Bioorganic & Medicinal Chemistry 20 (2012) 467–479

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The design, synthesis and biological evaluations of C-6 or C-7 substituted 2-hydroxyisoquinoline-1,3-diones as inhibitors of hepatitis C virus

Yue-Lei Chen [†], Jing Tang, Matthew J. Kesler, Yuk Y. Sham, Robert Vince, Robert J. Geraghty, Zhengqiang Wang [*]

Center for Drug Design, Academic Health Center, University of Minnesota, 516 Delaware St. SE, MMC 204, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 26 August 2011
Revised 13 October 2011
Accepted 18 October 2011
Available online 24 October 2011

Keywords:
2-Hydroxyisoquinoline-1,3-dione
Hepatitis C virus (HCV)
NS5B polymerase

ABSTRACT

C7-Substituted 2-hydroxyisoquinoline-1,3-diones inhibit the strand transfer of HIV integrase (IN) and the reverse-transcriptase-associated ribonuclease H (RNH). Hepatitis C virus (HCV) NS5B polymerase shares a similar active site fold to RNH and IN, suggesting that N-hydroxyimides could be useful inhibitor scaffolds of HCV via targeting the NS5B. Herein we describe the design, chemical synthesis, replicon and biochemical assays, and molecular docking of C-6 or C-7 aryl substituted 2-hydroxyisoquinoline-1,3-diones as novel HCV inhibitors. The synthesis involved an improved and clean cyclization method, which allowed the convenient preparation of various analogs. Biological studies revealed that the C-6 analogs, a previously unknown chemotype, consistently inhibit both HCV replicon and recombinant NS5B at low micromolar range. Molecular modeling studies suggest that these inhibitors may bind to the NS5B active site.

1. Introduction

Discovered as the causative agent of non-A/non-B hepatitis,[1] HCV infects an estimated 170 million people globally[2] and approximately 4 million in the USA.[3] Individuals with chronic HCV infection are at high risk of developing progressive liver injury, fibrosis, cirrhosis and hepatocellular carcinoma.[4,5] Current standard of care for HCV infection, the combination of subcutaneous pegylated interferon (peg-IFN) alpha and oral ribavirin (RBV),[6] has proved effective in only about 50% the patients infected with HCV genotype 1,[6,7] the predominant genotype in North America and Europe. In addition, the peg-IFN/RBV regimen is associated with severe adverse effects that are often found intolerable.[8] Therefore, there is an unmet clinical need to develop novel chemotherapy against HCV with better efficacy and tolerability. Experimental drugs specifically targeting HCV replication have consistently yielded improved sustained virological response (SVR) rates, suggesting that these direct-acting antivirals (DAAs) would provide a better treatment option for HCV infection.[9]

HCV is an enveloped RNA virus belonging to the Hepacivirus genus of the Flaviviridae family. Its genome is a 9.6 kb single-stranded positive sense RNA encoding a polyprotein precursor of about 3000 amino acids. This polyprotein is processed by host peptidases and viral proteases to yield four structural proteins (core protein, envelope proteins E1 and E2, and p7) and six nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). Among these proteins the NS3-4A serine-protease[10] and the NS5B viral RNA-dependent RNA polymerase[11] have been the primary viral targets for current efforts in HCV chemotherapy development. Major inhibitors in clinical development are highlighted in Figure 1. The two protease inhibitors, telaprevir (**1**)[12,13] and boceprevir (**2**)[14,15] (Fig. 1), were approved by FDA recently. These antivirals effect significantly improved SVR rates, though the response could be compromised by the quick development of drug resistance. Concerted efforts have been made to develop second-generation HCV protease inhibitors.[16–21] Similarly, the nonnucleoside inhibitors (NNIs)[22–24] of HCV NS5B polymerase are also confronted with the resistance issue as they bind to highly mutable allosteric sites. GS-9190[25] (**3**, Fig. 1), an NNI demonstrating exceptional antiviral activity in vitro, is currently in phase II clinical development. By contrast, nucleoside NS5B inhibitors, such as the uridine analog PSI-7977 (**4**)[26] and the guanosine analog PSI-938 (**5**)[27], target the highly conserved active site, thus have a high genetic barrier to resistance development and a pan-genotypic antiviral activity. Recently, a third HCV-encoded protein, NS5A, has emerged as a novel target for HCV chemotherapeutic intervention.[28–30] Although the exact role of NS5A remains unclear, the first-in-class inhibitor BMS-790052 (**6**)[28,30]

Abbreviations: IN, integrase; RNH, ribonuclease H; HCV, hepatitis C virus; peg-INF, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; DAA, direct-acting antiviral; NNI, nonnucleoside inhibitor; DKA, diketoacid; Luc, luciferase; 2 mA, 2′-C-methyl adenosine; SAR, Structure–activity-relationship.

* Corresponding author. Tel.: +1 612 626 7025; fax: +1 612 625 8154.
E-mail address: wangx472@umn.edu (Z. Wang).
† Present address: Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Room 5407, 555 Zuchongzhi Road, Shanghai 201203, PR China.

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.10.058

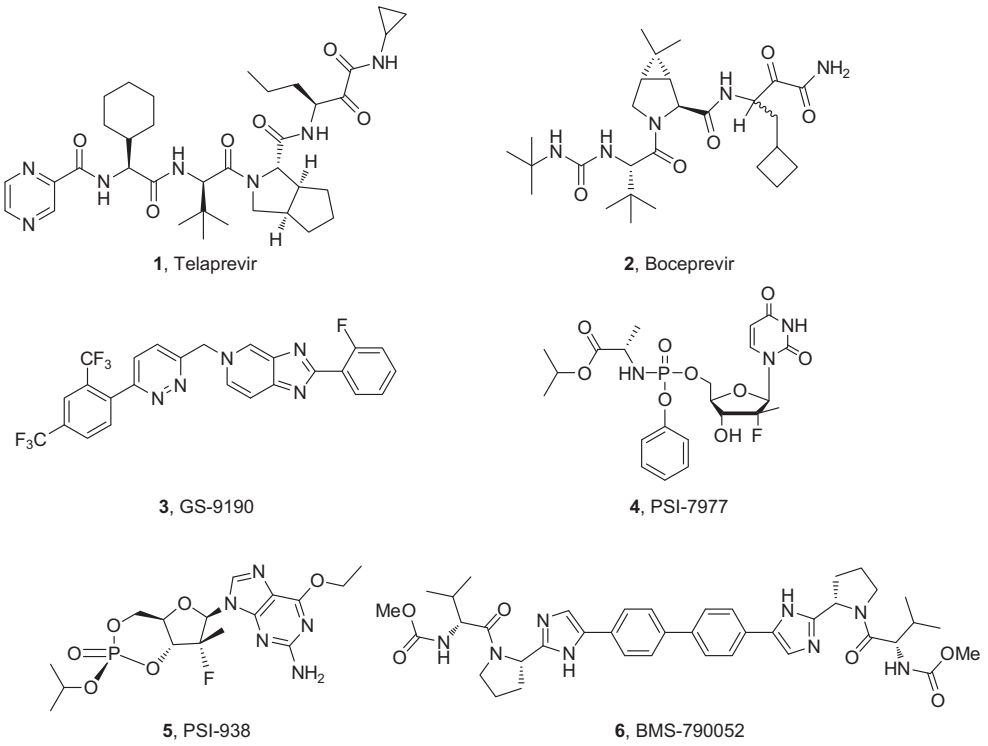


Figure 1. Representative HCV inhibitors: **1** and **2**, protease inhibitors; **3**, nonnucleoside polymerase inhibitor; **4** and **5**, nucleoside polymerase inhibitors; **6**, NS5A inhibitor.

exhibits exceptional in vitro potency and is currently under clinical development. Nevertheless, even if some of these DAAs progress to market, continued search for novel HCV inhibitor scaffolds is warranted to combat all genotypes and resistant viral strains.

Intriguingly, HCV NS5B polymerase shares a similar active site fold to RNH and IN,[31] which is manifested by the resemblance among their inhibitor pharmacophores (Fig. 2). Particularly, compounds **7**,[32] **8**[33] and **9**[34] featuring a diketoacid (DKA) chelating functionality inhibit all three enzymes. Similarly, 5-hydroxy-pyrimidinone compounds **10**[35] and **11**[36] containing a chelating triad similar to DKA were found to inhibit both HIV IN and HCV NS5B. A similar chemotype, 2-hydroxyisoquinoline-1,3-diones, is a well-known inhibitor scaffold for HIV RNH.[37] Of particular interest is the recent report of C-7-substituted 2-hydroxyisoquinoline-1,3-diones (**12** and **13**) as dual inhibitors of HIV RNH and IN.[38–40] Based on these observations, we hypothesized that *N*-hydroxyimides **14** could inhibit HCV replication by targeting the NS5B (Fig. 2).

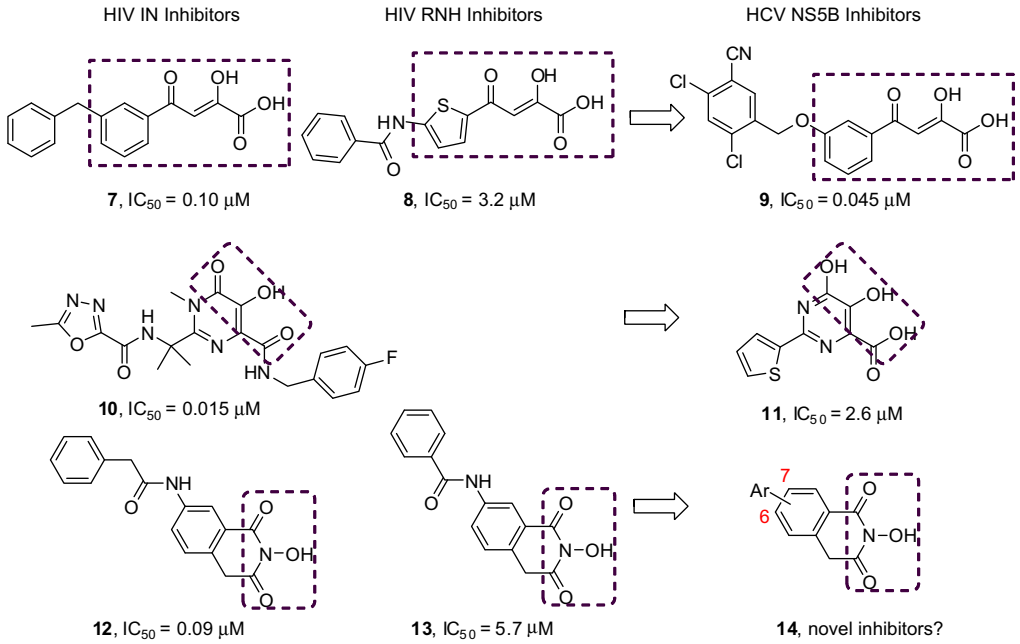


Figure 2. Design of novel NS5B inhibitor scaffold **14** based on the pharmacophore resemblance among HIV IN inhibitors, HIV RNH inhibitors and HCV NS5B inhibitors.

Notably, NS5B inhibitors with a chelating triad generally also have an aromatic ring directly connected to the chelating core, for example, the thiophene ring of compound **11**. This prompted us to synthesize analogs with a C-7 aromatic substitution (**14**, substitution at C-7). Additionally, aromatic substitution at C-6 position would generate a similar chemotype (**14**, substitution at C6) with a different overall molecular shape which is of medicinal chemistry interest. This latter chemotype has never been reported in literature, possibly due to the synthetic challenge posed by the unfavored directing effects from both the carbonyl and the methylene groups.

2. Results and discussion

2.1. Chemistry

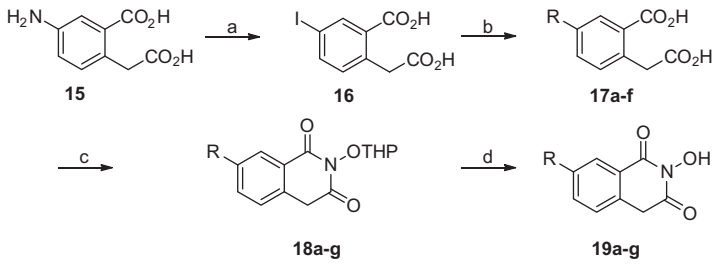
2-Hydroxyisoquinoline-1,3-diones with a substitution at C-7 position were prepared based on a reported procedure[38] as depicted in Scheme 1. Diacid **15** was subjected to standard Sandmeyer conditions for iodo substitution. After the addition of excessive urea, the diazonium salt intermediate was found particularly sensitive to temperature, hence a careful monitoring of the mixture temperature is recommended. Suzuki coupling of the common intermediate **16** with various aromatic boronic acids was found to proceed smoothly with a combination of $Pd(PPh_3)_4$, K_2CO_3, and H_2O: EtOH (v/v = 1:1) under microwave. For the synthesis of target structure **19**, the reported method[38] used $BnONH_2$ to cyclize with diacids **17** under azeotropic condition followed by the removal of the Bn protection with BBr_3. However, attempts to effect this reaction sequence with our substrates were confronted with inconsistent yields. Moreover, the forcing deprotection conditions would also compromise our ability to install substituents of various natures potentially useful for medicinal chemistry.

To optimize this reaction sequence, commercially available *O*-silyl and *O*-THP protected hydroxylamines were employed due largely to their easy deprotection. However, the cyclization of diacid **16** with hydroxylamines containing acid labile groups turned out to be particularly problematic. Many conventional dehydrating acylation conditions with activating reagents and the reported azeotropic condition all failed to achieve a workable cyclization. Through extensive reaction condition screening, we finally discovered that adding a dehydrating reagent, preferably carbonyldiimidazole (CDI), into a refluxing mixture of diacid **17** and $THPONH_2$ in chlorobenzene provided an extremely rapid access to **18**. The reaction typically completed in 30 s by TLC and the purification proved particularly easy. Filtering the mixture through a short silica gel plug and evaporation of solvents typically yielded essentially pure intermediates **18a–g**, which were used directly for the final deprotection. Removal of *O*-THP was effected under a catalytic amount of *p*-TSA monohydrate yielding desired compounds **19a–g** in pure form. Although this synthetic sequence is still compromised by the low yield of the cyclization step, it does provide a rapid and broadly applicable access to chemotype **19** due to the high functional group compatibility and ease of product separation.

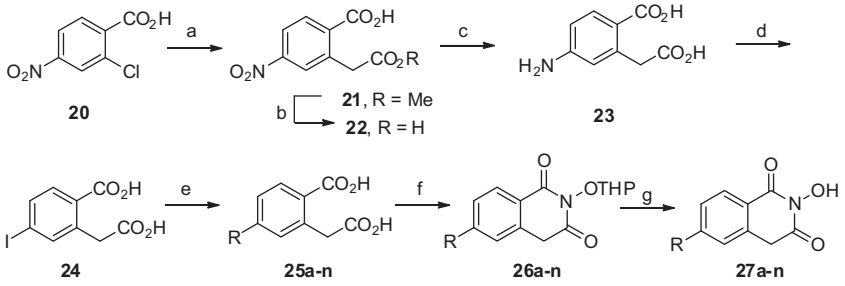
This cyclization method also allowed us to prepare the unprecedented C-6 substituted 2-hydroxyisoquinoline-1,3-diones (Scheme 2). In this case, the requisite amino diacid intermediate **23** was synthesized starting from commercially available chloride **20**. A nucleophilic aromatic substitution reaction and a subsequent saponification produced the nitro substituted diacid **22** which was reduced via a catalytic hydrogenation furnishing the desired amino diacid **23**.

2.2. HCV replicon assay

Until recently, detection of a complete HCV replication cycle in cell culture was not possible. To study more limited aspects of HCV replication, subgenomic viral RNAs (replicons) capable of self-replication in human liver cells were engineered.[41,42] An HCV genotype 1b replicon stably maintained in Huh7 liver cells



Scheme 1. Synthesis of C-7 substituted 2-hydroxyisoquinoline-1,3-diones. Reagents and conditions: (a) 4 N aq sulfuric acid, $NaNO_2$, −10 °C, then urea, −10 to 0 °C, KI, rt overnight, 44%; (b) boronic acid, $Pd(PPh_3)_4$, K_2CO_3, H_2O: EtOH (v/v = 1:1), microwave, 150 °C, 30 min, 87%-quant; (c) $THPONH_2$, ClPh, CDI, reflux 30 s; (d) *p*-TSA hydrate, MeOH, 6–22% over two steps.



Scheme 2. Synthesis of C-6 substituted 2-hydroxyisoquinoline-1,3-diones. Reagents and conditions: (a) dimethyl malonate, CuBr, NaOMe, 92%; (b) NaOH, MeOH, rt overnight, 49%; (c) 10% Pd/C, H_2, EtOH, 85%; (d) 4 N aq sulfuric acid, $NaNO_2$, −10 °C, then urea, −10 to 0 °C, KI, rt overnight, 58%; (e) boronic acid, $Pd(PPh_3)_4$, K_2CO_3, H_2O: EtOH (v/v = 1:1), microwave, 150 °C, 30 min, 59%-quant.; (f) $THPONH_2$, ClPh, CDI, reflux 30 s; (g) *p*-TSA hydrate, MeOH, 10–24% over two steps.

(Huh-7/HCV1b-Rluc) was used for this study. The replicon RNA expresses renilla luciferase (Luc) and the Luc activity can be measured as a surrogate for measuring the level of replicon RNA.

All newly synthesized 2-hydroxylisoquinoline-1,3-dione analogs were first tested at a single concentration of 10 μM in this replicon assay with RBV and 2′-C-methyl adenosine (2 mA)[43] as control compounds. Replicon cells receiving vehicle alone (DMSO) and cell culture medium were also included in the assay. The cell viability was measured using an MTS-based colorimetric assay. Compounds consistently producing excellent Luc reduction (⩾72%) and high viability (⩾86%) at 10 μM compared to DMSO alone were chosen for dose response studies. Both the EC_{50} and CC_{50} values were determined from these experiments (Table 1).

As clearly shown in Table 1, except for **19d**, all 2-hydroxyisoquinoline-1,3-diones with a C-7 aryl substituent showed significant cytotoxicity (viability ⩽37%) in Huh-7/HCV1b-Rluc cells, rendering them unsuitable as an inhibitor scaffold for HCV. Interestingly, when the C-7 aromatic group is replaced with iodine, the compound (**19g**) becomes a viable HCV inhibitor with an EC_{50} of 3.9 μM and a decent selectivity ratio. This iodo compound serves as a valuable handle for further structure–activity-relationship (SAR) explorations. On the other hand, relocating the aromatic substituent from C-7 to C-6 position generates an isochemotype which shows drastically improved antiviral activity as well as cytotoxicity profile. This is exemplified with compounds **27a** and **27f**, where a furan or thiophene substitution at C-6 position confers low micromolar (1.9 and 1.7 μM) inhibitory activity with good therapeutic index (6.8 and 11), while their corresponding C-7 regio-isomers (**19a** and **19b**) were found toxic (21% viability at 10 μM for both). These differences in activity and toxicity between regio-isomers may reflect a fundamental change in their binding mode to the protein target as their molecular shape can be significantly different. Nevertheless, these significant improvements led to the synthesis and testing of numerous analogs differing primarily in the aromatic substituent. In the end, one C-7 analog (**19g**) and four C-6 isomers (**27a**, **27b**, **27f** and **27l**) were found considerably more potent than RBV, validating this scaffold as a potentially useful HCV inhibitor type.

2.3. Mechanism of action

Our analogs were designed based on the binding mode and pharmacophore model of NS5B inhibitors. As mentioned earlier, 2-hydroxyisoquinoline-1,3-diones are known to inhibit HIV IN and RNH,[38–40] two enzymes sharing a similar fold of active conformation to HCV NS5B. Therefore, we hypothesized that the inhibitory activity of our compounds against HCV replicon can be attributed to NS5B inhibition. To test this hypothesis, active compounds selected from the replicon assay were further evaluated against recombinant NS5B using a scintillation proximity assay. The known DKA compound **9**[34] was used as a control compound.

As shown in Table 2, all selected compounds were found active against NS5B at low μM concentrations and **27a**, which has a C-6 furan, demonstrated a much higher potency (IC_{50} = 1.3 μM) than the C7 regio-isomer **19a** (IC_{50} = 18 μM). These observations correlate positively with the observed inhibitory activity in HCV replicon, strongly supporting NS5B as the enzyme target of our inhibitors.

2.4. Mode of binding

Superpositioning of all available NS5B X-ray crystallographic structures bound with known inhibitors revealed that besides the active site where endogenous substrates and nucleoside inhibitors bind, this enzyme also has at least four allosteric sites[22,44] (thumb I, thumb II, palm I and palm II) for the binding of various NNIs (Fig. 3). Our inhibitors contain a chelating triad capable of binding to both Mg^{2+} ions at the active site, thus could represent a rare type of active site binding NNIs. Similar to nucleoside inhibitors, active site binding NNIs would be less prone to resistance development and may have the advantage of pan-genotypic activity when compared to typical NNIs.

The most active compound observed in our biochemical assay, **27a**, was docked into the binding pocket of NS5B active site. The standard protocol for docking using the divalent metal cofactors as a required constraint identified the expected chelating geometry of the triad to the two metal cofactors (Fig. 4). This observed mode of binding suggested R48 and K155 may play a crucial role in the recognition of the 6- or 7- substitutions. Interestingly, both R48 ad K155 residues were also observed to form crucial salt bridge interactions with the terminal phosphate of UTP in the original X-ray crystallographic structure.[45] For compound **27a**, this mode of binding allows the furan oxygen to form a critical H-bond to either K155 or R48 depending on its orientation. For 6-thiophene analog (**27f**), this H-bond interaction was expected to be weaker due to the lower electronegativity of the sulfur atom, resulting in slight decrease in the inhibitory activity. Modeling of compounds **19a** and **27c** further showed the loss of this crucial H-bond interaction and could also explain the relative decrease of their inhibitory activity. Substitution at the C-7 position for compounds **19a** and **19d** led to the loss of this crucial H-bond. For compound **19d**, the presence of the dihalo-substituted phenyl group at the C-7 position resulted in optimal overlap of its aromatic ring with the guanidinium side chain of R48 (not shown), resulting in likely favorable π–π overlapping interactions for inhibitory binding.

3. Conclusions

Based on the similar fold of active site shared among HIV IN, RNH and HCV NS5B and the pharmacophore resemblance among their inhibitors, we designed C-6 and C-7 aromatic substituted 2-hydroxyisoquinoline-1,3-diones as a novel inhibitor scaffold for HCV NS5B. Difficulties presented in the synthesis of these analogs prompted us to discover an improved synthetic method for the key cyclization step with greater functional group compatibility and easier product separation. All synthetic analogs were tested in a primary screening assay using HCV replicon 1b Huh-7/HCV1b-Rluc cells and the C-6 isomers consistently showed significantly better inhibitory activity than RBV at low micromolar range. Biochemical assay against recombinant NS5B confirmed that these analogs target viral polymerase with low micromolar IC_{50}. Pharmacophore model and molecular docking suggested that these new NS5B inhibitors may bind to the active site. Collectively these studies have led to the identification of a molecular scaffold potentially useful as HCV inhibitors, and laid a foundation for further SAR to diversify the C-6 or C-7 substituents.

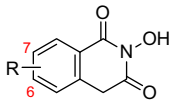
4. Experimental

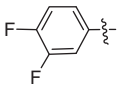
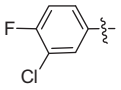
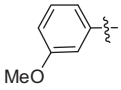
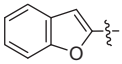
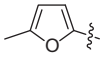
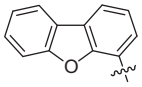
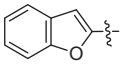
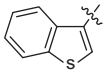
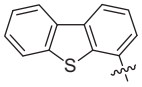
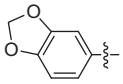
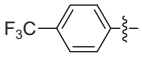
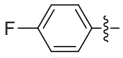
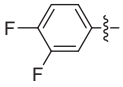
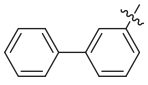
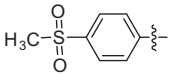
4.1. Chemistry

4.1.1. General procedures

All commercial chemicals were used as supplied unless otherwise indicated. Dry solvents (THF, Et_2O, CH_2Cl_2 and DMF) were dispensed under argon from an anhydrous solvent system with two packed columns of neutral alumina or molecular sieves. Flash chromatography was performed on a Teledyne Combiflash RF-200 with RediSep columns (silica) and indicated mobile phase. All reactions were performed under inert atmosphere of ultra-pure argon with oven-dried glassware. ^1H and ^{13}C NMR spectra were recorded on a Varian 600 MHz or a Varian 400 MHz spectrometer. Mass data

Table 1
Assay results for C6- or C7-substituted analogs in HCV replicon genotype 1b



Compd	Substitution position	R	Single concentration (10 μM)		Dose response		
			Luc reduction (%)[a]	Cell viability (%)	EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
19a	7		51	21	–	–	–
19b	7		99	21	–	–	–
19c	7		100	33	–	–	–
19d	7		30	83	–	–	–
19e	7		54	37	–	–	–
19f	7		83	33	–	–	–
19g	7	I	79	86	3.9 ± 1.5	27	6.9
27a	6		89	96	1.9 ± 0.81	13 ± 0.58	6.8
27b	6		76	87	4.3 ± 0.35	11 ± 2.6	2.6
27c	6		100	52	–[e]	–	–
27d	6		98	28	–	–	–
27e	6		73	46	–	–	–
27f	6		93	100	1.7 ± 0.46	18 ± 8.0	11
27g	6		72	100	44 ± 32	98	2.2
27h	6		95	34	–	–	–
27i	6		95	32	–	–	–
27j	6		28	79	–	–	–
27k	6		93	29	–	–	–
27l	6		73	90	9.6 ± 2.5	11 ± 2.7	1.2
27m	6		78	34			
27n	6		95	23	–	–	–
27o	6		3	100	–	–	–

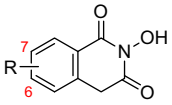
(*continued on next page*)

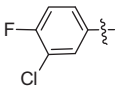
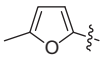
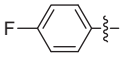
Table 1 (*continued*)

Compd	Substitution position	R	Single concentration (10 μM)		Dose response		
			Luc reduction (%)[a]	Cell viability (%)	EC_{50} (μM)[b]	CC_{50} (μM)[c]	TI[d]
RBV	—	—	—	—	14 ± 4.5	32	2.3
2 mA	—	—	—	—	0.20 ± 0.11	55 ± 15	270

[a] Reduction of Luc activity, indicating inhibitory activity of a compound.
[b] Concentration inhibiting virus replication by 50%, mean value ± standard deviation from at least two independent determinations.
[c] Concentration resulting in 50% cell death.
[d] Therapeutic index, defined by CC_{50}/EC_{50}.
[e] Not determined.

Table 2
Biochemical assay results against recombinant HCV NS5B



Compd	Substitution position	R	IC_{50} (μM)[a]
19a	7		18 ± 7.2
19d	7		7.1 ± 0.7
19g	7	I	15 ± 1.4
27a	6		1.3 ± 1.0
27b	6		18 ± 13
27c	6		11 ± 4.0
27f	6		7.3 ± 3.3
27g	6		9.0 ± 3.7
27l	6		20 ± 12
9[b]	—	—	0.015 ± 0.0070

[a] Concentration inhibiting enzyme by 50%, mean value ± standard deviation from at least two independent determinations.
[b] Reported IC_{50} = 0.045 μM.[34]

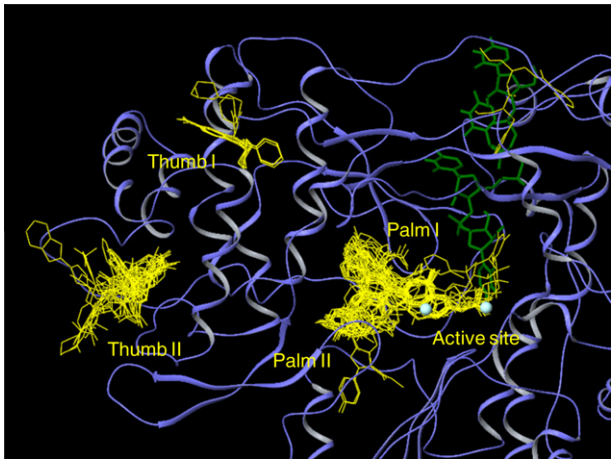


Figure 3. Binding sites of HCV NS5B inhibitors.

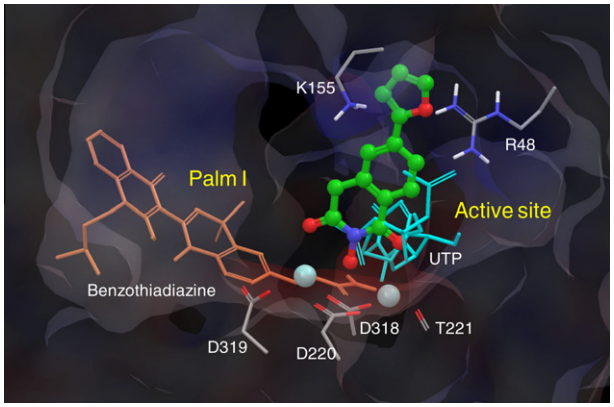


Figure 4. Docking of **27a** (green) in HCV NS5B polymerase active site. The chelation of both Mg^{2+} ions (light blue sphere) allows the placement of the C-6 furan ring in close proximity to R48 and K155, allowing the formation key H-bond for potent inhibition. Both UTP (cyan) (taken from PDB: 1NB6[45]) and benzothiadiazine (brown) (taken from PDB:3HHK[46]) are shown to highlight the unique mode of binding of compound **27a**.

were acquired on an Agilent TOF II TOS/MS spectrometer capable of ESI and APCI ion sources. Analysis of sample purity was performed on a Varian Prepstar SD-1 HPLC system with a Varian Microsorb-MW 100-5 C18 column (250 mm × 4.6 mm). HPLC conditions: solvent A = H_2O, solvent B = MeCN; flow rate = 1.0 mL/min; Gradient (B%): 0–13 min (1–5); 13–20 min (95); 20–23 min (95–10); 23–25 min (10). All tested compounds have a purity ⩾96%.

4.1.1.1. 2-(Carboxymethyl)-5-iodobenzoic acid (16)[38]. Ten grams (52 mmol) of amine **15** was dissolved in 100 mL of 4 N aq sulfuric acid and cooled to −15 °C. To this, a solution of $NaNO_2$ (10 g, 145 mmol) in 100 mL of water was added dropwise. The mixture was then stirred between −10 and 0 °C for 1 h, before a solution of 10 g of urea in 30 mL of water was introduced dropwise (internal temperature must be maintained below 0 °C). Upon ceasing of nitrogen generation, a solution of KI (15 g, 90 mmol) in 30 mL of water was added, and the mixture was warmed to rt gradually and stirred at rt overnight. The precipitate was separated by filtration, washed with cold water several times and dried at rt overnight to give 6.9 g (22.5 mmol, 44%) of product as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (br s, 2H, 2 × COOH), 8.14 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.83 (dd, J = 8.0, 2.0 Hz, 1H, H_{Ar}), 7.12 (d, J = 8.1 Hz, 1H, H_{Ar}), 3.87 (s, 2H, CH_2).

4.1.1.2. 2-(2-Methoxy-2-oxoethyl)-4-nitrobenzoic acid (21). A solution of 9.0 g (44.8 mmol) 2-chloro-4-nitrobenzoic acid **20** in 100 mL of dimethyl malonate was bubbled with Ar for 10 min, followed by addition of 500 mg of CuBr (3.5 mmol) and 6.0 g (111 mmol) of NaOMe. The mixture was stirred in a 100 °C

oil bath overnight. It was observed that the mixture turned first into solid and then gradually became a dark brown suspension. The resulting mixture was cooled to rt, mixed with 100 mL of water and 100 mL of hexanes, and filtered. The water phase was then separated, washed first with hexanes, and then with toluene several times. The resulting water phase was acidified (to pH 1) by 2 N aq HCl and extracted with DCM several times. Combined organic phases were evaporated to dryness, and the residue was co-evaporated with toluene several times to give a yellow solid as crude product (9.9 g, 41 mmol, 92%) which is sufficiently pure for the following reaction. The crude product was recrystallized from diethyl ether and hexanes for an analytically pure sample. R_f 0.82 (MeOH:DCM = 1:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (br s, 1H, COOH), 8.29 (d, J = 8.6 Hz, 1H, H_{Ar}), 8.26–8.21 (m, 1H, H_{Ar}), 8.16 (d, J = 2.1 Hz, 1H, H_{Ar}), 4.17 (s, 2H, CH_2), 3.73 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9 ($ArCH_2COOH$), 170.0 (Ar-*C*OOH), 150.1($C_{q,Ar}$), 138.5 ($C_{q,Ar}$), 134.2 ($C_{q,Ar}$), 133.0 (CH_{Ar}), 127.1 (CH_{Ar}), 122.4 (CH_{Ar}), 52.4 (CH_3), 40.3 (CH_2); HRMS (ESI−) calcd for $C_{10}H_8NO_6^-$[M−H]$^-$ 238.0357, found 238.0383; Mp: 100–101 °C (diethylether-hexanes); HPLC t_R 2.9 min.

4.1.1.3. 2-(Carboxymethyl)-4-nitrobenzoic acid (22). The crude product **21** (9.9 g, 41 mmol) from last step was stirred with a solution of 12 g (0.3 mol) of NaOH in 100 mL of MeOH at rt overnight. The mixture was then acidified (to pH 1) with conc. aq HCl, evaporated to dryness and co-evaporated with toluene several times. The residue was extracted with 20% MeOH in DCM several times. Combined organic phases were evaporated to dryness and the residue was recrystallized with minimum amount of EtOAc to give the product 4.5 g (20 mmol, 49%) of off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (d, J = 2.4 Hz, 1H, H_{Ar}), 8.19 (dd, J = 8.6, 2.4 Hz, 1H, H_{Ar}), 8.08 (d, J = 8.6 Hz, 1H, H_{Ar}), 4.08 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2 (C═O), 167.4 (C═O), 149.2 ($C_{q,Ar}$), 138.8 ($C_{q,Ar}$), 137.2 ($C_{q,Ar}$), 132.0 (CH_{Ar}), 127.1 ($_{CH}Ar$), 122.4 (CH_{Ar}), 39.8 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_9H_6NO_6^-$[M−H]$^-$ 224.0201, found 224.0201; Mp: 171–172 °C (EtOAc); HPLC t_R 2.2 min.

4.1.1.4. 4-Amino-2-(carboxymethyl)benzoic acid (23). Fifteen grams (66.7 mmol) of starting material **22** and 1.5 g of 10% Pd/C (purchased from Aldrich) were suspended 100 mL of EtOH and subjected to hydrogenation at 6 bar pressure for 3 h at rt. The resulting suspension was mixed with 100 mL of water, refluxed for 10 min, and filtered through Celite 545 (Aldrich) while hot. The filtrate was then evaporated to a minimum amount of solvent and the resulting solid was collected by filtration. The solid was washed with water, dried at rt overnight and weights at 11.1 g (56.9 mmol, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (d, J = 8.5 Hz, 1H, H_{Ar}), 6.49–6.30 (m, 2H, H_{Ar}), 5.79 (br s, 2H), 3.76 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1 (C═O), 168.4 (C═O), 152.7 ($C_{q,Ar}$), 139.3 ($C_{q,Ar}$), 133.4 (CH_{Ar}), 117.2 (CH_{Ar}), 116.5 ($C_{q,Ar}$), 111.7 (CH_{Ar}), 41.0 (CH_2); HRMS (ESI−) calcd for $C_9H_8NO_4^-$[M−H]$^-$ 194.0459, found 194.0459; Mp: 208–209 °C (water-ethanol); HPLC t_R 2.2 min.

4.1.1.5. 2-(Carboxymethyl)-4-iodobenzoic acid (24). Starting from 8.0 g (41 mmol) of amine **23**, the same procedure as described for the preparation of compound **16** gave 7.29 g (23.8 mmol, 58%) of yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (br s, 2H, 2 × COOH), 7.76–7.73 (m, 2H, 2 × H_{Ar}), 7.63–7.61 (m, 1H, H_{Ar}), 3.89 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6 (C═O), 168.3 (C═O), 141.2 (CH_{Ar}), 139.2 ($C_{q,Ar}$), 136.4 (CH_{Ar}), 132.5 (CH_{Ar}), 130.6 ($C_{q,Ar}$), 100.1 ($C_{q,Ar}$), 40.0 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI+) calcd for $C_9H_6IO_4^-$[M−H]$^-$ 304.9316, found 304.9321; Mp: 198–199 °C (EtOAc-hexnaes); HPLC t_R 2.4 min.

General procedure for Suzuki coupling: 450 mg (1.47 mmol) of iodo derivative **16** or **24**, boronic acid (2.4 mmol, 1.6 equiv), 40 mg (0.035 mmol) of $Pd(PPh_3)_4$ and 750 mg (5.4 mmol) of K_2CO_3 were mixed with 3 mL of aq EtOH (v/v = 1:1) and subjected to microwave reactor at 150 °C for 30 min. The resulting mixture was then evaporated to remove EtOH. The residue as an aqueous solution was filtered through glass wool while hot. The glass wool was washed several times with boiling aq K_2CO_3 (10% w/v). Combined filtrates were acidified (to pH 1) with 2 N aq HCl to give precipitate, which was collected by filtration, washed with water and dried overnight at rt.

4.1.1.6. 2-(Carboxymethyl)-5-(furan-2-yl)benzoic acid (17a). Yield 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.64 (br s, 2H, 2 × COOH), 8.17 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.79 (dd, J = 8.0, 2.0 Hz, 1H, H_{Ar}), 7.75 (dd, J = 1.8, 0.7 Hz, 1H, H_{furan}), 7.36 (d, J = 8.0 Hz, 1H, H_{Ar}), 6.99 (dd, J = 3.4, 0.7 Hz, 1H, H_{furan}), 6.59 (dd, J = 3.4, 1.8 Hz, 1H, H_{furan}), 3.93 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C═O), 168.5 (C═O), 152.5 ($C_{q,Ar}$), 143.7 (CH_{furan}), 135.9 ($C_{q,Ar}$), 133.5 (CH_{Ar}), 131.8 ($C_{q,Ar}$), 129.6 ($C_{q,Ar}$), 126.8 (CH_{Ar}), 125.5 (CH_{Ar}), 112.6 (CH_{Ar}), 106.8 (CH_{Ar}), 40.2 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{13}H_9O_5^-$[M−H]$^-$ 245.0455, found 245.0457; Mp: 216–218 °C; HPLC t_R 2.7 min.

4.1.1.7. 2-(Carboxymethyl)-5-(thiophen-2-yl)benzoic acid (17b). Quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.66 (br s, 2H, 2 × COOH), 8.08 (d, J = 2.2 Hz, 1H, H_{Ar}), 7.78 (dd, J = 8.0, 2.2 Hz, 1H, H_{Ar}), 7.55 (ddd, J = 4.8, 4.3, 1.2 Hz, 2H, 2 × $H_{thiophene}$), 7.36 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.14 (dd, J = 5.1, 3.6 Hz, 1H, $H_{thiophene}$), 3.92 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C═O), 168.4 (C═O), 142.6 ($C_{q,Ar}$), 136.1 ($C_{q,Ar}$), 133.7 (CH_{Ar}), 133.0 ($C_{q,Ar}$), 132.0 ($C_{q,Ar}$), 129.1 ($CH_{thiophene}$), 128.8 (CH_{Ar}), 127.5 (CH_{Ar}), 126.6 ($CH_{thiophene}$), 124.6 ($CH_{thiophene}$), 40.1 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{13}H_9O_4S^-$[M−H]$^-$ 261.0227, found 261.0235; Mp: 227–228 °C; HPLC t_R 3.2 min.

4.1.1.8. 4-(Carboxymethyl)-3',4'-difluorobiphenyl-3-carboxylic acid (17c). Yield 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (br s, 2H, 2 × COOH), 8.10 (d, J = 2.2 Hz, 1H, H_{Ar}), 7.82–7.74 (m, 2H, H_{Ar} and $H_{difluorophenyl}$), 7.54–7.49 (m, 2H, 2 × $H_{difluorophenyl}$), 7.41 (d, J = 8.0 Hz, 1H, H_{Ar}), 3.95 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C═O), 168.6 (C═O), 150.2 (dd, J = 245.4, 12.8 Hz, CF), 149.7 (dd, J = 246.5, 12.7 Hz, CF), 137.2 ($C_{q,Ar}$), 136.6 ($C_{q,Ar}$), 133.5 (CH_{Ar}), 131.9 ($C_{q,Ar}$), 130.3 (CH_{Ar}), 129.2 (d, J = 11.8 Hz, $C_{q,Ar}$), 128.9 (CH_{Ar}), 123.9 (dd, J = 6.5, 3.2 Hz, $CH_{difluorophenyl}$), 118.4 (d, J = 17.1 Hz, $CH_{difluorophenyl}$), 116.2 (d, J = 17.8 Hz, $CH_{difluorophenyl}$), 40.0 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −131.5 (m), −133.8 (m); HRMS (ESI−) calcd for $C_{15}H_9F_2O_4^-$[M−H]$^-$ 291.0474, found 291.0506; Mp: 192–195 °C; HPLC t_R 3.4 min.

4.1.1.9. 4-(Carboxymethyl)-3'-chloro-4'-fluorobiphenyl-3-carboxylic acid (17d). Quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (br s, 2H, 2 × COOH), 8.09 (d, J = 2.1 Hz, 1H, H_{Ar}), 7.89 (dd, J = 7.1, 2.3 Hz, 1H, $H_{chlorofluorophenyl}$), 7.79 (dd, J = 8.0, 2.1 Hz, 1H, H_{Ar}), 7.68 (ddd, J = 8.6, 4.6, 2.3 Hz, 1H, $H_{chlorofluorophenyl}$), 7.53–7.45 (m, 1H, $H_{chlorofluorophenyl}$), 7.41 (d, J = 8.0 Hz, 1H, H_{Ar}), 3.95 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C═O), 168.6 (C═O), 157.4 (d, J = 247.3 Hz, CF), 137.4 (d, J = 3.7 Hz, $C_{q,Ar}$), 137.0 ($C_{q,Ar}$), 136.6 ($C_{q,Ar}$), 133.5 (CH_{Ar}), 132.1 ($C_{q,Ar}$), 130.3 (CH_{Ar}), 129.1 ($CH_{chlorofluorophenyl}$), 129.0 (CH_{Ar}), 127.8 (d, J = 7.5 Hz, $CH_{chlorofluorophenyl}$), 120.6 (d, J = 17.8 Hz, $C_{q,Ar}$), 117.8 (d, J = 21.0 Hz, $CH_{chlorofluorophenyl}$), 40.1 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.7 (m); HRMS (ESI−) calcd for $C_{15}H_9ClFO_4^-$[M−H]$^-$ 307.0179, found 307.0206; Mp: 186–188 °C; HPLC t_R 5.5 min.

4.1.1.10. 4-(Carboxymethyl)-3'-methoxybiphenyl-3-carboxylic acid (17e). Quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (br s, 2H, 2 × COOH), 8.10 (d, J = 2.1 Hz, 1H, H_{Ar}), 7.78 (dd, J = 7.9, 2.1 Hz, 1H, H_{Ar}), 7.43–7.34 (m, 2H, H_{Ar} and $H_{methoxyphenyl}$), 7.25–7.19 (m, 1H, $H_{methoxyphenyl}$), 7.20–7.15 (m, 1H, $H_{methoxyphenyl}$), 6.95 (ddd, J = 8.2, 2.5, 0.8 Hz, 1H, $H_{methoxyphenyl}$), 3.95 (s, 2H, CH_2), 3.81 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 168.7 (C=O), 160.3 ($C_{q,Ar}$), 141.1 ($C_{q,Ar}$), 139.2 ($C_{q,Ar}$), 136.2 ($C_{q,Ar}$), 133.4 (CH_{Ar}), 130.6 ($CH_{methoxyphenyl}$), 130.4 (CH_{Ar}), 128.9 (CH_{Ar}), 119.4 ($CH_{methoxyphenyl}$), 113.8 ($CH_{methoxyphenyl}$), 112.6 ($CH_{methoxyphenyl}$), 55.6 (OMe), 40.1 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{16}H_{13}O_5^-$[M−H]$^-$ 285.0786, found 285.0795; Mp: 167–170 °C; HPLC t_R 3.5 min.

4.1.1.11. 5-(Benzofuran-2-yl)-2-(carboxymethyl)benzoic acid (17f). Yield 87%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (br s, 2H, 2 × COOH), 8.39 (d, J = 2.0 Hz, 1H, H_{Ar}), 8.03 (dd, J = 8.0, 2.0 Hz, 1H, H_{Ar}), 7.68–7.62 (m, 2H, 2 × $H_{benzofuran}$), 7.50 (d, J = 0.9 Hz, 1H, $H_{benzofuran}$), 7.46 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.35–7.29 (m, 1H, $H_{benzofuran}$), 7.29–7.23 (m, 1H, $H_{benzofuran}$), 3.98 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7 (C=O), 168.3 (C=O), 154.7 ($C_{q,Ar}$), 154.6 ($C_{q,Ar}$), 137.5 ($C_{q,Ar}$), 133.7 (CH_{Ar}), 131.9 ($C_{q,Ar}$), 129.2 ($C_{q,Ar}$), 129.0 ($C_{q,Ar}$), 128.2 (CH_{Ar}), 126.7 (CH_{Ar}), 125.3 ($CH_{benzofuran}$), 123.8 ($CH_{benzofuran}$), 121.7 ($CH_{benzofuran}$), 111.7 ($CH_{benzofuran}$), 103.0 ($CH_{benzofuran}$), 40.2 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{17}H_{11}O_5^-$[M−H]$^-$ 295.0612, found 295.0623; Mp: 237–240 °C; HPLC t_R 5.4 min.

4.1.1.12. 2-(Carboxymethyl)-4-(furan-2-yl)benzoic acid (25a). Yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br s, 2H, 2 × COOH), 7.93 (d, J = 8.8 Hz, 1H, H_{Ar}), 7.80 (dd, J = 1.8, 0.7 Hz, 1H, H_{furan}), 7.67 (m, 2H, 2 × H_{Ar}), 7.07 (dd, J = 3.4, 0.7 Hz, 1H, H_{furan}), 6.63 (dd, J = 3.4, 1.8 Hz, 1H, H_{furan}), 3.97 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C=O), 168.2 (C=O), 152.4 ($C_{q,Ar}$), 144.4 (CH_{furan}), 138.0 ($C_{q,Ar}$), 133.5 ($C_{q,Ar}$), 131.8 (CH_{Ar}), 129.4 ($C_{q,Ar}$), 127.5 (CH_{Ar}), 122.1 (CH_{Ar}), 112.9 (CH_{furan}), 108.4 (CH_{furan}), 40.5 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{13}H_9O_5^-$[M−H]$^-$ 245.0455, found 245.0458; Mp: 202–204 °C; HPLC t_R 2.8 min.

4.1.1.13. 2-(Carboxymethyl)-4-(5-methylfuran-2-yl)benzoic acid (25b). Yield 80%. ^{1}H NMR (600 MHz, CD_3OD-d_4) δ 8.02 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.60 (d, J = 8.4, 1.2 Hz, 1H, H_{Ar}), 7.55 (s, 1H_{Ar}), 6.77 (d, J = 3.6 Hz, 1H_{furan}), 6.10 (d, J = 2.4 Hz, H_{furan}), 4.03 (s, 2H, CH_2), 2.36 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.3 (C=O), 169.2 (C=O), 153.1 (CH_{furan}), 150.9 ($C_{q,Ar}$), 136.7 ($C_{q,Ar}$), 134.4 ($C_{q,Ar}$), 131.6 ($C_{q,Ar}$), 128.2 (CH_{Ar}), 126.3, (CH_{Ar}), 121.2 (CH_{Ar}), 108.2 (CH_{furan}), 107.9 (CH_{furan}), 40.4 (CH_2), 12.4 (CH_3); MS (ESI−) [M−H]$^-$ 259.25.

4.1.1.14. 2-(Carboxymethyl)-4-(furan-3-yl)benzoic acid (25c). Yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (br s, 2H, 2 × COOH), 8.27 (dd, J = 1.5, 0.9 Hz, 1H, H_{furan}), 7.93–7.88 (m, 1H, H_{Ar}), 7.77–7.74 (m, 1H, H_{furan}), 7.60 (m, 2H, 2 × H_{Ar}), 7.00 (dd, J = 1.9, 0.9 Hz, 1H, H_{furan}), 3.95 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 168.4 (C=O), 145.1 (CH_{furan}), 141.0 (CH_{furan}), 137.9 ($C_{q,Ar}$), 135.8 ($C_{q,Ar}$), 131.7 (CH_{Ar}), 129.8 (CH_{Ar}), 129.1 ($C_{q,Ar}$), 125.3 ($C_{q,Ar}$), 124.3 (CH_{Ar}), 109.0 (CH_{furan}), 40.6 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{13}H_9O_5^-$[M−H]$^-$ 245.0455, found 245.0462; Mp: 208–209 °C; HPLC t_R 2.8 min.

4.1.1.15. 2-(Carboxymethyl)-4-(dibenzo[*b*,*d*]furan-4-yl)benzoic acid (25d). Quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (br s, 2H, 2 × COOH), 8.21–8.15 (m, 2H, 2 × $H_{dibenzofuran}$), 8.05 (d, J = 8.1 Hz, 1H, H_{Ar}), 7.93 (dd, J = 8.1, 1.9 Hz, 1H, H_{Ar}), 7.84 (d, J = 1.9 Hz, 1H, H_{Ar}), 7.75–7.70 (m, 2H, 2 × $H_{dibenzofuran}$), 7.52 (ddd, J = 15.4, 8.5, 4.5 Hz, 2H, 2 × $H_{dibenzofuran}$), 7.44–7.38 (m, 1H, $H_{dibenzofuran}$), 4.05 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 168.6 (C=O), 155.9 ($C_{q,Ar}$), 153.0 ($C_{q,Ar}$), 139.2 ($C_{q,Ar}$), 137.5 ($C_{q,Ar}$), 132.5 (CH_{Ar}), 131.4 (CH_{Ar}), 128.3 ($CH_{dibenzofuran}$), 127.5 (2C, $CH_{dibenzofuran}$ and CH_{Ar}), 125.0 ($C_{q,Ar}$), 124.2 ($CH_{dibenzofuran}$), 124.2 ($C_{q,Ar}$), 123.8 ($CH_{dibenzofuran}$), 123.8 ($C_{q,Ar}$), 121.7 ($CH_{dibenzofuran}$), 121.6 ($C_{q,Ar}$), 121.6 ($CH_{dibenzofuran}$), 112.3 ($CH_{dibenzofuran}$), 40.8 (CH_2); HRMS (ESI−) calcd for $C_{21}H_{13}O_5^-$[M−H]$^-$ 345.0768, found 345.0778; Mp: 207–208 °C; HPLC t_R 6.9 min.

4.1.1.16. 4-(Benzofuran-2-yl)-2-(carboxymethyl)benzoic acid (25e). Yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.88 (br s, 2H, 2 × COOH), 7.90 (d, J = 8.1 Hz, 1H, H_{Ar}), 7.86–7.81 (m, 2H, 2 × H_{Ar}), 7.69–7.61 (m, 2H, 2 × $H_{benzofuran}$), 7.52 (d, J = 0.9 Hz, 1H, $H_{benzofuran}$), 7.37–7.30 (m, 1H, $H_{benzofuran}$), 7.30–7.23 (m, 1H, $H_{benzofuran}$), 3.89 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 169.2 (C=O), 154.9 ($C_{q,Ar}$), 154.6 ($C_{q,Ar}$), 136.9 ($C_{q,Ar}$), 132.1 ($C_{q,Ar}$), 131.7 (CH_{Ar}), 129.2 ($C_{q,Ar}$), 129.1 ($C_{q,Ar}$), 127.8 (CH_{Ar}), 125.5 ($CH_{benzofuran}$), 123.8 ($CH_{benzofuran}$), 123.3 (CH_{Ar}), 121.9 ($CH_{benzofuran}$), 111.7 ($CH_{benzofuran}$), 104.0 ($CH_{benzofuran}$), 41.9 (CH_2); HRMS (ESI−) calcd for C − 17$H_{11}O_5^-$[M−H]$^-$ 295.0612, found 295.0622; Mp: 242–243 °C; HPLC t_R 5.4 min.

4.1.1.17. 2-(Carboxymethyl)-4-(thiophen-2-yl)benzoic acid (25f). Yield 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (br s, 2H, 2 × COOH), 7.92 (d, J = 8.7 Hz, 1H, H_{Ar}), 7.66–7.62 (m, 3H, H_{Ar}, 2 × $H_{thiophene}$), 7.61 (s, 1H, H_{Ar}), 7.16 (dd, J = 4.8, 3.9 Hz, 1H, $H_{thiophene}$), 3.98 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (C=O), 168.2 (C=O), 142.4 ($C_{q,Ar}$), 138.2 ($C_{q,Ar}$), 137.2 ($C_{q,Ar}$), 132.0 (CH_{Ar}), 129.6 ($C_{q,Ar}$), 129.5 ($CH_{thiophene}$), 129.2 ($CH_{thiophene}$), 127.6 (CH_{Ar}), 125.6 (CH_{Ar}), 124.0 ($CH_{thiophene}$), 40.4 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{13}H_9O_4S^-$[M−H]$^-$ 261.0227, found 261.0238; Mp: 209–210 °C; HPLC t_R 3.3 min.

4.1.1.18. 2-(Carboxymethyl)-4-(thiophen-3-yl)benzoic acid (25g). Yield 83%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (2 × COOH peak is not presented) 7.93–7.91 (m, 1H, H_{Ar}), 7.71–7.73 (m, 1H, H_{Ar}), 7.64–7.62 (m, 1H, H_{Ar}), 7.60–7.58 (m, 1H, H_{Ar}), 7.57–7.55 (m, 2H, H_{Ar}), 3.70 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2 (C=O), 170.5 (C=O), 141.0 ($C_{q,Ar}$), 136.8 ($C_{q,Ar}$), 135.7 (2C, $C_{q,Ar}$), 131.5 (CH_{Ar}), 128.5 (CH_{Ar}), 127.7 (CH_{Ar}), 126.6 (CH_{Ar}), 124.5 (CH_{Ar}), 122.3 (CH_{Ar}), 43.3 (CH_2).

4.1.1.19. 4-(Benzo[b]thiophen-3-yl)-2-(carboxymethyl)benzoic acid (25h). Yield 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (br s, 2H, 2 × COOH), 8.08 (m, 1H, $H_{benzothiophene}$), 8.03 (d, J = 7.9 Hz, 1H, H_{Ar}), 7.95–7.89 (m, 2H, H_{Ar} and $H_{benzothiophene}$), 7.64–7.58 (m, 2H, H_{Ar} and $H_{benzothiophene}$), 7.50–7.39 (m, 2H, 2 × $H_{benzothiophene}$), 4.04 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 168.5 (C=O), 140.6 ($C_{q,Ar}$), 138.9 ($C_{q,Ar}$), 137.8 ($C_{q,Ar}$), 137.3 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 132.6 ($CH_{benzothiophene}$), 131.6 (CH_{Ar}), 130.1 ($C_{q,Ar}$), 127.2 (CH_{Ar}), 126.4 (CH_{Ar}), 125.3 ($CH_{benzothiophene}$), 125.2 ($CH_{benzothiophene}$), 123.8 ($CH_{benzothiophene}$), 122.8 ($CH_{benzothiophene}$), 40.0 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{17}H_{11}O_4S^-$[M−H]$^-$ 311.0384, found 311.0403; Mp: 197–198 °C; HPLC t_R 5.8 min.

4.1.1.20. 2-(Carboxymethyl)-4-(dibenzo[*b*,*d*]thiophen-4-yl)benzoic acid (25i). Yield 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (br s, 2H, 2 × COOH), 8.45–8.35 (m, 2H, 2 × $H_{dibenzothiophene}$), 8.07 (d, J = 8.0 Hz, 1H, H_{Ar}), 8.04–7.97 (m, 1H, $H_{dibenzothiophene}$), 7.76 (dd, J = 8.0, 1.8 Hz, 1H, H_{Ar}), 7.71 (d, J = 1.8 Hz, 1H, H_{Ar}), 7.68–7.56 (m, 2H, 2 × $H_{dibenzothiophene}$), 7.56–7.48 (m, 2H, 2 × $H_{dibenzothiophene}$), 4.05 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ

172.8 (C=O), 168.4 (C=O), 143.4 ($C_{q,Ar}$), 138.8 ($C_{q,Ar}$), 137.9 ($C_{q,Ar}$), 137.7 ($C_{q,Ar}$), 136.4 ($C_{q,Ar}$), 135.6 ($C_{q,Ar}$), 135.5 ($C_{q,Ar}$), 132.3 (CH_{Ar}), 131.6 (CH_{Ar}), 130.9 ($C_{q,Ar}$), 127.8 ($CH_{dibenzothiophene}$), 127.6 ($CH_{dibenzothiophene}$), 126.8 (CH_{Ar}), 126.1 ($CH_{dibenzothiophene}$), 125.4 ($CH_{dibenzothiophene}$), 123.3 ($CH_{dibenzothiophene}$), 122.7 ($CH_{dibenzothiophene}$), 122.2 ($CH_{dibenzothiophene}$), 40.5 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{21}H_{13}O_4S^-$[M−H]$^-$ 361.0540, found 361.0531; Mp: 187–188 °C; HPLC t_R 7.5 min.

4.1.1.21. 4-(Benzo[*d*][1,3]dioxol-5-yl)-2-(carboxymethyl)benzoic acid (25j). Yield 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (br s, 2H, 2 × COOH), 7.92 (d, J = 8.8 Hz, 1H, H_{Ar}), 7.60 (m, 2H, 2 × H_{Ar}), 7.30 (d, J = 1.8 Hz, 1H, $H_{methylenedioxyphenyl}$), 7.21 (dd, J = 8.1, 1.8 Hz, 1H, $H_{methylenedioxyphenyl}$), 7.01 (d, J = 8.1 Hz, 1H, $H_{methylenedioxyphenyl}$), 6.06 (s, 2H, CH_2-methylenedioxyphenyl), 3.98 (s, 2H, CH_2-hydroximide); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 168.5 (C=O), 148.5 ($C_{q,Ar}$), 147.9 ($C_{q,Ar}$), 143.3 ($C_{q,Ar}$), 137.8 ($C_{q,Ar}$), 133.4 ($C_{q,Ar}$), 131.6 (CH_{Ar}), 130.7 (CH_{Ar}), 129.3 ($C_{q,Ar}$), 125.1 (CH_{Ar}), 121.1 ($CH_{methylenedioxyphenyl}$), 109.2 ($CH_{methylenedioxyphenyl}$), 107.6 ($CH_{methylenedioxyphenyl}$), 101.8 ($CH_2$-methylenedioxyphenyl), 40.6 (CH_2, overlapped with CH_3-DMSO); HRMS (ESI−) calcd for $C_{16}H_{11}O_6^-$[M−H]$^-$ 299.0561, found 299.0577; Mp: 229–230 °C; HPLC t_R 3.4 min.

4.1.1.22. 3-(Carboxymethyl)-4′-(trifluoromethyl)biphenyl-4-carboxylic acid (25k). Yield 81%. ^{1}H NMR (600 MHz, CD_3OD-d_6) δ 8.03 (d, J = 7.8 Hz, 1H, H_{Ar}), 7.72 (d, J = 7.2 Hz, 2H$_{Ar}$), 7.63(d, J = 7.8 Hz, 1H, H_{Ar}), 7.54 (d, J = 7.2 Hz, 1H, H_{Ar}), 7.49 (s, 1H, H_{Ar}), 4.00 (s, 2H, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.1 (C=O), 168.7 (C=O), 143.3 ($C_{q,Ar}$), 142.9 (CH_{Ar}), 137.4 ($C_{q,Ar}$), 131.6 ($C_{q,Ar}$), 130.9 (CH_{Ar}), 129.9 (CH_{Ar}), 127.4 (CH_{Ar}), 125.5 (CH_{Ar}), 125.4 (CH_{Ar}), 125.3 ($C_{q,Ar}$), 40.0 (CH_2); MS (ESI−) [M−H]$^-$ 323.25.

4.1.1.23. 3-(Carboxymethyl)-4′-fluorobiphenyl-4-carboxylic acid1 (25l). Yield 78%. ^{1}H NMR (600 MHz, CD_3OD-d_4) δ 8.03 (d, J = 7.8 Hz, 1H, H_{Ar}), 7.62 (td, J = 8.4, 3.0 Hz, 2H, 2 × H_{Ar}), 7.53 (d, J = 7.8 Hz, 1H, H_{yl}), 7.47 (s, 1H, H_{Ar}), 7.13 (t, J = 8.4 Hz, 2H, 2 × H_{Ar}), 4.03 (s, 2H, CH_2); ^{13}C NMR (150 MHz,CD_3OD) δ 174.1 (C=O), 168.7 (C=O), 163.7 (CF), 162.1 (CH_{Ar}), 143.7 (CH_{Ar}), 137.3 ($C_{q,Ar}$), 135.9 ($C_{q,Ar}$), 131.5 ($C_{q,Ar}$), 130.5 (CH_{Ar}), 128.7 (d, J = 8.0 Hz, $C_{q,Ar}$), 125.1 (CH_{Ar}), 115.4(d, J = 6.8 Hz, CH_{Ar}), 40.2 (CH_2); MS (ESI−) [M−H]$^-$ 273.24.

4.1.1.24. 3-(Carboxymethyl)-3′,4′-difluorobiphenyl-4-carboxylic acid (25m). Yield 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.58 (br s, 2H, 2 × COOH), 7.88 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.81 (ddd, J = 12.3, 7.8, 2.1 Hz, 1H, $H_{difluorophenyl}$), 7.67–7.60 (m, 2H, 2 × H_{Ar}), 7.60–7.48 (m, 2H, 2 × $H_{difluorophenyl}$), 3.89 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9 (C=O), 169.1 (C=O), 150.2 (dd, J = 245.3, 12.7 Hz, CF), 149.8 (dd, J = 245.4, 14.3 Hz, CF), 140.6 ($C_{q,Ar}$), 137.4–136.6 (m, 3C, 3 × $C_{q,Ar}$), 131.6 (CH_{Ar}), 130.4 (CH_{Ar}), 125.4 (CH_{Ar}), 124.1 (dd, J = 6.6, 3.2 Hz, $CH_{difluorophenyl}$), 118.5 (d, J = 17.1 Hz, $CH_{difluorophenyl}$), 116.4 (d, J = 17.9 Hz, $CH_{difluorophenyl}$), 41.5 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −131.55(m), −133.34(m); HRMS (ESI−) calcd for $C_{15}H_9F_2O_4^-$[M−H]$^-$ 291.0474, found 291.0499; Mp: 188–190 °C; HPLC t_R 3.5 min.

4.1.1.25. 3-(Carboxymethyl)-3′-(phenyl)biphenyl-4-carboxylic acid (25n). Yield 82%. ^{1}H NMR (600 MHz, CD_3OD-d_4) δ 8.15 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.84 (s, 1H), 7.66 (m, 3H, H_{Ar}), 7.62 (d, J = 7.8 Hz, 2H), 7.58 (s, 1H), 7.53 (t, J = 7.8 Hz, 1H, H_{Ar}), 7.46 (t, J = 7.8 Hz, 2H$_{Ar}$), 7.37 (t, J = 7.2 Hz, 1H$_{Ar}$), 4.12 (s, 2H, CH_2); ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.2 (C=O), 169.0 (C=O), 144.9 ($C_{q,Ar}$), 141.9 ($C_{q,Ar}$), 140.8 ($C_{q,Ar}$), 140.2 ($C_{q,Ar}$), 137.1 ($C_{q,Ar}$), 131.7 ($C_{q,Ar}$), 130.9 (CH_{Ar}), 129.2 (CH_{Ar}), 128.9 (CH_{Ar}), 128.6 (CH_{Ar}), 127.3 (CH_{Ar}), 126.9 (CH_{Ar}), 126.6 (CH_{Ar}), 125.9 (CH_{Ar}), 125.7 (CH_{Ar}), 125.6 (CH_{Ar}), 40.3 (CH_2); MS (ESI−) [M−H]$^-$ 331.24.

4.1.1.26. 3-(Carboxymethyl)-4′-(methylsulfonyl)biphenyl-4-carboxylic acid (25o). Yield 80%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.09 (d, J = 6.6 Hz, 2H$_{Ar}$), 8.05 (d, J = 7.2 Hz, 2H$_{Ar}$), 7.99 (d, J = 7.2 Hz, 1H$_{Ar}$), 7.78 (d, J = 7.2 Hz, 1H, H_{Ar}), 7.77(s, 1H), 3.97 (s, 2H, CH_2); MS (ESI−) [M−H]$^-$ 333.34.

General procedure for cyclization and deprotection: 0.8 mmol of diacid, 1.7 mmol (2.1 equiv) of $THPONH_2$ and 3 mL of PhCl were refluxed in a 130 °C oil bath for several minutes with vigorous stirring. To the mixture, a solution of CDI in DCM (3 mL, 0.4 M, 1.2 mmol) was added dropwise. The suspension turned immediately to clear and the reflux was kept for another 30 s. The resulting solution was evaporated to dryness, re-dissolved in DCM and loaded to a silica gel plug, which was then rinsed with diethyl ether for several times. The filtrates were combined and evaporated to dryness. The residue could be purified by silica gel chromatography with EtOAc and hexanes. Otherwise it was directly stirred with 50 mg of *p*-TSA hydrate and 3 mL MeOH. Upon disappearance of starting material by TLC, the mixture was again evaporated to dryness under vacuum below 40 °C. The residue was triturated with DCM and water to give the precipitate, which was collected by filtration and washed with DCM and water several times, and dried at rt overnight.

4.1.1.27. 7-(Furan-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (19a). Yield 20% (for two steps from **17a**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H, –NOH), 8.25 (d, J = 1.9 Hz, 1H, H_{Ar}), 7.95 (dd, J = 8.2, 1.9 Hz, 1H, H_{Ar}), 7.78 (dd, J = 1.7, 0.5 Hz, 1H, H_{furan}), 7.42 (d, J = 8.2 Hz, 1H, H_{Ar}), 7.08 (dd, J = 3.3, 0.5 Hz, 1H, H_{furan}), 6.61 (dd, J = 3.3, 1.7 Hz, 1H, H_{furan}), 4.24 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 162.0 (C=O), 152.1 ($C_{q,Ar}$), 143.9 (CH_{furan}), 134.0 ($C_{q,Ar}$), 129.9 ($C_{q,Ar}$), 128.9 (CH_{Ar}), 128.6 (CH_{Ar}), 126.1 ($C_{q,Ar}$), 122.2 (CH_{Ar}), 112.7 (CH_{furan}), 107.3 (CH_{furan}), 37.2 (CH_2); HRMS (ESI−) calcd for $C_{13}H_8NO_4^-$[M−H]$^-$ 242.0459, found 242.0466; Mp: 203–208 °C (decomp.); HPLC t_R 9.9 min.

4.1.1.28. 2-Hydroxy-7-(thiophen-2-yl)isoquinoline-1,3(2*H*,4*H*)-dione (19b). Yield 21% (for two steps from **17b**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H, –NOH), 8.16 (d, J = 1.9 Hz, 1H, H_{Ar}), 7.94 (dd, J = 8.0, 1.9 Hz, 1H, H_{Ar}), 7.60 (dd, J = 7.3, 4.3 Hz, 2H, 2 × $H_{thiopene}$), 7.42 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.15 (dd, J = 5.0, 3.7 Hz, 1H, $H_{thiopene}$), 4.25 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 162.0 (C=O), 142.2 ($C_{q,Ar}$), 134.2 ($C_{q,Ar}$), 133.3 ($C_{q,Ar}$), 130.6 (CH_{Ar}), 129.2 ($CH_{thiophene}$), 129.1 (CH_{Ar}), 126.9 ($CH_{thiophene}$), 126.2 ($C_{q,Ar}$), 125.0 ($CH_{thiophene}$), 124.1 (CH_{Ar}), 37.2 (CH_2); HRMS (ESI−) calcd for $C_{13}H_8NO_3S^-$[M−H]$^-$ 258.0230, found 258.0230; Mp: 211–219 °C (decomp.); HPLC t_R 10.0 min.

4.1.1.29. 7-(3,4-Difluorophenyl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (19c). Yield 22% (for two steps from **17c**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H, –NOH), 8.22 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.96 (dd, J = 8.0, 2.1 Hz, 1H, H_{Ar}), 7.85 (m, 1H, $H_{difluorophenyl}$), 7.54 (m, 3H, H_{Ar} and 2 × $H_{difluorophenyl}$), 4.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 162.0 (C=O), 150.3 (dd, J = 245.7, 12.6 Hz, CF), 149.8 (dd, J = 246.8, 12.5 Hz, CF), 137.5 ($C_{q,Ar}$), 136.8 (dd, J = 6.2, 3.7 Hz, 1C, $C_{q,Ar}$), 134.8 ($C_{q,Ar}$), 132.1 (CH_{Ar}), 129.0 (CH_{Ar}), 126.1 ($C_{q,Ar}$), 125.9 (CH_{Ar}), 124.1 (dd, J = 6.6, 3.3 Hz, 1C, $CH_{difluorophenyl}$), 118.5 (d, J = 17.2 Hz, 1C, $CH_{difluorophenyl}$), 116.4 (d, J = 18.1 Hz, 1C, $CH_{difluorophenyl}$), 37.2 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −131.42(m), −133.48(m); HRMS (ESI−) calcd for $C_{15}H_8F_2NO_3^-$[M−H]$^-$ 288.0478, found 288.0497; Mp: 196–198 °C; HPLC t_R 11.7 min.

4.1.1.30. 7-(3-Chloro-4-fluorophenyl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (19d). Yield 21% (for two steps from **17d**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H, –NOH), 8.21 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.99–7.91 (m, 2H, H_{Ar} and $H_{chlorofluorophenyl}$), 7.73 (m, 1H, $H_{chlorofluorophenyl}$), 7.49 (m, 2H, H_{Ar} and $H_{chlorofluorophenyl}$), 4.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6 (C=O), 162.0 (C=O), 157.6 (d, J = 247.7 Hz, CF), 137.3 ($C_{q,Ar}$), 137.01 (d, J = 3.7 Hz, $C_{q,Ar}$), 134.8 ($C_{q,Ar}$), 132.2 (CH_{Ar}), 129.2 (CH_{Ar}), 129.0 ($CH_{chlorofluorophenyl}$), 127.9 (d, J = 7.5 Hz, $CH_{chlorofluorophenyl}$), 126.1 ($C_{q,Ar}$), 125.9 (CH_{Ar}), 120.7 (d, J = 17.8 Hz, $C_{q,Ar}$), 117.9 (d, J = 21.1 Hz, $CH_{chlorofluorophenyl}$), 37.2 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.34(m); HRMS (ESI−) calcd for $C_{15}H_8ClFNO_3^-$[M−H]$^-$ 304.0182, found 304.0191; Mp: 220–224 °C (decomp.); HPLC t_R 12.0 min.

4.1.1.31. 2-Hydroxy-7-(3-methoxyphenyl)isoquinoline-1,3(2*H*,4*H*)-dione (19e). Yield 12% (for two steps from **17e**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (br s, 1H, –NOH), 8.21 (d, J = 2.0 Hz, 1H, H_{Ar}), 7.96 (dd, J = 8.0, 2.0 Hz, 1H, H_{Ar}), 7.47 (d, J = 8.0 Hz, 1H, H_{Ar}), 7.44–7.37 (m, 1H, $H_{methoxyphenyl}$), 7.31–7.24 (m, 1H, $H_{methoxyphenyl}$), 7.23–7.19 (m, 1H, $H_{methoxyphenyl}$), 6.97 (ddd, J = 8.2, 2.5, 0.9 Hz, 1H, $H_{methoxyphenyl}$), 4.28 (s, 2H, CH_2), 3.82 (s, 3H, OMe); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 162.1 (C=O), 160.3 ($C_{q,Ar}$), 140.7 ($C_{q,Ar}$), 139.6 ($C_{q,Ar}$), 134.4 ($C_{q,Ar}$), 132.2 (CH_{Ar}), 130.7 ($CH_{methoxyphenyl}$), 128.9 (CH_{Ar}), 126.0 ($C_{q,Ar}$), 125.8 (CH_{Ar}), 119.4 ($CH_{methoxyphenyl}$), 114.1 ($CH_{methoxyphenyl}$), 112.6 ($CH_{methoxyphenyl}$), 55.7 (OMe), 37.2 (CH_2); HRMS (ESI−) calcd for $C_{16}H_{12}NO_4^-$[M−H]$^-$ 282.0772, found 282.0780; Mp: 132–137 °C (decomp.); HPLC t_R 11.0 min.

4.1.1.32. 7-(Benzofuran-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (19f). Yield 6% (for two steps from **17f**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.52 (br s, 1H, –NOH), 8.47 (d, J = 1.8 Hz, 1H, H_{Ar}), 8.19 (dd, J = 8.1, 1.8 Hz, 1H, H_{Ar}), 7.67 (d, J = 8.1 Hz, 2H, 2 × $H_{benzofuran}$), 7.58 (s, 1H, $H_{benzofuran}$), 7.52 (d, J = 8.1 Hz, 1H, H_{Ar}), 7.34 (dd, J = 11.2, 4.4 Hz, 1H, $H_{benzofuran}$), 7.27 (t, J = 7.8 Hz, 1H, $H_{benzofuran}$), 4.30 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6 (C=O), 162.1 (C=O), 154.7 ($C_{q,Ar}$), 154.2 ($C_{q,Ar}$), 135.5 (CH_{Ar}), 129.8 ($C_{q,Ar}$), 129.4 (CH_{Ar}), 129.1 ($C_{q,Ar}$), 129.1 ($C_{q,Ar}$), 126.4 ($C_{q,Ar}$), 125.5 ($CH_{benzofuran}$), 123.9 ($CH_{benzofuran}$), 123.5 (CH_{Ar}), 121.8 ($CH_{benzofuran}$), 111.7 ($CH_{benzofuran}$), 103.5 ($CH_{benzofuran}$), 37.4 (CH_2); HRMS (ESI−) calcd for $C_{17}H_{10}NO_4^-$[M−H]$^-$ 292.0615, found 292.0631; Mp: 241–249 °C (decomp); HPLC t_R 12.0 min.

4.1.1.33. 2-Hydroxy-7-iodoisoquinoline-1,3(2*H*,4*H*)-dione (19g). Yield 11% (for two steps from **16**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (br s, 1H, –NOH), 8.24 (d, J = 1.8 Hz, 1H, H_{Ar}), 7.96 (dd, J = 8.1, 1.8 Hz, 1H, H_{Ar}), 7.18 (d, J = 8.1 Hz, 1H, H_{Ar}), 4.18 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4 (C=O), 161.0 (C=O), 142.0 (CH_{Ar}), 136.0 (CH_{Ar}), 134.8 ($C_{q,Ar}$), 130.3 (CH_{Ar}), 127.5 ($C_{q,Ar}$), 92.9 ($C_{q,Ar}$), 37.2 (CH_2); HRMS (ESI−) calcd for $C_9H_5INO_3^-$[M−H]$^-$ 301.9320, found 301.9317; Mp: 166–174 °C (decomp.); HPLC t_R 9.3 min.

4.1.1.34. 2-((Tetrahydro-2*H*-pyran-2-yl)oxy)-6-(thiophen-2-yl)isoquinoline-1,3(2*H*,4*H*)-dione (26f). Yield 20% from compound **25f**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.77 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.68 (m, 3H, H_{Ar} and 2 × $H_{thiophene}$), 7.19 (dd, J = 5.0, 3.7 Hz, 1H, $H_{thiophene}$), 5.19 (s, 1H, H^2_{THP}), 4.31 (pseudo-s, 3H, H^{6a}_{THP} and CH_2-hydroximide), 3.50 (d, J = 10.9 Hz, 1H, H^{6b}_{THP}), 1.92 (m, 1H, H^{3a}_{THP}), 1.85–1.65 (m, 2H, H^{3b}_{THP} and H^{4a}_{THP}), 1.58 (m, 3H, H^{4b}_{THP} and $H^{5a,b}_{THP}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6 (C=O), 161.8 (C=O), 141.9 ($C_{q,Ar}$), 138.9 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 129.4 ($CH_{thiophene}$), 129.3 (CH_{Ar}), 128.4 ($CH_{thiophene}$), 126.4 (CH_{Ar}), 124.6 (CH_{Ar}), 124.3 ($CH_{thiophene}$), 124.2 ($C_{q,Ar}$), 102.0 (C^2_{THP}), 62.0 (C^6_{THP}), 37.9 (CH_2-hydroximide), 28.0 (C^3_{THP}), 25.0 (C^5_{THP}), 18.0 (C^4_{THP}); HRMS (ESI+) calcd for $C_{18}H_{17}NNaO_4S^+$[M+Na]$^+$ 366.0770, found 366.0781; R_f 0.60 (EtOAc:hexanes = 1:1); Mp: 148–149 °C; HPLC t_R 14.6 min.

4.1.1.35. 6-(Furan-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27a). Yield 22% (for two steps from **25a**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H, –NOH), 8.04 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.85 (d, J = 1.4 Hz, 1H, H_{furan}), 7.78 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.69 (s, 1H, H_{Ar}), 7.17 (d, J = 3.3 Hz, 1H, H_{furan}), 6.66 (dd, J = 3.3, 1.4 Hz, 1H, H_{furan}), 4.29 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 161.9 (C=O), 152.0 ($C_{q,Ar}$), 144.9 (CH_{furan}), 136.0 ($C_{q,Ar}$), 134.8 ($C_{q,Ar}$), 128.9 (CH_{Ar}), 124.0 ($C_{q,Ar}$), 122.6 (CH_{Ar}), 122.3 (CH_{Ar}), 113.0 (CH_{furan}), 109.4 (CH_{furan}), 37.4 (CH_2); HRMS (ESI−) calcd for $C_{13}H_8NO_4^-$[M−H]$^-$ 242.0459, found 242.0466; Mp: 190–193 °C (decomp.); HPLC t_R 10.0 min.

4.1.1.36. 2-Hydroxy-6-(5-methylfuran-2-yl)isoquinoline-1,3(2*H*,4*H*)-dione (27b). Yield 22% (for two steps from **25b**). ^{1}H NMR (600 MHz, DMSO) δ 10.41 (s, 1H, –NOH), 8.00 (d, J = 7.8 Hz, 1H, H_{Ar}), 7.72 (d, J = 7.8 Hz, 1H, H_{furan}), 7.61 (s, 1H, H_{Ar}), 7.04 (d, J = 3.0 Hz, H_{furan}), 6.27 (d, J = 2.4 Hz, 1H, H_{furan}), 4.26 (s, 2H, CH_2), 2.35 (s, 3H, CH_3); HRMS (ESI−) calcd. for $C_{14}H_{11}NO_4$ [M−H]$^-$ 256.0610, found 256.0569; HPLC t_R 13.1 min.

4.1.1.37. 6-(Furan-3-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27c). Yield 14% (for two steps from **25c**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H, –NOH), 8.34 (s, 1H, H_{Ar}), 7.99 (d, J = 8.2 Hz, 1H, H_{furan}), 7.79 (s, 1H, H_{Ar}), 7.72 (d, J = 8.2 Hz, 1H, H_{furan}), 7.63 (s, 1H, H_{furan}), 7.03 (s, 1H, H_{Ar}), 4.24 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 162.0 (C=O), 145.3 (CH_{Ar}), 141.6 (CH_{Ar}), 137.3 ($C_{q,Ar}$), 135.8 ($C_{q,Ar}$), 128.8 (CH_{furan}), 125.2 ($C_{q,Ar}$), 124.8 (CH_{furan}), 124.6 (CH_{Ar}), 123.8 ($C_{q,Ar}$), 109.0 (CH_{furan}), 37.4 (CH_2); HRMS (ESI−) calcd for $C_{13}H_8NO_4^-$[M−H]$^-$ 242.0459, found 242.0456; Mp: 194–198 °C (decomp.); HPLC t_R 9.7 min.

4.1.1.38. 6-(Dibenzo[*b*,*d*]furan-4-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27d). Yield 24% (for two steps from **25d**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H, –NOH), 8.26–8.14 (m, 3H, H_{Ar}, 2 × $H_{dibenzofuran}$), 8.03 (dd, J = 8.2, 1.5 Hz, 1H, H_{Ar}), 7.92 (d, J = 1.5, 1H, H_{Ar}), 7.78–7.70 (m, 2H, 2 × $H_{dibenzofuran}$), 7.59–7.49 (m, 2H, 2 × $H_{dibenzofuran}$), 7.43 (td, J = 7.5, 0.9 Hz, 1H, $H_{dibenzofuran}$), 4.37 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8 (C=O), 162.0 (C=O), 155.9 ($C_{q,Ar}$), 153.0 ($C_{q,Ar}$), 140.9 ($C_{q,Ar}$), 135.6 ($C_{q,Ar}$), 128.6 (CH_{Ar}), 128.4 ($CH_{dibenzofuran}$), 127.9 (CH_{Ar}), 127.9 (CH_{Ar}), 127.6 ($CH_{dibenzofuran}$), 125.1 ($C_{q,Ar}$), 124.9 ($C_{q,Ar}$), 124.3 ($CH_{dibenzofuran}$), 123.9 ($CH_{dibenzofuran}$), 123.9 ($CH_{dibenzofuran}$), 123.7 ($C_{q,Ar}$), 122.0 ($C_{q,Ar}$), 121.8 ($CH_{dibenzofuran}$), 112.3 ($CH_{dibenzofuran}$), 37.6 (CH_2); HRMS (ESI−) calcd for $C_{21}H_{12}NO_4^-$[M−H]$^-$ 342.0772, found 342.0754; Mp: 185–195 °C (decomp.); HPLC t_R 13.6 min.

4.1.1.39. 6-(Benzofuran-2-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27e). Yield 22% (for two steps from **25e**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (br s, 1H, –NOH), 8.10 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.99 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.90 (pseudo-s, 1H, H_{Ar}), 7.72–7.60 (m, 3H, 3 × $H_{benzofuran}$), 7.37 (dd, J = 11.1, 4.2 Hz, 1H, $H_{benzofuran}$), 7.28 (t, J = 7.3 Hz, 1H, $H_{benzofuran}$), 4.32 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6 (C=O), 161.8 (C=O), 155.0 ($C_{q,Ar}$), 154.0 ($C_{q,Ar}$), 136.1 ($C_{q,Ar}$), 134.3 ($C_{q,Ar}$), 129.0 ($C_{q,Ar}$), 128.9 (CH_{Ar}), 126.0 ($CH_{benzofuran}$), 125.2 ($C_{q,Ar}$), 124.0 ($CH_{benzofuran}$), 123.9 (CH_{Ar}), 123.7 (CH_{Ar}), 122.2 ($CH_{benzofuran}$), 111.7 ($CH_{benzofuran}$), 105.3 ($CH_{benzofuran}$), 37.5 (CH_2); HRMS (ESI−) calcd for $C_{17}H_{10}NO_4^-$[M−H]$^-$ 292.0615, found 292.0614; Mp: 262–265 °C (decomp.); HPLC t_R 12.0 min.

4.1.1.40. 2-Hydroxy-6-(thiophen-2-yl)isoquinoline-1,3(2*H*,4*H*)-dione (27f). Yield: 72% (from **26f**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.39 (s, 1H, –NOH), 8.01 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.75 (dd, J = 8.3, 1.6 Hz, 1H, H_{Ar}), 7.67 (m, 3H, H_{Ar}, 2 × $H_{thiophene}$), 7.18 (dd, J = 4.8, 4.0 Hz, 1H, $H_{thiophene}$), 4.27 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 161.8 (C=O), 142.0 ($C_{q,Ar}$), 138.6 ($C_{q,Ar}$), 136.1 ($C_{q,Ar}$), 129.3 ($CH_{thiophene}$), 129.1 (CH_{Ar}), 128.3 ($CH_{thiophene}$), 126.3 (CH_{Ar}), 124.6 (CH_{Ar}), 124.3 ($CH_{thiophene}$), 124.2 ($C_{q,Ar}$), 37.4 (CH_2); HRMS (ESI−) calcd for $C_{13}H_8NO_3S^-$[M−H]$^-$ 258.0230, found 258.0230; Mp: 202–210 °C (decomp.); HPLC t_R 10.6 min.

4.1.1.41. 2-Hydroxy-6-(thiophen-3-yl)isoquinoline-1,3(2*H*,4*H*)-dione (27g). Yield: 6% (from **25g**) ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H, –NOH), 8.07 (m, 1H, H_{Ar}), 8.01 (m, 1H, H_{Ar}), 7.83 (m, 1H, H_{Ar}), 7.75 (m, 1H, H_{Ar}), 7.69 (m, 1H, H_{Ar}), 7.63 (m, 1H, H_{Ar}), 4.27 (s, 2H, CH_2).

4.1.1.42. 6-(Benzo[*b*]thiophen-3-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27h). Yield 16% (for two steps from **25h**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (br s, 1H, –NOH), 8.14 (d, J = 8.1 Hz, 1H, H_{Ar}), 8.11–8.07 (m, 1H, $H_{benzothiophene}$), 7.99 (s, 1H, $H_{benzothiophene}$), 7.98–7.91 (m, 1H, $H_{benzothiophene}$), 7.72 (d, J = 8.1 Hz, 1H, H_{Ar}), 7.65 (s, 1H, H_{Ar}), 7.50–7.41 (m, 2H, 2 × $H_{benzothiophene}$), 4.35 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8 (C=O), 162.0 (C=O), 140.7 ($C_{q,Ar}$), 140.4 ($C_{q,Ar}$), 137.1 ($C_{q,Ar}$), 136.0 ($C_{q,Ar}$), 135.8 ($C_{q,Ar}$), 128.8 (CH_{Ar}), 127.8 ($CH_{benzothiophene}$), 127.7 ($CH_{benzothiophene}$), 127.1 ($CH_{benzothiophene}$), 125.4 ($CH_{benzothiophene}$), 125.3 ($CH_{benzothiophene}$), 124.6 ($C_{q,Ar}$), 123.8 ($CH_{benzothiophene}$), 122.8 ($CH_{benzothiophene}$), 37.5 (CH_2); HRMS (ESI−) calcd for $C_{17}H_{10}NO_3S^-$[M−H]$^-$ 308.0387, found 308.0377; Mp: 210–215 °C; HPLC t_R 12.7 min.

4.1.1.43. 6-(Dibenzo[*b*,*d*]thiophen-4-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27i). Yield 16% (for two steps from **25i**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H, –NOH), 8.43–8.32 (m, 2H, H_{Ar}, $H_{dibenzothiophene}$), 8.14 (d, J = 8.1 Hz, 1H, H_{Ar}), 8.00–7.93 (m, 1H, $H_{dibenzothiophene}$), 7.82 (d, J = 8.2 Hz, 1H, H_{Ar}), 7.70 (s, 1H, $H_{dibenzothiophene}$), 7.61 (t, J = 7.6 Hz, 1H, $H_{dibenzothiophene}$), 7.55 (d, J = 7.1 Hz, 1H, $H_{dibenzothiophene}$), 7.48 (dd, J = 6.0, 3.1 Hz, 2H, 2 × $H_{dibenzothiophene}$), 4.30 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 161.9 (C=O), 144.9 ($C_{q,Ar}$), 138.7 ($C_{q,Ar}$), 137.6 ($C_{q,Ar}$), 136.5 ($C_{q,Ar}$), 136.0 ($C_{q,Ar}$), 135.5 ($C_{q,Ar}$), 135.2 ($C_{q,Ar}$), 128.9 (CH_{Ar}), 127.9 ($CH_{dibenzothiophene}$), 127.7 ($CH_{dibenzothiophene}$), 127.6 ($CH_{dibenzothiophene}$), 127.3 (CH_{Ar}), 126.2 ($CH_{dibenzothiophene}$), 125.4 ($CH_{dibenzothiophene}$), 125.3 ($C_{q,Ar}$), 123.3 ($CH_{dibenzothiophene}$), 122.8 (CH_{Ar}), 122.5 ($CH_{dibenzothiophene}$), 37.5 (CH_2); HRMS (ESI−) calcd for $C_{21}H_{12}NO_3S^-$[M−H]$^-$ 358.0543, found 358.0546; Mp: 190–193 °C (decomp.); HPLC t_R 14.5 min.

4.1.1.44. 6-(Benzo[*d*][1,3]dioxol-5-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27j). Yield 10% (for two steps from **25j**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (br s, 1H, –NOH), 8.02 (d, J = 8.3 Hz, 1H, H_{Ar}), 7.71 (d, J = 8.2 Hz, 1H, H_{Ar}), 7.64 (s, 1H, H_{Ar}), 7.32 (s, 1H, $H_{methylenedioxyphenyl}$), 7.24 (d, J = 8.2 Hz, 1H, $H_{methylenedioxyphenyl}$), 7.03 (d, J = 8.1 Hz, 1H, $H_{methylenedioxyphenyl}$), 6.07 (s, 2H, CH_2-methylenedioxyphenyl), 4.27 (s, 2H, CH_2-hydroximide); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8 (C=O), 162.0 (C=O), 148.6 ($C_{q,Ar}$), 148.3 ($C_{q,Ar}$), 144.8 ($C_{q,Ar}$), 135.7 ($C_{q,Ar}$), 133.0 ($C_{q,Ar}$), 128.7 (CH_{Ar}), 125.7 (2C, 2 × CH_{Ar}), 123.9 ($C_{q,Ar}$), 121.4 ($CH_{methylenedioxyphenyl}$), 109.3 ($CH_{methylenedioxyphenyl}$), 107.6 ($CH_{methylenedioxyphenyl}$), 101.9 ($CH_2$-methylenedioxyphenyl), 37.5 (CH_2-hydroximide); HRMS (ESI−) calcd for $C_{16}H_{10}NO_5^-$[M−H]$^-$ 296.0564, found 296.0556; Mp: 232–235 °C (decomp.); HPLC t_R 10.5 min.

4.1.1.45. 6-(4-Fluorophenyl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27k). Yield 5% (for two steps from **25k**). ^{1}H NMR (600 MHz, CD_3OD-d_4) δ 8.10 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.65 (m, 3H, H_{Ar}), 7.55 (s, 1H), 7.14 (t, J = 8.4 Hz, 2H, H_{Ar}), 4.38 (s, 2H, CH_2); HRMS (ESI−) calcd. for $C_{15}H_{10}FNO_3^-$[M−H]$^-$ 270.0566, found 270.0544; HPLC t_R 11.9 min.

4.1.1.46. 2-Hydroxy-6-(4-(trifluoromethyl)phenyl)isoquinoline-1,3(2*H*,4*H*)-dione (27l). 10% (for two steps from **25l**). ^{1}H NMR (600 MHz, CD_3OD) δ 8.27 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.88 (d, J = 8.4 Hz, 2H, H_{Ar}), 7.81 (m, 3H, H_{Ar}), 7.71 (s, 1H, H_{Ar}), 4.80 (s, 2H, CH_2). HRMS (ESI−) calcd. for $C_{16}H_{10}F_3NO_3^-$[M−H]$^-$ 320.0535, found 320.0524; HPLC t_R 12.2 min.

4.1.1.47. 6-(3,4-Difluorophenyl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27m). Yield 11% (for two steps from **25m**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (br s, 1H, NOH), 8.06 (d, J = 8.2 Hz, 1H, H_{Ar}), 7.91–7.82 (m, 1H, $H_{difluorophenyl}$), 7.80 (d, J = 8.2 Hz, 1H, H_{Ar}), 7.74 (s, 1H, H_{Ar}), 7.58 (m, 2H, 2 × $H_{difluorophenyl}$), 4.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7 (C=O), 161.9 (C=O), 150.3 (dd, J = 246.1, 7.7 Hz, CF), 150.1 (dd, J = 248.2, 6.9 Hz, CF), 142.8 ($C_{q,Ar}$), 136.5 (dd, J = 6.0, 3.9 Hz, $C_{q,Ar}$), 135.8 ($C_{q,Ar}$), 128.8 (CH_{Ar}), 126.3 (CH_{Ar}), 126.1 (CH_{Ar}), 124.9 ($C_{q,Ar}$), 124.4 (dd, J = 6.8, 3.2 Hz, $CH_{difluorophenyl}$), 118.6 (d, J = 17.1 Hz, $CH_{difluorophenyl}$), 116.6 (d, J = 18.1 Hz, $CH_{difluorophenyl}$), 37.5 (CH_2); ^{19}F NMR (376 MHz, DMSO-d_6) δ −131.30(m), −132.47(m); HRMS (ESI−) calcd for $C_{15}H_8F_2NO_3^-$[M−H]$^-$ 288.0478, found 288.0483 Mp:149–156 °C (decomp.); HPLC t_R 11.4 min.

4.1.1.48. 6-(Biphenyl-3-yl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione (27n). 13% (for two steps from **25n**). ^{1}H NMR (600 MHz, CD_3Cl_3) δ 8.31 (d, J = 8.4, 1H, H_{Ar}), 7.81 (s, 1H, H_{Ar}), 7.79 (d, J = 8.4, 1H, H_{Ar}), 7.68 (m, 3H, H_{Ar}), 7.60 (m, 3H, H_{Ar}), 7.50 (t, J = 8.4, 2H, H_{Ar}), 7.41(t, J = 7.2, 1H, H_{Ar}), 4.28 (s, 2H, CH_2); HRMS (ESI−) calcd. for $C_{21}H_{15}NO_3^-$[M−H]$^-$ 328.0974, found 328.0957; HPLC t_R 18.5 min.

4.1.1.49. 2-Hydroxy-6-(3-(methylsulfonyl)phenyl)isoquinoline-1,3(2*H*,4*H*)-dione (27o). 5% (for two steps from **25o**). ^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.21 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.97 (d, J = 8.4 Hz, 2H, H_{Ar}), 7.78 (d, J = 7.8 Hz, 2H, H_{Ar}), 7.67 (d, J = 8.4 Hz, 1H, H_{Ar}), 7.50 (s, 1H, H_{Ar}), 4.17 (s, 2H, CH_2), 3.02 (s, 3H, Me); HRMS (ESI−) calcd. for $C_{16}H_{13}NO_5S^-$[M−H]$^-$ 330.0436, found 330.0425; HPLC t_R 15.2 min.

4.2. Biology

4.2.1. Cells and recombinant protein

HCV genotype 1b Huh-7/HCV1b-Rluc replicon cells (obtained from G. Luo, University of Kentucky) were maintained in DME supplemented with 10% fetal bovine serum (fbs), 500 μgm/mL G418 (Invitrogen), 100 IU streptomycin and 100 IU penicillin, 1× nonessential amino acids (Invitrogen), and 1 mM sodium pyruvate (Invitrogen). The Huh-7/HCV1b-Rluc replicon RNA expresses neomycin phosphotransferase to promote retention in the Huh7 cells by selection with G418. The replicon also expresses renilla luciferase that can be measured to determine the level of replicon RNA production. The HCV genotype 1b NS5B polymerase missing the C-terminal 55 amino acids (NS5BΔ55) was prepared by expressing the plasmid pET-21d(+)-NS5BΔ55 in bacteria as described[47] except the heparin-agarose chromatography was omitted. The enzyme was stored at −80 °C in storage buffer (50 mM Tris pH 7.5, 50% glycerol, 1 mM β-ME).

4.2.2. HCV replicon assay

Replicon cells were plated out at 6 × 10^3 cells per well in a white opaque 96-well tissue culture plate (BD Falcon) in the absence of G418. The next day, the cells were incubated at 37 °C/5%

CO_2 in culture medium containing compound (dissolved in DMSO), DMSO alone, or nothing added. Three days later, the culture medium was removed from the cells and renilla luciferase ViVi-Ren Live Cell Substrate (Promega) was added in a 1:1000 dilution in DME without phenol red and 10% fbs. Luminescence was immediately measured on a SpectraMax E5 spectrometer (Molecular Devices). Any given compound concentration or control was performed in triplicate and each experiment was performed independently at least twice. For single concentration experiments, ribavirin (10 μM) and 2 mA (500 nM) were included as controls. The 50% effective concentration (EC_{50}) was defined as the concentration of compound that reduced luciferase activity by 50%. The EC_{50} was determined by comparing luciferase activity for eight serial dilutions of compound and vehicle treated cells using GraphPad Prism software.

4.2.3. Cell proliferation assay

Replicon cells were plated out at 6×10^3 cells per well in a clear 96-well tissue culture plate (Corning) in the absence of G418. The next day, the cells were incubated at 37 °C/5% CO_2 in culture medium containing compound (dissolved in DMSO), DMSO alone, or nothing added for three days. CellTiter 96 AQ_{ueous} One Solution Cell Proliferation reagent (Promega) was added according to manufacturer's instructions and viability measured by spectrometry at 450 nm with a SpectraMax E5 (Molecular Devices). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of compound that reduced cell proliferation by 50%. The CC_{50} was determined by comparing absorbance readings from eight serial dilutions of compound and vehicle treated cells using GraphPad Prism software.

4.2.4. HCV NS5B assay

Primer-dependent NS5BΔ55 polymerase activity was measured using a scintillation proximity assay (SPA). Purified recombinant NS5BΔ55 (2 nM) was added to Mix 1 (20 mM Tris pH 7.5, 50 mM NaCl, 5 mM KCl, 0.5 mM $MnCl_2$, 4 U RNasin (Promega), 1 mM DTT, 1 μL compound in DMSO or 1 μL DMSO alone). Next Mix 2 was added (450 nM Oligo-U12 primer, 30 nM UTP, 75 nM Poly(A) template, 0.2 μCi H^3-labeled UTP) and the 41 μL reaction incubated in 0.7 mL tubes at room temperature for 3 h. The reaction was stopped by adding 350 μL of 1.2 mg streptavidin-coated SPA beads (PerkinElmer) in 0.15 M EDTA. The samples were analyzed immediately for 1 min in a scintillation counter. Each compound concentration was performed in duplicate and repeated at least two independent times. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of compound that reduced polymerase activity by 50%. The IC_{50} was determined by comparing counts from eight serial dilutions of compound and vehicle alone reactions using GraphPad Prism software.

4.2.5. Modeling and docking

The study was carried out using Schrodinger modeling suite.[48] The X-ray crystallographic structure of HCV NS5B in complex with Mn^{2+} ions and UTP (PDB:1NB6[45]) was taken from Protein Data Bank. The crystallographic Mn^{2+} ions were replaced with Mg^{2+} ions. All missing hydrogen atoms were added by standard protein preparation protocol within Maestro followed by energy minimization using OPLS 2005 forcefield[49] to optimize all hydrogen bonding networks. Docking of the most active compound **27a** was carried out using Glide with standard precision protocol[50] with the Mg^{2+} ions defined as a required constraints. The van der Waals radii of nonpolar atoms for each of the ligands were scaled by a factor of 0.8 to account for structure variability to specific ligand binding. The ionized form of compound **27a** was used based on the estimated pK_a of its hydroxyl group of 7.6 by ACDLabs pK_a predictor (Advanced Chemistry Development, Inc., Toronto, Canada) and the presence of the two divalent metal ions within the active site for binding. Based on our earlier studies of HIV-1 integrase strand transfer inhibition[51], the highest scored conformation involving the chelating triad to the divalent metal ions bichelation was selected as the most probable mode of inhibition. All compounds within Table 2 were modeled based on this observed mode of binding to rationalize the changes in the inhibition activity. To account for local protein flexibility, each of the final docked complexes was further refined by locally restrained energy minimization under implicit solvent condition.

Acknowledgment

This research was supported by the Center for Drug Design at the University of Minnesota. We thank Dr. Guangxiang Luo at University of Kentucky for providing the Huh-7/HCV1b-Rluc replicon cells, Dr. Riccado Petrelli at University of Camerino, Italy, for providing 2 mA and the Minnesota Supercomputing Institute for computing resources.

Supplementary data

Supplementary data (Spectral data.) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.10.058.

References and notes

1. Choo, Q. L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359.
2. Shepard Colin, W.; Finelli, L.; Alter Miriam, J. *Lancet Infect. Dis.* **2005**, *5*, 558.
3. Rustgi Vinod, K. *J. Gastroenterol.* **2007**, *42*, 513.
4. Amarapurkar, D. *J. Gastroenterol. Hepatol.* **2000**, *15*, E105.
5. Thomas, D. L.; Astemborski, J.; Rai, R. M.; Anania, F. A.; Schaeffer, M.; Galai, N.; Nolt, K.; Nelson, K. E.; Strathdee, S. A.; Johnson, L.; Laeyendecker, O.; Boitnott, J.; Wilson, L. E.; Vlahov, D. *JAMA* **2000**, *284*, 450.
6. Neukam, K.; Macias, J.; Mira, J. A.; Pineda, J. A. *Expert Opin. Pharmacother.* **2009**, *10*, 417.
7. Herrine, S. K.; Rossi, S.; Navarro, V. *J. Clin. Exp. Med.* **2006**, *6*, 20.
8. Hashemi, N.; Rossi, S.; Navarro, V. J.; Herrine, S. K. *Expert Opin. Drug Saf.* **2008**, *7*, 771.
9. Shiffman Mitchell, L. *Curr. Gastroenterol. Rep.* **2010**, *12*, 70.
10. Chen Kevin, X.; Njoroge, F. G. *Curr. Opin. Investig. Drugs* **2009**, *10*, 821.
11. Beaulieu, P. L. *Expert Opin. Ther. Pat.* **2009**, *19*, 145.
12. Lin, C.; Kwong, A. D.; Perni, R. B. *Infect. Disord.: Drug Targets* **2006**, *6*, 3.
13. Perni, R. B.; Almquist, S. J.; Byrn, R. A.; Chandorkar, G.; Chaturvedi, P. R.; Courtney, L. F.; Decker, C. J.; Dinehart, K.; Gates, C. A.; Harbeson, S. L.; Heiser, A.; Kalkeri, G.; Kolaczkowski, E.; Lin, K.; Luong, Y.-P.; Rao, B. G.; Taylor, W. P.; Thomson, J. A.; Tung, R. D.; Wei, Y.; Kwong, A. D.; Lin, C. *Antimicrob. Agents Chemother.* **2006**, *50*, 899.
14. Malcolm, B. A.; Liu, R.; Lahser, F.; Agrawal, S.; Belanger, B.; Butkiewicz, N.; Chase, R.; Gheyas, F.; Hart, A.; Hesk, D.; Ingravallo, P.; Jiang, C.; Kong, R.; Lu, J.; Pichardo, J.; Prongay, A.; Skelton, A.; Tong, X.; Venkatraman, S.; Xia, E.; Girijavallabhan, V.; Njoroge, F. G. *Antimicrob. Agents Chemother.* **2006**, *50*, 1013.
15. Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; Agrawal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madison, V.; Broske, L.; Cui, X.; Cheng, K.-C.; Hsieh, T. Y.; Brisson, J.-M.; Prelusky, D.; Korfmacher, W.; White, R.; Bogdanowich-Knipp, S.; Pavlovsky, A.; Bradley, P.; Saksena, A. K.; Ganguly, A.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. *J. Med. Chem.* **2006**, *49*, 6074.
16. Arasappan, A.; Bennett, F.; Bogen, S. L.; Venkatraman, S.; Blackman, M.; Chen, K. X.; Hendrata, S.; Huang, Y.; Huelgas, R. M.; Nair, L.; Padilla, A. I.; Pan, W.; Pike, R.; Pinto, P.; Ruan, S.; Sannigrahi, M.; Velazquez, F.; Vibulbhan, B.; Wu, W.; Yang, M.; Saksena, A. K.; Girijavallabhan, V.; Shih, N.-Y.; Kong, J.; Meng, T.; Jin, Y.; Wong, J.; McNamara, P.; Prongay, A.; Madison, V.; Piwinski, J. J.; Cheng, K.-C.; Morrison, R.; Malcolm, B.; Tong, X.; Ralston, R.; Njoroge, F. G. *ACS Med. Chem. Lett.* **2010**, *1*, 64.
17. Arasappan, A.; Padilla, A. I.; Jao, E.; Bennett, F.; Bogen, S. L.; Chen, K. X.; Pike, R. E.; Sannigrahi, M.; Soares, J.; Venkatraman, S.; Vibulbhan, B.; Saksena, A. K.; Girijavallabhan, V.; Tong, X.; Cheng, K.-C.; Njoroge, F. G. *J. Med. Chem.* **2009**, *52*, 2806.

18. Bennett, F.; Huang, Y.; Hendrata, S.; Lovey, R.; Bogen, S. L.; Pan, W.; Guo, Z.; Prongay, A.; Chen, K. X.; Arasappan, A.; Venkatraman, S.; Velazquez, F.; Nair, L.; Sannigrahi, M.; Tong, X.; Pichardo, J.; Cheng, K.-C.; Girijavallabhan, V. M.; Saksena, A. K.; Njoroge, F. G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2617.
19. Bogen, S. L.; Arasappan, A.; Velazquez, F.; Blackman, M.; Huelgas, R.; Pan, W.; Siegel, E.; Nair, L. G.; Venkatraman, S.; Guo, Z.; Doll, R.; Shih, N.-Y.; George Njoroge, F. *Bioorg. Med. Chem.* **2010**, *18*, 1854.
20. Chen, K. X.; Nair, L.; Vibulbhan, B.; Yang, W.; Arasappan, A.; Bogen, S. L.; Venkatraman, S.; Bennett, F.; Pan, W.; Blackman, M. L.; Padilla, A. I.; Prongay, A.; Cheng, K.-C.; Tong, X.; Shih, N.-Y.; Njoroge, F. G. *J. Med. Chem.* **2009**, *52*, 1370.
21. Nair, L. G.; Bogen, S.; Ruan, S.; Pan, W.; Pike, R.; Tong, X.; Cheng, K.-C.; Guo, Z.; Doll, R. J.; Njoroge, F. G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1689.
22. Li, H.; Shi, S. T. *Future Med. Chem.* **2010**, *2*, 121.
23. Liu, Y.; He, Y.; Molla, A. *Antiviral Res.* **2009**, 137.
24. Beaulieu, P. L. *Curr. Opin. Invest. Drugs* **2007**, *8*, 614.
25. Li, H.; Tatlock, J.; Linton, A.; Gonzalez, J.; Jewell, T.; Patel, L.; Ludlum, S.; Drowns, M.; Rahavendran, S. V.; Skor, H.; Hunter, R.; Shi, S. T.; Herlihy, K. J.; Parge, H.; Hickey, M.; Yu, X.; Chau, F.; Nonomiya, J.; Lewis, C. *J. Med. Chem.* **2009**, *52*, 1255.
26. Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M. M.; Niu, C.; Otto, M. J.; Furman, P. A. *J. Med. Chem.* **2010**, *53*, 7202.
27. Reddy, P. G.; Chun, B.-K.; Zhang, H.-R.; Rachakonda, S.; Ross, B. S.; Sofia, M. J. *J. Org. Chem.* **2011**, *23*, 23. doi:10.1021/jo200060f.
28. Gao, M.; Nettles, R. E.; Belema, M.; Snyder, L. B.; Nguyen, V. N.; Fridell, R. A.; Serrano-Wu, M. H.; Langley, D. R.; Sun, J.-H.; O'Boyle, D. R., II; Lemm, J. A.; Wang, C.; Knipe, J. O.; Chien, C.; Colonno, R. J.; Grasela, D. M.; Meanwell, N. A.; Hamann, L. G. *Nature* **2010**, *465*, 96.
29. Lemm, J. A.; O'Boyle, D., II; Liu, M.; Nower, P. T.; Colonno, R.; Deshpande, M. S.; Snyder, L. B.; Martin, S. W.; St. Laurent, D. R.; Serrano-Wu, M. H.; Romine, J. L.; Meanwell, N. A.; Gao, M. *J. Virol.* **2009**, *84*, 482.
30. Romine, J. L.; St. Laurent, D. R.; Leet, J. E.; Martin, S. W.; Serrano-Wu, M. H.; Yang, F.; Gao, M.; O'Boyle, D. R.; Lemm, J. A.; Sun, J.-H.; Nower, P. T.; Huang, X.; Deshpande, M. S.; Meanwell, N. A.; Snyder, L. B. *ACS Med. Chem. Lett.* **2011**, *2*, 224.
31. Nowotny, M. *EMBO Rep.* **2009**, *10*, 144.
32. Wai, J. S.; Egbertson, M. S.; Payne, L. S.; Fisher, T. E.; Embrey, M. W.; Tran, L. O.; Melamed, J. Y.; Langford, H. M.; Guare, J. P.; Zhuang, L.; Grey, V. E.; Vacca, J. P.; Holloway, M. K.; Naylor-Olsen, A. M.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Schleif, W. A.; Gabryelski, L. J.; Young, S. D. *J. Med. Chem.* **2000**, *43*, 4923.
33. Kirschberg, T. A.; Balakrishnan, M.; Squires, N. H.; Barnes, T.; Brendza, K. M.; Chen, X.; Eisenberg, E. J.; Jin, W.; Kutty, N.; Leavitt, S.; Liclican, A.; Liu, Q.; Liu, X.; Mak, J.; Perry, J. K.; Wang, M.; Watkins, W. J.; Lansdon, E. B. *J. Med. Chem.* **2009**, *52*, 5781.
34. Summa, V.; Petrocchi, A.; Pace, P.; Matassa, V. G.; De Francesco, R.; Altamura, S.; Tomei, L.; Koch, U.; Neuner, P. *J. Med. Chem.* **2004**, *47*, 14.
35. Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Gonzalez Paz, O.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteagudo, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.
36. Koch, U.; Attenni, B.; Malancona, S.; Colarusso, S.; Conte, I.; Di Filippo, M.; Harper, S.; Pacini, B.; Giomini, C.; Thomas, S.; Incitti, I.; Tomei, L.; De Francesco, R.; Altamura, S.; Matassa, V. G.; Narjes, F. *J. Med. Chem.* **2006**, *49*, 1693.
37. Klumpp, K.; Mirzadegan, T. *Curr. Pharm. Des.* **2006**, *12*, 1909.
38. Billamboz, M.; Bailly, F.; Barreca, M. L.; De Luca, L.; Mouscadet, J.-F.; Calmels, C.; Andreola, M.-L.; Witvrouw, M.; Christ, F.; Debyser, Z.; Cotelle, P. *J. Med. Chem.* **2008**, *51*, 7717.
39. Billamboz, M.; Bailly, F.; Lion, C.; Calmels, C.; Andreola, M.-L.; Witvrouw, M.; Christ, F.; Debyser, Z.; De Luca, L.; Chimirri, A.; Cotelle, P. *Eur. J. Med. Chem.* **2011**, *46*, 535.
40. Billamboz, M.; Bailly, F.; Lion, C. d.; Touati, N.; Vezin, H.; Calmels, C.; Andreola, M.-L.; Christ, F.; Debyser, Z.; Cotelle, P. *J. Med. Chem.* **2011**, *54*, 1812.
41. Lohmann, V.; Korner, F.; Koch, J. O.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.
42. Blight, K. J.; Norgard, E. A. *Hepat. C Viruses* **2006**, 311.
43. Tomassini, J. E.; Getty, K.; Stahlhut, M. W.; Shim, S.; Bhat, B.; Eldrup, A. B.; Prakash, T. P.; Carroll, S. S.; Flores, O.; MacCoss, M.; McMasters, D. R.; Migliaccio, G.; Olsen, D. B. *Antimicrob. Agents Chemother.* **2005**, *49*, 2050.
44. Pauwels, F.; Mostmans, W.; Quirynen, L. M.; van der Helm, L.; Boutton, C. W.; Rueff, A. S.; Cleiren, E.; Raboisson, P.; Surleraux, D.; Nyanguile, O.; Simmen, K. A. *J. Virol.* **2007**, *81*, 6909.
45. O'Farrell, D.; Trowbridge, R.; Rowlands, D.; Jager, J. *J. Mol. Biol.* **2003**, *326*, 1025.
46. Shaw, A. N.; Tedesco, R.; Bambal, R.; Chai, D.; Concha, N. O.; Darcy, M. G.; Dhanak, D.; Duffy, K. J.; Fitch, D. M.; Gates, A.; Johnston, V. K.; Keenan, R. M.; Lin-Goerke, J.; Liu, N.; Sarisky, R. T.; Wiggall, K. J.; Zimmerman, M. N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4350.
47. Ivanov, A. V.; Korovina, A. N.; Tunitskaya, V. L.; Kostyuk, D. A.; Rechinsky, V. O.; Kukhanova, M. K.; Kochetkov, S. N. *Protein Expr. Purif.* **2006**, *48*, 14.
48. Schrodinger, L., Maestro 9.0, Glide v5.5, Prime v2.1, Macromodel v9.7. New York, NY.
49. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
50. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1139.
51. Tang, J.; Maddali, K.; Dreis, C. D.; Sham, Y. Y.; Vince, R.; Pommier, Y.; Wang, Z. *ACS Med. Chem. Lett.* **2011**, *2*, 63.