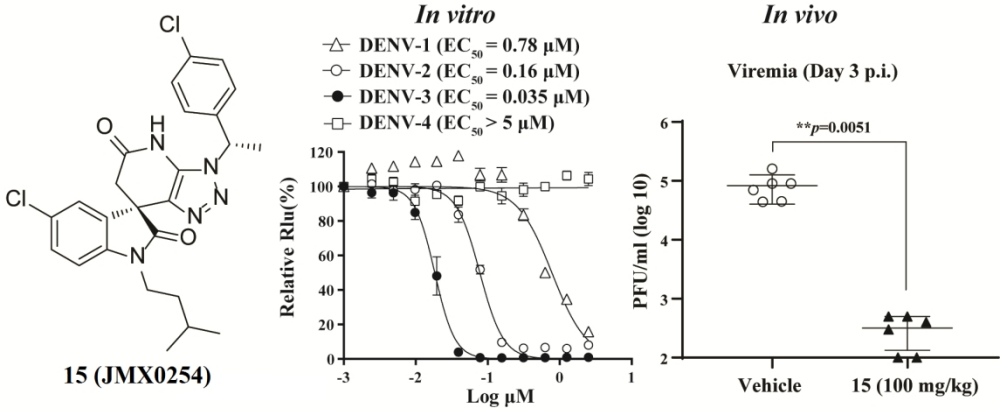
56. Xie, X.; Zou, J.; Shan, C.; Yang, Y.; Kum, D. B.; Dallmeier, K.; Neyts, J.; Shi, P.-Y. Zika Virus Replicons for Drug Discovery. *EBioMedicine*. **2016,** *12*, 156-160.

57. Xie, X.; Zou, J.; Wang, Q. Y.; Noble, C. G.; Lescar, J.; Shi, P. Y. Generation and Characterization of Mouse Monoclonal Antibodies against NS4B Protein of Dengue Virus. *Virology*. **2014,** *450-451*, 250-257.

58. Xie, X.; Yang, Y.; Muruato, A. E.; Zou, J.; Shan, C.; Nunes, B. T.; Medeiros, D. B.; Vasconcelos, P. F.; Weaver, S. C.; Rossi, S. L.; Shi, P. Y. Understanding Zika Virus Stability and Developing a Chimeric Vaccine through Functional Analysis. *MBio*. **2017,** *8*, 1-14.

Table of Contents (TOC) Graphic





TOC

157x65mm (300 x 300 DPI)