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A new series of HCV inhibitors based on 2-(thieno[2, 3-b]pyridin-2-yl)-1,3,4-oxadiazole scaffold

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A new series of HCV inhibitors based on the 2-(thieno[2,3-b]pyridin-2-yl)-1,3,4-oxadiazole scaffold was developed. Detailed SAR investigations revealed the HCV inhibitory activity was sensitive to the size of C5, C6-fused ring, the size and flexibility of C5' cycloalkane, which led to the identification of several compounds with potent inhibitory activity against HCV genotype 1b replicon. The most potent compound **10d** showed ~100-fold improvement in potency compared with compound **1**, with an EC_{50} of 0.039 μM, while without obvious cytotoxicity in *vitro*.

Introduction

Hepatitis C virus (HCV) infection is a global health issue, with more than 170 million affected people worldwide[1]. Generally, after a latency period of 10–20 years, 70%~80% of infected patients progress to a chronic state, of which 10%~20% will develop into liver-destroying cirrhosis or hepatocellular carcinoma and there are approximately 350,000 deaths each year due to diseases related to hepatitis C virus (HCV) infection[2]. In addition, chronic hepatitis C virus (HCV) infection is the leading cause of liver transplantation in the US[3]. Owing to significant advances in understanding of the replication of HCV and the role of viral non-structural proteins[4], treatment for HCV infection is progressing from the combination of pegylated IFN-α and ribavirin[5] to triple-therapies and recently approved all-oral combination regimens. Combinating one of the FDA approved direct-acting antivirals (DAAs), such as NS3/4A protease inhibitors (Boceprevir[6], Telaprevir[7], Simeprevir[8]), NS5B polymerase inhibitor (Sofosbuvir[9]) or NS5A inhibitor (Daclatasvir[10]), with pegylated IFN-α and ribavirin[11], the so-called triple-therapies, can increase the rates of sustained virologic response (SVR), but the side effects associated with PEG-IFN-α and RBV cannot be eliminated and these treatment strategies just exhibited limited efficacy against HCV genotypes beyond genotype 1a and 1b[12]. Thus the development of more effective, tolerable and interferon-free therapies is ongoing[13, 14]. In 2014, Harvoni (Ledipasvir and Sofosbuvir)[15] and Viekira Pak (Ombitasvir, Paritaprevir, Ritonavir and Dasabuvir)[16] were approved by the U.S. Food and Drug Administration, which made more than 95% patients with difficult-to-treat chronic HCV genotype 1 infection achieve SVR in a treatment duration of as short as 8~12 weeks and could avoid the side effects associated with interferon. These new all-oral treatments (AOTs) opened a new era for HCV treatment across all genotypes.

Recent study reported by our group had described thieno[2,3-b]pyridine analogue **1** (**Fig. 1**) as an HCV inhibitor with an EC_{50} of 3.3μM[17]. As our efforts to pursue new HCV inhibitors, we designed a novel scaffold which combined thieno[2,3-b]pyridine[17] with oxadiazole. Here we reported the design, synthesis and SAR studies of this new series of HCV inhibitors based on the 2-(thieno[2,3-b]pyridin-2-yl)-1,3,4-oxadiazole scaffold for the purpose of seeking more potent compounds against HCV genotype 1b replicon, which led to the discovery of a nanomolar HCV inhibitor **10d (Fig. 1)**.

Results and discussion

Chemistry

Synthetic routine to the target molecules is shown in **Scheme 1**. Compound **3**, which was prepared as our previous study[17], reacted with hydrazine hydrate in ethanol under reflux to afford the key intermediate acyl hydrazides **4**. This building block was then coupled with acyl chloride in THF at 0 ℃ and cyclizated in $POCl_3$ to produce 1, 3, 4-oxadiazole derivatives **5**. While compound **6** were prepared by coupling of acyl hydrazides **4** with isocyanate in DMSO at room temperature followed by cyclization step under alky conditions. The title compounds **10** and **11** were synthesized via a similar routine as shown in **Scheme 2**. Compound **11** was subsequently methylated to give **12**, and **13** was prepared by deamination of **10** with tert-butyl nitrite in DMF as our previous report[17]. The structures of all the title compounds examined in this study were fully characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS analysis.

SAR analysis and preliminary biological evaluation

Our initial efforts mainly focused on C5' position of 2-(thieno[2,3-b]pyridin-2-yl)-1,3,4-oxadiazole scaffold. In previous study[17] with respect to thieno[2,3-b]pyridine HCV inhibitors had revealed that the phenyl and cyclopropyl in C6 position showed comparable potency. Two series of C6-phenyl and cyclopropyl derivatives listed in **Table 1** were firstly synthesized to survey the

SAR of substituents on C5' position. In the C6-phenyl substituted series, different size of cycloalkane **(5a-5d)** were investigated, but only one of the resulting compounds **5d** exhibited moderate anti-HCV activity, with an EC_{50} of 7.60 μM against the HCV GT-1b replicon. Interestingly, replacement of the cycloalkane with cycloalkaneamino afforded two more active derivatives **6a** and **6b** with EC_{50}s of 3.22μM and 3.51μM. However, when cycloalkane was replaced by rigid phenyl-ring (**6c**, EC_{50} > 10 μM), a dramatic decline of the potency was observed, implying that aromatic-ring in this position might be disadvantageous. It is worth noting that, in the C6-cyclopropyl series, compounds **5e-5h**, **6d-6e** displayed enhanced inhibitory potency against the replicon compared with corresponding C6-phenyl series. A similar investigation of substituents on C5' position revealed that introducing different size of cycloalkane from cyclopropyl (**5e**, EC_{50} = 3.54 μM) to cyclohexyl (**5h**, EC_{50} = 2.43 μM) was well tolerated, of which the cyclohexyl was the most suitable substituents. The C5'-cycloalkaneamino substituted derivatives **6d** (EC_{50} = 3.11 μM) and **6e** (EC_{50} = 4.70 μM) was slightly less potent than the corresponding cycloalkane derivatives, suggesting that conformational change caused by amino inserting would be tolerated in C6-cyclopropyl series. Replacing C6-cyclopropyl by C6-cyclopentyl (**5i**, EC_{50} = 6.8 μM) resulted in a sharp loss of replicon activity, implying that a bulky group in C6 position might be disadvantageous, which could also be concluded from the C6-phenyl series. Together, these results seemed to imply that the size of C6 substituent were critical to its HCV inhibitory activity. Preliminary SAR investigation revealed that cycloalkane or cycloalkaneamino on C5'position exhibiting moderate anti-HCV activity in the C6-cyclopropyl series, which became the group of choice for further optimization.

Subsequently, we incorporated fused-rings strategies on the regions of C5 and C6 position by replacement of a cyclopropyl with a fused alkane ring. A set of compounds with cyclohexa[b]thieno[3,2-e]pyridin core were synthesized and evaluated. As shown in **Table 2**, in contrast with C6-single substituted series, the size of cycloalkane substituted on C5' position had substantial impaction on C5, C6-fused-ring series compared with C6-cyclopropyl series. In the cyclopenta[b]thieno[3,2-e]pyridin series, increased replicon activity was observed as the size of C5'-cycloalkane increased from cyclopropyl to cyclohexyl, with EC_{50}s of >10 μM for cyclopropyl deravitive **10a** to 0.039 μM for cyclohexyl derivative **10d**, the most potent compound in this series. However, replacement of cyclohexyl with cyclohexylamino was detrimental to Gt-1b potency, which could be concluded from the cyclohexylamino derivatives **11a** (EC_{50} = 3.60 μM), **11b** (EC_{50} = 5.72 μM) and their cyclohexyl congeners **10d** (EC_{50} = 0.039 μM), **10c** (EC_{50} = 2.20 μM), respectively. On the basis of SAR on C5' position in cyclohexa[b]thieno[3,2-e]pyridine series, we concluded that HCV inhibitory activity was more sensitive to the size and flexibility of C5' cycloalkane in C5, C6-fused-ring series. Having identified the cyclohexyl or cyclohexylamino as the favorable substituents on C5' position, subsequent modification focused on the size of fused-ring in C5, C6-position to seek for the best fused ring in C5, C6 position. As shown in **Table 2**, replacement of cyclopenta[b]thieno[3,2-e]pyridine with cyclohexa- or cyclohepta-thieno[3,2-e]pyridine provided a series of six to seven-membered ring-fused derivatives **10e**, **10f**, **11c**, **11d**. It was observed that, as the size of C5,C6-fused-rings increased from five to seven-membered, a sharp loss of potency could be observed, for which cyclohexyl- (**10e**, EC_{50} = 4.30 μM) and cycloheptyl-fused derivatives (**10f**, EC_{50} = 6.71 μM) showed about 100-fold decrease in potency compared with their cyclopenty congener. Similar trend would also be observed in the cyclohexylamino substituted series, which could be illustrated by compounds **11c** (EC_{50} = 6.70 μM) and **11d** (EC_{50} = 8.11 μM). Together, these results indicated that the size of C5, C6-fused-rings has a significant impact on replicon activity, of which a five-membered fused ring derivatives **10d** (EC_{50} = 0.039 μM) was the optimal. Methylating amino giving compound **12a** (EC_{50} > 10 μM), **12b** (EC_{50} > 10μM), **12c** (EC_{50} > 10 μM), which exhibited no anti-HCV activity, implying that conformational change caused by the introduction of methyl group was harmful to the anti-HCV activity. The importance of amino group on C3 position was revealed by the dramatic decrease of the potency in compound **13a** (EC_{50} = 0.66 μM), with a 17-fold decrease in potency comparing with its C3-amino congener. The replacement of trifluoromethyl at the C4-position by pentafluoroeth (**10g**, EC_{50} = 0.87 μM) resulted in a 22-fold decrease in potency, indicating that introduction of smaller hydrophobic group in C4 position was more potent. Interestingly, when introduction a methyl to the fused cyclopentyl ring (**10h**, EC_{50} > 10μM), a sharp loss of replicon activity was observed, which could also be explained by the observation that the size of C5, C6-fused ring is crucial to the potency against HCV.

As several compounds with promising anti-HCV activity were discovered through the SAR study, the cytotoxicity of selected potent compounds were evaluated against normal cell lines. All compounds showed no obvious cytotoxicity against the cell line HEK293 by MTT assay in an extensive concentration range up to 100 μM, indicating that these inhibitors showed a good safety profile in vitro.

To investigate the precise target of this kind of HCV inhibitors, compound **1** was tested against a panel of HCV replicons containing mutations mapped to NS3/4A protease, NS4B, NS5A, and NS5B polymerase. Two strains bearing NS4B mutations were found to be significantly resistant to this compound related to GT-1b wild type replicon, implying that compound **1** could target at NS4B. But this resistant profile could not be reproduced with respect to our optimized compound **10d**, which was equipotent to both the mutant strains bearing NS4B mutations and GT-1b wild type replicon. These results suggested that compound **1** and **10d** would target at different proteins of HCV. Subsequent resistant replicon selection study is under going to identify the precise target.

Conclusions

In summary, we have successfully developed a novel series of HCV Gt-1b inhibitors with potent anti-HCV activity based on a 2-(thieno[2,3-b]pyridin-2-yl)-1,3,4-oxadiazole scaffold. The SAR studies on the C3, C4, C5, C6 and C5'-position were carried out aimed at seeking new HCV inhibitors with improve potency. Optimization at C5' position indicated that the HCV inhibitory activity was sensitive to the size of C5'-cycloalkane in C5, C6-fused-ring series, of which the cyclohexyl was the most suitable

substituent. In addition, we incorporated fused-rings on C5 and C6 position, which revealed that five-membered ring-fused thieno[3,2-e]pyridine was the optimal. The selected compound **10d**, a novel HCV inhibitor, showed low-nanomolar activity against the HCV GT-1b replicon without obvious cytotoxicity. Resistance mapping studies revealed that compound **10d** and **1** might target at different proteins of HCV, indicating **10d** wound act by new mechanisms of action. Further effort is needed to explore their specific targets and detailed mechanism.

Experimental

Chemistry

All materials were from commercial suppliers and used without purification. We used DMSO-d6 or CDCl3 as the solvent of all products for the 1H and 13C NMR assays, which were recorded on a Bruker AVANCEш 400 spectrometer. Chemical shifts (δ) were reported in ppm relative to Me4Si (internal standard) and coupling constants (J) were reported in Hz. Mass Spectra (MS) were performed on a Waters Q-TOF Premier mass spectrometer. Thin layer chromatography (TLC) and Column chromatography were used Qingdao Haiyang Silica gel F-254 plates and Qingdao Haiyang Silica gel 60 (300-400 mesh). HPLC analysis was performed on an UltiMate3000 HPLC system (Dionex, USA). The purity of all target products was ≧90%, detecting by HPLC under UV 254 nm wavelength, NMR and ESI-MS.

General procedure for step a-j.

Step **a,b,c**. The synthesis of compounds **3** (or **8**) from ketone **2**(or **7**) was performed as our previous reported[17].

Step **d**. A solution of compound **3** (or **8**) (1.0 equiv) in ethanol was added hydrazine hydrate (1.2 equiv). The reaction mixture was stirred at 100 ℃ for 6~8 h until the starting materials were consumed, as determined by TLC. Then water was added to the mixture so that the desire product could be precipitated. The precipitate was collected by filtration and washed with distilled water, then recrystallized from appropriate solvent to give the desire product. Yield: 80-100%.

Step **e**. A solution of compound **4** (or **9**) (1.0 equiv) in THF was added dropwise with a solution of acylchloride (1.0 equiv) in THF at 0 ℃. The reaction mixture was stirred at 0 ℃ for 0.5~2 h until the starting materials were consumed, as determined by TLC. Then the solvent was evaporated under reduced pressure. After dried in *vacuo*, the product can be straight used for step **f**.

Step **f**. A solution of the intermediate from step **e** was stirred in POCl3 at 100 ℃ for 1 h until the starting materials were consumed, as determined by TLC. The solvent was evaporated under reduced pressure, after cooled to room temperature. Then ice water was added to the mixture so that the desire product can be precipitated. The precipitate was collected by filtration and washed with distilled water, then recrystallized from appropriate solvent to give the desire product. Yield: 40-60%. In some cases, such as **5g**, the product cannot be precipitated after the addition of water, then an extraction procedure is needed to transfer the product from water phase to the EA phase, after by a subsequent column chromatography separation procedure to purify the product.

Step **g**. A solution of compound **4** (or **9**) (1.0 equiv) in DMSO was added isocyanate. The resulting mixture was stirred for 1~2 h at room temperature. Then water was added to the mixture so that the desire product can be precipitated. The precipitate was collected by filtration and washed with distilled water, then recrystallized from appropriate solvent to give the desire product. Yield: >90%. In some cases, the product cannot be precipitated after the addition of water, then an extraction procedure is needed to transfer the product from DMSO/water phase to the EA phase, after by a subsequent column chromatography separation procedure to purify the product.

Step **h**. A solution of the intermediate from step **g** in NMP was added p-TsCl (1.2equiv) and triethylamine (2.0 equiv). The reaction mixture was stirred at room temperature for 2~4 h until the starting materials were consumed, as determined by TLC. Then water was added to the mixture so that the desire product can be precipitated. The precipitate was collected by filtration and washed with distilled water, then recrystallized from appropriate solvent to give the desire product. Yield: 60-80%. In some cases, such as **11b**, the product cannot be precipitated after the addition of water, then an extraction procedure is needed to transfer the product from NMP/water phase to the EA phase, after by a subsequent column chromatography separation procedure to purify the product.

Step **i**. A solution of compound **11** (1.0 equiv) in DMF was added TEA followed by the addition of MeI (2 equiv) at room temperature. The resulting mixture was stirred for 6~10 h at room temperature. Then water was added to the mixture so that the desire product can be precipitated. The precipitate was collected by filtration and washed with distilled water, then recrystallized from appropriate solvent to give the desire product. Yield: 60%~80%. In some cases such as **12c**, the product cannot be precipitated after the addition of water, then an extraction procedure is needed to transfer the product from DMF/water phase to the EA phase, after by a subsequent column chromatography separation procedure to purify the product.

Step **j**. A solution of compound **10** (1.0 equiv) in DMF was added dropwise with a solution of t-BuONO (2.0 equiv) in DMF (5 mL) at 60 ℃. After stirring for 0.5 h, the reaction mixture was poured into hydrochloric acid (1 N) and extracted with ethyl acetate. The organic phase was washed with brine and dried with MgSO4, filtered and concentrated in *vacuo*. Purified by column chromatography gave the title compound. Yield: 15-30%.

2-(5-cyclopropyl-1,3,4-oxadiazol-2-yl)-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5a**). Yield: 28%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 6.8 Hz, 2H), 8.03 (s, 1H), 7.55-7.49 (m, 3H), 6.28 (sbr, 2H), 2.26-2.20 (m, 1H), 1.28-1.18 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.53, 162.84, 161.38, 156.30, 140.38, 137.11, 132.28 (q, J = 33.0 Hz), 130.38, 129.10, 127.30, 123.08 (d, J = 272.0 Hz), 118.47, 113.38 (q, J = 6.3 Hz), 94.65, 8.64, 6.31. ESI-MS: m/z 403.1 $[M + H]^+$.

2-(5-cyclobutyl-1,3,4-oxadiazol-2-yl)-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5b**). Yield: 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 6.0 Hz, 2H), 7.97 (s, 1H), 7.48-7.44 (m, 3H), 6.02 (sbr, 2H), 3.77-3.73 (m, 1H), 2.50-2.43 (m, 4H), 2.13-2.04 (m, 2H). ^{13}C NMR (100MHz, $CDCl_3$) δ 167.20, 162.71, 161.97, 156.30, 140.60, 136.92, 132.33 (q, J = 33.0 Hz), 130.5, 129.15, 127.38, 123.46 (d, J = 272.0 Hz), 118.60, 113.48 (q, J = 6.2 Hz), 94.77, 30.37, 27.11, 18.92. ESI-MS: m/z 417.1 $[M + H]^+$.

2-(5-cyclopentyl-1,3,4-oxadiazol-2-yl)-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5c**). Yield: 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 6.1 Hz, 2H), 7.98 (s, 1H), 7.49-7.45 (m, 3H), 5.98 (sbr, 2H), 3.38-3.21 (m, 1H), 2.12-2.10 (m, 2H), 1.98-1.93 (m, 2H), 1.81-1.79 (m, 2H), 1.70-1.67 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.26, 162.79, 162.01, 156.31, 140.52, 137.02, 132.36 (q, *J* = 33.0 Hz), 130.47, 129.15, 127.37, 123.49 (d, *J* = 272.0 Hz), 118.43, 113.32 (q, *J* = 6.2 Hz), 94.75, 35.91, 31.22, 25.50. ESI-MS: m/z 431.1 $[M + H]^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5d**). Yield: 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 6.2 Hz, 2H), 7.92 (s, 1H), 7.45-7.41 (m, 3H), 6.21 (sbr, 2H), 3.12-2.91 (m, 1H), 2.10-2.06 (m, 2H), 1.81-1.78 (m, 2H), 1.68-1.57 (m, 3H), 1.37-1.25 (m, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ 167.99, 162.85, 161.75, 156.25, 140.49, 137.06, 132.26 (q, *J* = 33.0 Hz), 130.36, 129.07, 127.27, 123.08 (d, *J* = 272.0 Hz), 118.60, 113.51 (q, *J* = 6.2 Hz), 95.01, 35.09, 30.16, 25.60, 25.42. ESI-MS: m/z 445.2 $[M + H]^+$.

5-(3-amino-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-2-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**6a**). Yield: 20%. ^{1}H NMR (400 MHz, $CDCl_3$)) δ 8.05 (d, *J* = 6.1 Hz, 2H), 7.96 (s, 1H), 7.45-7.41 (m, 3H), 6.23 (s, 1H), 6.02 (sbr, 2H), 3.75-3.60 (m, 1H), 2.06-1.98 (m, 2H), 1.74-1.59 (m, 2H), 1.35-1.18 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.36, 162.81, 162.43, 155.99, 139.42, 137.16, 132.65 (q, *J* = 33.0 Hz), 130.33, 129.11, 12155.74, 139.49, 137.06, 132.46 (q, *J* = 33.0 Hz), 130.41, 129.12, 127.41, 123.18 (d, *J* = 271.0 Hz), 118.45, 113.59 (q, *J* = 6.2 Hz), 99.93, 53.43, 33.03, 29.76, 24.55. ESI-MS: m/z 458.3 $[M - H]^+$.

5-(3-amino-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-2-yl)-N-cyclopentyl-1,3,4-oxadiazol-2-amine (**6b**). Yield: 23%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 6.1 Hz, 2H), 7.95 (s, 1H), 7.46-7.42 (m, 3H), 6.13 (s, 1H), 6.03 (sbr, 2H), 3.95-3.84 (m, 1H), 1.97-1.93 (m, 2H), 1.65-1.75 (m, 2H), 1.53-1.62 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 166.36, 162.81, 162.43, 155.99, 139.42, 137.16, 132.65 (q, *J* = 33.0 Hz), 130.33, 129.11, 127.26, 123.30 (d, *J* = 270.0 Hz), 118.57, 113.40 (q, *J* = 6.2 Hz), 55.83, 33.24, 29.71, 23.60. ESI-MS: m/z 446.2 $[M + H]^+$.

5-(3-amino-6-phenyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-2-yl)-N-phenyl-1,3,4-oxadiazol-2-amine (**6c**). Yield: 20%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.36 (s, 1H), 8.29 (d, J = 7.6 Hz, 2H), 7.63(d, J = 7.5 Hz, 2H), 7.40-7.37 (m, J = 8.0 Hz, 3H), 7.06-7.02 (m, 3H), 6.39 (sbr, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.14, 158.10, 155.24, 155.19, 139.25, 138.38, 136.24, 132.74 (q, *J* = 33.0 Hz), 130.49, 129.05, 127.24, 124.13, 123.50 (d, *J* = 271.0 Hz), 121.95, 118.11, 117.03, 113.73 (q, *J* = 6.2 Hz), 93.50. ESI-MS m/z 476.3$[M + Na]^+$.

6-cyclopropyl-2-(5-cyclopropyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5e**). Yield: 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 6.21 (sbr, 2H), 2.17-2.25 (m, 2H), 1.12-1.25 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.29, 163.54, 162.78, 161.53, 140.54, 131.30 (q, *J* = 33.0 Hz), 122.93(q, *J* = 272.0 Hz), 117.48, 114.97, 92.88, 17.74, 11.50, 8.55, 6.29. ESI-MS m/z 367.1 $[M + H]^+$.

2-(5-cyclobutyl-1,3,4-oxadiazol-2-yl)-6-cyclopropyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5f**) Yield: 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 6.26 (sbr, 2H), 3.82-3.75 (m, 1H), 2.58-2.47 (m, 4H), 2.22-2.05 (m, 3H), 1.22-1.11 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.96, 163.60, 162.86, 162.14, 140.75, 131.36 (q, *J* = 33.0 Hz), 123.08 (q, *J* = 272.0 Hz), 117.49, 114.96, 92.95, 30.37, 27.10, 18.90, 17.75, 11.52. ESI-MS m/z 381.1$[M + H]^+$.

2-(5-cyclopentyl-1,3,4-oxadiazol-2-yl)-6-cyclopropyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5g**). Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 6.24 (sbr, 2H), 3.42-3.34 (m, 1H), 2.22-1.70 (m, 9H), 1.25-1.14 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.99, 163.55, 162.85, 162.16, 140.67, 131.33 (q, *J* = 33.0 Hz), 123.09 (q, *J* = 272.0 Hz), 117.49, 114.95, 93.00, 35.88, 31.18, 25.48, 17.75, 11.50. ESI-MS m/z 395.1 $[M + H]^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-6-cyclopropyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5h**). Yield: 22%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 6.16 (sbr, 2H), 3.05-2.92 (m, 1H), 2.29-2.09 (m, 3H), 1.94-1.76 (m, 2H), 1.75-1.57 (m, 3H), 1.56-1.24 (m, 3H), 1.23-1.04 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.84, 163.45, 162.44, 161.86, 140.62, 131.55 (q, *J* = 33.0 Hz), 123.00 (q, *J* = 272.0 Hz), 117.68, 114.89, 93.17, 35.07, 30.16, 25.59, 25.40, 17.67, 11.66. ESI-MS m/z 409.2 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-6-cyclopentyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-3-amine (**5i**). Yield: 30%. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.48 (s, 1H), 6.27 (s, 2H), 3.40-3.32 (m, 1H), 3.02-2.95 (m, 1H), 2.17-2.15 (m, 4H), 1.95-1.82 (m, 6H), 1.81-1.61 (m, 5H), 1.49-1.31 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 167.86, 166.01, 162.40, 161.90, 140.59, 131.68 (q, *J* = 33.0 Hz), 123.10 (q, *J* = 272.0 Hz), 117.93, 115.15, 93.56, 35.07, 33.57, 30.16, 29.69, 25.86, 25.59, 25.40. ESI-MS m/z 437.2 $(M + H)^+$.

5-(3-amino-6-cyclopropyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-2-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**6d**). Yield: 20%. ^{1}H NMR (400 MHz, DMSO-d_6) *δ* 7.87 (d, *J* = 7.2, 1H), 7.82 (s, 1H), 6.16 (sbr, 2H), 3.49-3.38 (m, 1H), 2.46-2.40 (m, 1H), 2.05-1.92 (m, 2H), 1.72-1.67 (m, 2H), 1.42-1.25 (m, 6H), 1.19-1.04 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) *δ* 163.00, 161.08, 160.91, 154.82, 138.34, 129.93 (q, *J* = 33.0 Hz), 122.79 (q, *J* = 272.0 Hz), 117.10, 115.53, 92.45, 51.83, 32.11, 25.14, 24.24, 16.92, 11.39. ESI-MS m/z 424.2 $(M + H)^+$.

5-(3-amino-6-cyclopropyl-4-(trifluoromethyl)thieno[2,3-b]pyridin-2-yl)-N-cyclopentyl-1,3,4-oxadiazol-2-amine (**6e**). Yield: 22%. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.35 (s, 1H), 6.03 (sbr, 2H), 4.05(s, 1H), 2.17-1.96 (m, 2H), 1.80-1.51(m, 4H), 1.29-1.11(m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 162.01, 161.28, 159.57, 155.04, 138.32, 129.94 (q, *J* = 33.0 Hz), 122.06 (q, *J* = 272.0 Hz), 116.62, 113.90, 92.19, 54.64, 32.17, 28.68, 22.57, 16.64, 10.38. ESI-MS m/z 410.1 $(M + H)^+$.

2-(5-cyclopropyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10a**). Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 6.05 (sbr, 2H), 3.23 (sbr, 2H), 3.10 (t, *J* = 7.6 Hz, 2H), 2.18 (m, 3H), 1.15-1.11 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 167.92, 166.32, 161.49, 160.82, 140.84, 132.29, 127.83 (q, *J* = 33.0 Hz), 124.02 (q, *J* = 273.0 Hz), 118.59, 93.46, 33.80, 31.09, 23.00, 8.57, 6.29. ESI-MS m/z 367.1 $(M + H)^+$.

2-(5-cyclobutyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10b**). Yield: 21%; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 6.33 (sbr, 2H), 3.87-

3.72 (m, 1H), 3.33-3.07 (m, 4H), 2.59-2.41 (m, 4H), 2.28-2.05 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.28, 166.94, 162.21, 161.44, 141.13, 132.04, 127.59 (q, J = 33.0 Hz), 124.14 (q, J = 273.0 Hz), 118.31, 93.38, 33.95, 31.10, 30.38, 27.09, 23.00, 18.91. ESI-MS m/z 381.1 $(M + H)^+$.

2-(5-cyclopentyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10c**). Yield: 20%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (sbr, 2H), 3.42-3.24 (m, 3H), 3.15 (m, 2H), 2.32-1.64 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.21, 167.93, 162.19, 161.39, 141.01, 131.99, 127.54 (q, J = 33.0 Hz), 124.11(q, J = 273.0 Hz), 118.28, 93.40, 35.87, 33.92, 31.15, 31.03, 25.46, 22.97. ESI-MS m/z 395.2 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10d**). Yield: 23%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (sbr, 2H), 3.18-3.22 (m, 3H), 2.95-2.89 (m, 2H), 2.28-2.09 (m, 4H), 1.82-1.49 (m, 4H), 1.41-1.18 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.90, 167.69, 161.83, 160.49, 140.94, 132.55, 127.34 (q, J = 33.0 Hz), 124.11(q, J = 273.0 Hz), 118.94, 93.85, 35.11, 33.88, 31.20, 30.18, 25.58, 25.40, 23.10. ESI-MS m/z 409.2 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-2-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**11a**). Yield: 22%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (d, J =7.6Hz, 1H), 6.19 (sbr, 2H), 3.29-3.20 (m, 2H), 3.07(t, J = 8.0 Hz, 2H), 2.20-2.10 (m, 2H), 2.00-1.95 (m, 2H), 1.75-1.69 (m, 2H), 1.59-1.53 (m, 2H), 1.37-1.28 (m, 5H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.71, 161.07, 159.32, 154.94, 138.69, 132.22, 126.02 (q, J = 34.0 Hz), 123.80 (q, J = 274.0 Hz), 117.76, 92.81, 51.82, 33.14, 32.13, 30.48, 25.14, 24.24, 22.46. ESI-MS m/z 424.2 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-2-yl)-N-cyclopentyl-1,3,4-oxadiazol-2-amine (**11b**). Yield: 22%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12 (sbr, 2H), 4.68 (d, J = 6.8 Hz, 1H), 4.10-4.16 (m, 1H), 3.32-3.26 (m, 2H), 3.17-3.10 (m, 2H), 2.27-2.18 (m, 2H), 2.15-2.05 (m, 2H), 1.83-1.60 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.53, 161.10, 158.48, 154.14, 138.41, 132.09, 126.12(q, J = 34.0 Hz), 123.96 (q, J = 274.0 Hz), 118.78, 92.03, 55.53, 33.88, 33.28, 31.05, 23.57, 23.00. ESI-MS m/z 410.1 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-2-yl)-N-cyclohexyl-N-methyl-1,3,4-oxadiazol-2-amine (**12a**). Yield: 28%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.10 (s, 2H), 3.92-3.86 (m, 1H), 3.32-3.28 (m, 2H), 3.16-3.12 (m, 2H), 3.05 (s, 3H), 2.27-2.17 (m, 2H), 1.89-1.86 (m, 4H), 1.75-1.70 (m, 2H), 1.59-1.39 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.10, 161.69, 159.69, 155.21, 137.89, 130.78, 126.06 (q, J = 34.0 Hz), 123.84 (q, J = 274.0 Hz), 117.75, 93.69, 56.90, 32.85, 30.06, 29.24, 28.70, 24.60, 24.41, 22.00. ESI-MS m/z 438.2 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinolin-3-amine (**10e**). Yield: 21%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (sbr, 2H), 3.12-3.01 (m, 4H), 2.92-2.86 (m, 1H), 2.13-2.02 (m, 2H), 1.85-1.78 (m, 6H), 1.68-1.56 (m, 3H), 1.40-1.22 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.79, 162.05, 159.36, 159.08, 141.25, 130.37 (q, J = 32.0 Hz), 127.85, 124.49 (q, J = 274.0 Hz), 119.51, 93.33, 35.06, 33.84, 30.15, 25.58, 25.40, 22.67, 21.67. ESI-MS m/z 423.2 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinolin-2-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**11c**). Yield: 21%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.17 (sbr, 2H), 4.75 (d, J = 6.8Hz, 1H), 3.69-6.41 (m, 1H), 2.15-2.13 (m, 3H), 2.05-1.70 (m, 9H), 1.70-1.1 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.92, 159.08, 158.39, 156.57, 139.61, 129.89 (q, J = 32.0 Hz), 127.65, 124.61 (q, J = 274.0 Hz), 119.81, 94.14, 52.72, 33.90, 33.11, 27.00, 25.45, 24.58, 22.74, 21.78. ESI-MS m/z 438.2 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinolin-2-yl)-N-cyclohexyl-N-methyl-1,3,4-oxadiazol-2-amine (**12b**). Yield: 22%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.18 (sbr, 2H), 3.12 (s, 3H), 2.20-2.07 (m, 1H), 1.96-1.70 (m, 10H), 1.40-1.18 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.45, 159.12, 158.41, 156.12, 139.66, 133.21, 128.89 (q, J = 32.0 Hz), 124.61 (q, J = 274.0 Hz), 119.83, 94.10, 63.81, 52.73, 33.30, 29.17, 28.43, 27.10, 25.47, 24.52, 22.73, 21.78. ESI-MS m/z 452.2 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-4-(trifluoromethyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[3,2-e]pyridin-3-amine (**10f**). Yield: 25%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (sbr, 2H), 3.22-2.88 (m, 5H), 2.10-2.06 (m, 3H), 1.82-1.61 (m, 10H), 1.60-1.43 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.89, 164.88, 162.00, 158.53, 141.51, 134.10, 128.24 (q, J = 31.0 Hz), 123.18 (q, J = 274.0 Hz), 119.65, 93.68, 38.46, 35.10, 31.05, 30.18, 29.89, 26.98, 26.39, 25.58, 25.42. ESI-MS m/z 437.2 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-4-(perfluoroethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10g**). Yield: 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.35 (s, 2H), 3.29-3.15 (m, 2H), 3.14-3.01 (m, 2H), 2.94-2.83 (m, 1H), 2.25-2.00 (m, 5H), 1.83-1.78 (m, 2H), 1.69-1.52 (m, 5H). ^{19}F NMR (100 MHz, CDCl3) δ -82.77, -103.92. ESI-MS m/z 459.1 $(M + H)^+$.

2-(5-cyclohexyl-1,3,4-oxadiazol-2-yl)-5-methyl-4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-3-amine (**10h**). Yield: 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.27 (s, 2H), 3.40-3.30 (m, 1H), 3.01-2.91 (m,1H), 2.22-2.06 (m, 4H), 1.96-1.81 (m, 6H), 1.81-1.61 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.87, 166.02, 162.41, 161.90, 140.59, 131.69 (q, J = 34.0 Hz), 123.10 (q, J = 272.0 Hz), 117.94, 115.15, 93.56, 48.05, 35.07, 33.58, 30.16, 29.70, 25.87, 25.59, 25.41. ESI-MS m/z 423.1 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[3,2-e]pyridin-2-yl)-N-cyclohexyl-1,3,4-oxadiazol-2-amine (**11d**). Yield: 26%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (d, J = 7.6 Hz, 1H), 6.29 (sbr, 2H), 3.25-3.17 (m, 2H), 3.10-2.98 (m, 2H), 2.01-1.88 (m, 3H), 1.82-1.63 (m, 7H), 1.56-1.16(m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.52, 161.05, 157.13, 155.08, 139.42, 133.82, 128.22 (q, J = 31.0 Hz), 123.10 (q, J = 274.0 Hz), 119.65, 92.63, 64.91, 53.30, 37.86, 32.11, 30.35, 29.07, 26.52, 25.88, 25.14, 24.23. ESI-MS m/z 452.2 $(M + H)^+$.

5-(3-amino-4-(trifluoromethyl)-6,7,8,9-tetrahydro-5H-cyclohepta[b]thieno[3,2-e]pyridin-2-yl)-N-cyclohexyl-N-methyl-1,3,4-oxadiazol-2-amine (**12c**). Yield: 24%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.05 (sbr, 2H), 3.82-3.76 (m, 1H), 3.16-3.13 (m, 2H), 2.96 (s, 3H), 2.05-1.54 (m, 12H), 1.51-1.05 (m, 6H). ^{13}C NMR

(100 MHz, $CDCl_3$) δ 163.22, 161.67, 157.36, 155.35, 138.45, 132.55, 128.24 (q, J = 31.0 Hz), 123.37 (q, J = 274.0 Hz), 118.57, 93.57, 56.93, 37.73, 30.08, 29.27, 28.81, 28.70, 26.03, 25.42, 24.61, 24.41. ESI-MS m/z 466.2 $(M + H)^+$.

2-cyclohexyl-5-(4-(trifluoromethyl)-6,7-dihydro-5H-cyclopenta[b]thieno[3,2-e]pyridin-2-yl)-1,3,4-oxadiazole (**13a**). Yield: 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (s, 1H), 3.24-3.20 (m, 2H), 3.18-3.00 (m, 3H), 2.25-2.11 (m, 2H), 2.09-2.07 (m, 2H), 1.87-1.73 (m, 2H), 1.63 (m, 3H), 1.53-1.36 (m, 2H), 1.37-1.27 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.20, 168.83, 160.96, 159.45, 133.15, 127.40 (q, J = 33.0 Hz), 125.48, 125.03, 124.31 (q, J = 273.0 Hz), 121.06, 34.18, 33.39, 29.47, 25.08, 24.64, 22.71. ESI-MS m/z 394.1 $(M + H)^+$.

HCV antiviral assay and cytotoxicity test.

The Huh 7 cell line was used for antiviral evaluation, which harbors a dicistronic self-replicating HCV RNA replicon with a firefly luciferase gene. The activity of the luciferase reporter is proportional to HCV RNA levels. Briefly, the replicon cells (8000 cells/well) were seeded into two identical sets of 96-well plates and cultured overnight, followed by treatment with various concentrations of compounds for 72 h, which were solubilized in DMSO and dilutions prepared in DMEM. Then, The Steady-Glo luciferase assay system (Promega, Madison, WI) was used to assess the replicon-derived luciferase activity of one set of the cells. In addition, a tetrazolium-based CytoTox-1 cell proliferation assay (Promega, Madison WI) was used to determine cytotoxicity of another set of the plates, and the EC50 and CC50 values were calculated. For cytotoxicity test, such as in HEK293 cell lines, the procedures were similar to that of Huh 7.

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Notes and references

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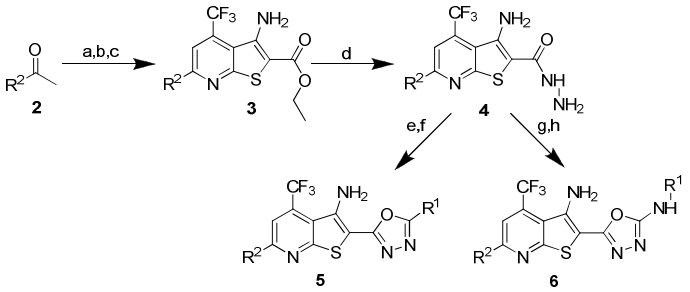
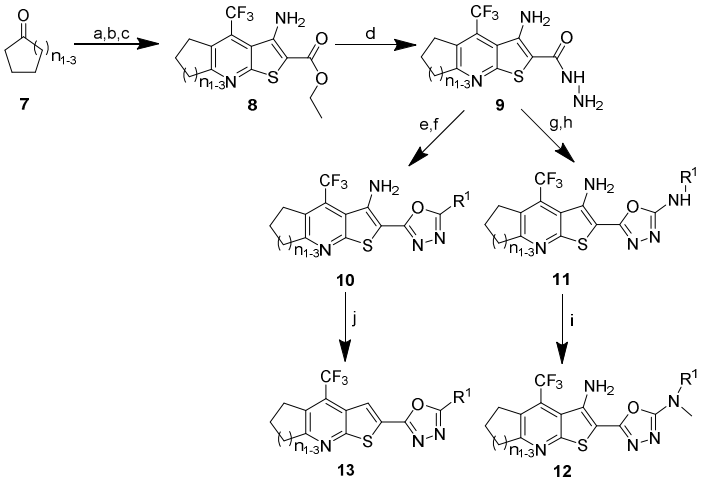
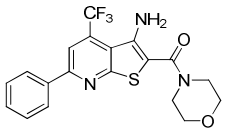
1. Manns, M.P.; Foster, G.R.; Rockstroh, J.K.; Zeuzem, S.; Zoulim, F.; Houghton, M. *Nat. Rev. Drug Discov*. 2007, 6, 991.
2. World Health Organization. Hepatitis C. http://www.who.int/mediacentre/factsheets/fs164/en/ (Accessed April 2014).
3. Shepard, C.W.; Finelli, L.; Alter, M.J. *Lancet Infect Dis*. **2005**, 5, 558.
4. Sapay, N.; Montserret, R.; Chipot, C.; Brass, V.; Moradpour, D.; Deleage, G.; Penin, F. *Biochemistry*. **2006**, 45, 2221.
5. Feld, J.J.; Hoofnagle, J.H. *Nature*. **2005**, 436, 967.
6. Venkatraman, S. *Trends Pharmacol. Sci.***2012**, 33, 289.
7. Kwong, A. D.; Kauffman, R. S.; Hurter, P.; Mueller, P. *Nat. Biotechnol.***2011**, 29, 993.
8. Rosenquist, Å.; Samuelsson, B.; Johansson, P. O.; Cummings, M. D.; Lenz, O.; Raboisson, P.; Simmen, K.; Vendeville, S.; de Kock, H.; Nilsson, M.; Horvath, A.; Kalmeijer, R.; de la Rosa, G.; Beaumont-Mauviel, M. *J. Med. Chem.***2014**, 57, 1802.
9. Rodríguez-Torres, M. *Expert Rev. Anti Infect. Ther*.**2013**, 11, 1269.
10. Belema, M.; Lopez, O. D.; Bender, J. A.; Romine, J. L.St.; Laurent, D. R.; Langley, D. R.; Lemm, J. A.; O'Boyle, D. R., 2nd; Sun, J. H.; Wang, C.; Fridell, R. A.; Meanwell, N. A. *J. Med. Chem.* **2014**, 57, 1643.
11. Sheridan, C. *Nat. Biotechnol.* **2011**, 29, 553.
12. Manns, M. P.; Wedemeyer, H.; Cornberg, M. *Gut*, **2006**, 55, 1350.
13. Liang, T. J.; Ghany, M. G. *N. Engl. J. Med*. **2013**, 368, 1907.
14. Kwong, A. D. *ACS Med. Chem. Lett*. **2014**, 5, 214
15. U.S. Food and Drug Administration. FDA approves first combination pill to treat hepatitis C. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm418365 (Accessed Oct 2014).
16. U.S. Food and Drug Administration. FDA approves Viekira Pak to treathepatitis C. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427530 (Accessed Dec 2014).
17. Wang, N. Y.; Zuo, W. Q.; Xu, Y.; Gao, C.; Zeng, X. X.; · Zhang, L. D.; You, X. Y.; Peng, C. T.; Shen, Y.; Yang, S. Y.; Wei, Y. Q.; Yu, L. T.; *Bioorg. Med. Chem. Lett*. **2014**, 24, 1581.



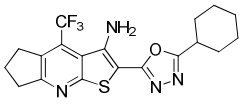
Scheme 1. Synthesis of compounds **5**. **6**. Reagents and conditions: (a) Ethyl trifluoroacetate, sodium methoxide, methanol/THF, r.t., 16~24 h, 40-90%; (b) Cyanothioacetamide, DABCO, ethanol, reflux, 8~12 h, 70-90%; (c) ethyl bromoacetate, KOH (10%), DMF, 4~6 h, 60-90%; (d) hydrazine hydrate, ethanol, 100 ℃, 10~12 h, 80-100%; (e) acyl chloride, THF, 0 ℃, 0.5~2 h, 80-100%; (f) $POCl_3$, 100 ℃, 1 h, 40-60%; (g) isocyanate, DMSO, r.t., 1~2 h, >90%; (h) p-tosyl chloride, TEA, NMP, r.t., 8~12 h, 40-60%.



Scheme 2. Synthesis of compounds **10**, **11**, **12** and **13**. Reagents and conditions: (a) Ethyl trifluoroacetate, NaH, THF, r.t., 40-90%; (b) Cyanothioacetamide, DABCO, ethanol, reflux, 8~12 h, 30-50%; (c) ethyl bromoacetate, KOH (10%), DMF, 4~6 h, 40-70%; (d) hydrazine hydrate, ethanol, 100 ℃, 10~12 h, 80-100%; (e) 1) acyl chloride, THF, 0 ℃, 0.5~2 h, 80-100% ; (f) $POCl_3$, 100 ℃, 1 h, 40-60%; (g) isocyanate, DMSO, r.t., 1~2 h, >90%; (h) p-tosylchloride, TEA, NMP, r.t., 8~12 h, 40-60%; (i) MeI, TEA, DMF, 6~10 h, 60%-80%; (j) tert-Butylnitrite, DMF, 0.5~1 h, 15%-30%.



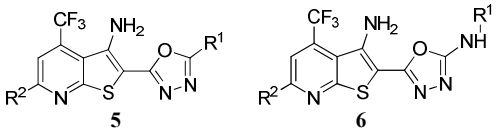
1, EC_{50} = 3.3 μM



10d, EC_{50} = 0.039 μM

Fig. 1. Structure of compound **1** and **10d**

Table 1. Structure -Activity of compound **5a-5i**, **6a-6e**.



5 **6**

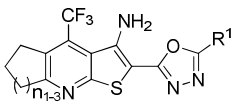
Compd	R^2	R^1	Cytotoxicity CC_{50}^{a} (μM)	GT-1b replicon EC_{50}^{a} (μM)
5a			>10	>10

5b			>10	>10
5c			>10	>10
5d			>10	7.60
5e			>10	3.54
5f			>10	3.71
5g			>10	3.41
5h			>10	2.43
5i			>10	6.8
6a			>10	3.22
6b			>10	3.51

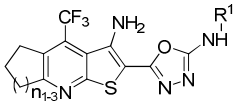
6c			>10	>10
6d			>10	3.11
6e			>10	4.70

[a] The cytotoxicity CC_{50} test and HCV replicon luciferase reporter assay were performed in the HCV 1b Replicon System, for details see supporting information;

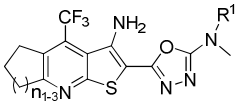
Table 2. Structure -Activity of compound **10a-10h**, **11a-11d**, **12a-12c**, **13a**.



10

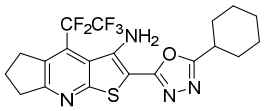
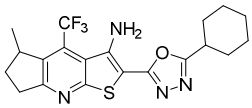


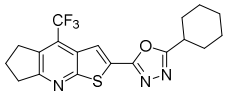
11



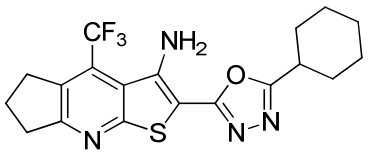
12

Compd	n	R^1	Cytotoxicity CC_{50}[a] (μM)	GT-1b replicon EC_{50}[a] (μM)
10a	1		>10	>10
10b	1		>10	8.55
10c	1		>10	2.20
10d	1		>10	0.039

10e	2		>10	4.30
10f	3		>10	6.71
10g			>10	0.87
10h			>10	>10
11a	1		>10	3.60
11b	1		>10	5.72
11c	2		>10	6.70
11d	3		>10	8.11
12a	1		>10	>10
12b	2		>10	>10

12c	3		>10	>10
13a			0.66	>10

[a] The cytotoxicity CC_{50} test and HCV replicon luciferase reporter assay were performed in the HCV 1b Replicon System, for details see supporting information;



10d HCV Gt-1b EC_{50} = 0.039 μM