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Discovery of imidazo[2,1-b]thiazole HCV NS4B inhibitors exhibiting synergistic effect with other direct-acting antiviral agents

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ABSTRACT

The design, synthesis and SARs studies of novel inhibitors of HCV NS4B which based on imidazo[2,1-b]thiazole scaffold were described. Optimization of potency with respect to genotype 1b resulted in the discovery of two potent leads **26f** (EC_{50}=16 nM) and **28g** (EC_{50}= 31 nM). The resistance profile studies revealed that **26f** and **28g** targeted at HCV NS4B, more precisely the second amphipathic α helix of NS4B (4BAH2). Cross-resistance between our 4BAH2 inhibitors and other direct-acting antiviral agents targeting at NS3/4A, NS5A and NS5B was not observed. For the first time, the synergism of a series of combinations based on 4BAH2 inhibitor was evaluated. The results demonstrated that our 4BAH2 inhibitor **26f** was synergistic with NS3/4A inhibitor Simeprevir, NS5A inhibitor Daclatasvir and NS5B inhibitor Sofosbuvir, and it could also reduce the dose of these drugs at almost all effect levels. Our study suggested that favorable effects could be achieved by combination 4BAH2 inhibitors like **26f** with these approved drugs and new all-oral antiviral combinations based on 4BAH2 inhibitors were worth developing to supplement or even replace current treatment regiments for curing HCV infection.

Introduction

Approximately 150 million people worldwide are tortured by chronic Hepatitis C (CHC) and its complications, which place tremendous economic burden on both the HCV-infected subjects and the healthcare system.[1, 2] Despite considerable effort on the understanding of Hepatitis C, there is still no preventive vaccine available to date.[3] Current standard of care (SoC) for HCV-infected subjects involves a combination of pegylated interferon alpha (PEG IFN-α), ribavirin (RBV) and one of the five approved direct-acting antivirals (DAAs), including Boceprevir,[4] Telaprevir,[5] Simeprevir,[6] Sofosbuvir[7] and Daclatasvir (**Figure 1**).[8] Although these triple therapies can achieve distinct improvement in the

cure rate compared with previous SoC just consisting of PEG IFN-α and RBV, the adverse events associated with interferon and RBV cannot be eliminated, and the treatment durations of these new regiments are still lengthy.[9,10] There is still a long way to go to achieve the final destination of eradication of hepatitis C virus globally.

The high mutation rate of HCV provides it the ability to generate drug resistance to almost all DAAs after a short-term treatment when a single drug is used exclusively, which makes interferon indispensable for traditional treatments. Interferon-sparing combination regimen, which comprises two or more DAAs, could effectively decrease the generation of resistant mutation and achieve high sustained virologic response (SVR) rates, especially when the combination treatments contain an antiviral agent with high resistance barrier such as nucleoside NS5B polymerase inhibitors or the second-generation NS3/4A protease inhibitors.[11] Such all-oral treatments (AOTs) have open a new era for HCV treatment as the approval of Gilead's two-agent combo Harvoni[12] and AbbVie's four-agent combo Viekira Pak[13]. Both of Harvoni and Viekira Pak can achieve SVR in 95%+ of difficult-to-treat genotype 1-infected subjects with duration as short as 8-12 weeks while avoid of the uncomfortable adverse events produced by IFN. These impressive results have spurred us to develop more novel and safe DAAs to supplement current treatment and further shorten the treatment duration to less than 8 weeks.

The HCV genome encodes a polyprotein precursor of about 3010 amino acids, which is processed to yield mature structural (Core, E1, E2, and p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins in the host cells. Almost all current clinical candidates and approved DAA drugs addressing HCV infection target at one of the three nonstructural proteins, including the NS3/4A protease, NS5A, and the RNA-dependent RNA polymerase NS5B. Compared to these nonstructural proteins, the nonstructural protein NS4B is a less well characterized 27 kDa protein which plays several essential roles in HCV replication.[14] And its inhibitors were not well developed or reported until

recently. NS4B could specifically recognize and bind to the 3' terminus of the negative viral strand, and the RNA binding function could be blocked by clemizole (**1a**), an H1 histamine receptor antagonist.[15] This compound, which was the first reported NS4B inhibitor, was found to substantially inhibit HCV replication and be highly synergistic with HCV protease inhibitors in *vitro*.[16] However, the clinical efficiency of clemizole is unavailable at present yet. The second amphipathic α helix of NS4B (4BAH2) could induce vesicle aggregation which is essential for the membranous web formation.[17] It is reported recently that small molecule like anguizole (**1b**) that disrupts the membrane association of 4BAH2 could alter the subcellular distribution pattern of the NS4B protein and thus inhibits the replication of HCV.[18] In addition to anguizole, a series of compounds with various chemotypes have been reported to target at NS4B.[19-24] Because all of these inhibitors shared similar key resistant mutants with anguizole, specifically H94N/R and V105L/M (genotype 1b),[18] to discriminate these NS4B inhibitors from RNA binding inhibitors like Clemizole, we thus termed this kind of NS4B inhibitors as 4BAH2 inhibitor in this study. Some of these 4BAH2 inhibitors exhibit high potency against genotype 1 in vitro and in PXB® mice,[20] the clinical efficiency of these compounds is yet to be verified. More importantly, expected as a part of future oral drug cocktail, the combination effects of this kind of NS4B (4BAH2) inhibitor with other DAAs are also required to be evaluated to provide us the feasible evidence of developing new all-oral anti-HCV therapies based on these 4BAH2 inhibitor.

Our research group was actively seeking new anti-HCV agents.[25] Herein we describe our efforts to develop novel 4BAH2 inhibitor candidates. A series of imidazo[2,1-b]thiazole derivatives were designed and synthesized based on the imidazo[1,2-a]pyridine NS4B inhibitors (**1c**) reported by GlaxoSmithKline.[22] Extensive structure−activity relationship (SAR) studies were performed leading to the discovery of several nanomolar HCV inhibitors and the resistance profile as well as cross-resistance of the most potent compounds **26f** and **28g** were then evaluated. Finally, the most potent compound **26f** was further selected to evaluate the synergism of drug-drug interaction as potential combination partner with other DAAs, including the NS3/4A inhibitor Simeprevir, NS5A inhibitor Daclatasvir, NS5B

inhibitor Sofosbuvir as well as the NS4B RNA binding inhibitor Clemizole, which provide the first evidence of the feasibility of developing new all-oral anti-HCV therapies based on 4BAH2 inhibitors.

Chemistry

The target compounds **26a-26k, 27a-27f, 28a-28g** for this study were prepared by coupling two crucial building blocks, the carboxylic acid segment **A** and the amine segment B, with EDCI and HOBt in dichloromethane (**Scheme 1**). Unless commercially available, the preparation of the important intermediates 2-aminothiazoles for the synthesis of carboxylic acid segment **A** are illustrated in **Scheme 2**. Reacting 1-cyclopropylethanone **2** with bromine in methanol furnished α-bromoketone **3**, which was cyclized with thiocarbamide in ethanol to afford 4-cyclopropylthiazol-2-amine **8f**. Starting from the common substrate 4-methylpentan-2-one, compound **8d** and **8g** were prepared after two collective steps with a ratio of 1:7 due to lack of chemoselectivity for bromination. For the synthesis of 5-isopropylthiazol-2-amine **8c**, bromination and cyclization were completed one pot due to the instability of 2-bromo-3-methylbutyraldehyde under room temperature.[26] Cyclization of various 2-aminothiazoles **8a-g** with ethyl bromopyruvate followed by chlorination of the resultant intermediates **9a-g** furnished imidazo[2,1-b]thiazole-6-carboxylic acid esters **10a-i**, which went through LiOH-mediated ester hydrolysis to produce carboxylic acids **11a-i (Scheme 3)**.

The tricyclic carboxylic acids were prepared from cyclic ketone or commercially available 2-benzothiazolamines through the sequence depicted in **Scheme 4**. Bromination of cyclopentanone **12a** through a literature procedure[27] followed by cyclization of the resultant 2-bromocyclopentanone produced 5,6-dihydro-4*H*-cyclopenta[d]thiazol-2-amine **14a**, The corresponding tricyclic carboxylic acid **16a** was then obtained from **14a** following our standard cyclization-chlorination-hydrolysis sequence. This method could also be applied to the synthesis of tricyclic carboxylic acids **16b-f (Experimental Section)**. Commercially available 2-benzothiazolamines **17a-e** proceeded standard cyclization-chlorination-hydrolysis steps to afford corresponding tricyclic carboxylic acids **18a-e**.

The amine building block **B** was synthesized as outlined in **Scheme 5**. Treatment glycine with 4-nitrobenzenesulfonyl chloride in sodium hydroxide aqueous produced 2-(4-nitrophenylsulfonamido)acetic acid **22**, which was condensed with various amines and cyclized with 1,2-dibromoethane to give the piperazinone intermediates **24a-c**.[28] Subsequently deprotection of **24a-c** by thiols like methyl 3-mercaptopropionate produced corresponding amines **25a-c**.

Results and Discussion

Potency of the compounds described in this study were determined using GT 1b subgenomic replicon assay.[29] Starting from compound **26a**, we first evaluated a number of simple substituents on the thiazole ring, and the results were summarized in **Table 1**. The size of the substituent has a substantial impact on replicon activity. Compound **26a** without substituent on the thiazole ring (EC_{50}>10 μM) exhibited no inhibitory activity against Gt-1b replicon and a small methyl substituted at C2- (**26b**) or C3-position (**26e**) just provided weak inhibitory activity, whereas a much bulkier group like isopropyl (**26c**, EC_{50}=1.32 μM) or cyclopropyl (**26f**, EC_{50}=0.016 μM) provided significant increase in replicon activity. However, the introduction of isobutyl at C3-position (**26g,** EC_{50}=0.74 μM) led to a 46-fold decrease in potency compared with its cyclopropyl counterpart **26f**, suggesting a bulky substituent over 4 carbon atoms at C3-position was unfavourable for the potency. Compound with two substituents on the thiazole ring (**26d**) was less active than its single substituent counterpart (**26c**), which can be further confirmed by comparing the potency of **26f** and **26g** with their C2-position chloro-substituted counterparts **26h** (EC_{50}=8.6 μM) and **26i** (EC_{50}=6.7 μM). As the best substituent on thiazole scaffold was found to be cyclopropyl at C3-position, variants of R3 in the context of 3-cyclopropylimidazo[2,1-b]thiazole were subsequently explored. Unfortunately, when cyclopentyl was replaced by hydrophilic trans-4-hydroxycyclohexyl in **26j** (EC_{50}=0.89 μM), a substantial loss of potency was observed, and attempt to increase potency by introduction of unsaturated five-membered ring (**26k,** EC_{50}=0.079 μM) was also unsuccessful.

Our initial effort have revealed that the imidazo[2,1-b]thiazole core could be a favorable scaffold for HCV inhibitor, and a single substituent at C3-position of thiazole ring with a size of 3 carbon atoms was optimal. We next questioned if a ring fused to the imidazo[2,1-b]thiazole scaffold was tolerant. A series of tricyclic analogues were thus synthesized and evaluated. As shown in **Table 2**, the introduction of various saturated rings fused to the imidazo[2,1-b]thiazole resulted in a series of less potent HCV inhibitors and the potency of the analogues declined as the size of the fused rings increasing (**27a**, EC_{50}=0.13 μM, **27b**, EC_{50}=0.19 μM, **27c**, EC_{50}=0.94 μM), consistant with the SAR observed in the bicyclic series. Among the six-membered fused ring analogues, compound without substituent on the fused ring (**27b**) proved to be the most potent. The introduction of a single methyl provided a slightly decrease in potency, while the gem-dimethyl variant showed a 6-fold loss of potency. The replacement of the hydrophobic cyclohexane ring in **27b** with a relative hydrophilic cycloketone ring resulted in an inactive analogue (**27f**), suggesting hydrophobic interactions between this region and the target existed. A series of rigid benzo[d]imidazo[2,1-b]thiazole derivatives were thus synthesized. When the saturated rings fused on the imidazo[2,1-b]thiazole scaffold were replaced by a benzene ring in **28a** (EC_{50}=0.092 μM), a slight improvement in potency was observed, whereas the introduction of substituents into benzene rings (**28b-28e**) were detrimental to the potency, regardless of their electronic and lipophilic effects, which might attribute to their size exceeding the optimal range, consistent with the trends in **27a-f**. The influence of R3 on the potency was also investigated. The replacement of cyclopentyl in **28a** by trans-4-hydroxycyclohexyl in **28f** (EC_{50}=0.33 μM) led to a 3-fold decrease in potency, while the introduction of unsaturation to the five-membered ring (**28g**, EC_{50}=0.031 μM) provided 3-fold increase in potency. **28g** combined with **26f** outstanding from the above series of imidazo[2,1-b]thiazole derivatives were selected for further biologic evaluation.

In order to verify that the synthesized imidazo[2,1-b]thiazole HCV inhibitors indeed target at NS4B, side-by-side transient transfection GT 1b assays with wild type (WT) and a panel of representative resistant replicons of NS4B were performed on **26f** and **28g,** with the results shown in

Table 3. Three of the five strains bearing NS4B mutations (H94R, F98C, V105M),[18,22] which have emerged as a hallmark of many 4BAH2 inhibitors such as anguizole and **1c**, showed significant resistance to **26f** and **28g**. Unexpectedly, the other two strains bearing NS4B mutations (H3R and Q26R) mapped to N-terminal region of NS4B, which were reported resistant to Bristol Myers Squibb's undisclosed NS4B inhibitor,[14] showed resistance to **26f** and **28g** as well. To preclude the possibility that our specific imidazo[2,1-b]thiazole scaffold endow their resistance to these resistant strains, the imidazo[1,2-a]pyridine 4BAH2 inhibitor **1c** was also evaluated for its potency against these two resistant strains. The results in Table **3** showed that **1c** exhibited significant shift in potency on both strains. Although other 4BAH2 inhibitors were not evaluated in this assay, our study proved that N-terminal region of NS4B was also important for the potency of some 4BAH2 inhibitor. The observation of cross resistance between our imidazo[2,1-b]thiazole HCV inhibitors and the reported 4BAH2 inhibitor **1c** had confirmed that the imidazo[2,1-b]thiazole derivatives in our study were NS4B inhibitors, more precisely 4BAH2 inhibitors.

We further investigated whether there was cross-resistance between our 4BAH2 inhibitors and other DAAs, including NS3/4A inhibitors, NS5A inhibitors, nucleoside and non-nucleoside NS5B inhibitors. The potency of **26f** and **28g** against a panel of HCV replicons containing representative mutations resistant to NS3/4A inhibitors (A156T),[30] NS5A inhibitors (Y93H),[31,32] nucleoside (S282T)[33] and non-nucleoside (M423I, P495A, Y448H)[34] NS5B inhibitors were tested, and all of these replicons were equally sensitive to **26f** and **28g** compared with the wild type 1b replicon (**Table 3**), suggesting that there was no cross-resistance between our 4BAH2 inhibitors and other DAAs. It also implied the potential of the combination of our 4BAH2 inhibitors with other DAAs to develop novel antiviral therapies.

Combination therapy is indispensable for chronic hepatitis C individual, and it has become increasingly clear that traditional interferon-based SoC will soon be replaced by all-oral therapies.

Previous studies have developed a series of all-oral anti-HCV regimens and the efficiency of which have been verified in clinic.[35-39] However, to our knowledge there is no report addressed on the possibility of developing novel all-oral anti-HCV regimens based on 4BAH2 inhibitor currently, even if in vitro. To investigate the drug-drug interaction between our 4BAH2 inhibitors and other DAAs, we next assessed the additive, antagonistic, or synergistic properties of combination therapies based on our 4BAH2 inhibitor **26f** in the HCV subgenomic GT 1b replicon system.

The Combination Index (CI) is considered to be the golden standard to define the synergism of drug-drug interaction.[40-42] CI values=1 always represents additive effect, while CI values <1 and >1 mean synergistic and antagonistic interactions, respectively. Thus, a low CI value stands for strong synergism, and vice versa. The Dose-Reduction Index (DRI) is another important parameter to assess the synergism of drug-drug interaction. It is a measurement of how many fold the dosage of each drug in a combination may be reduced at a given effect level compared with the dosage of each drug used alone.[41] DRI<1 indicates unfavorable dose-reduction, whereas DRI>1 indicates favorable dose-reduction, which is expected in drug combination therapies. Three representative marketed DAAs, the NS3/4A inhibitor Simeprevir, the NS5A inhibitor Daclatasvir, the NS5B inhibitor Sofosbuvir as well as the NS4B RNA binding inhibitor Clemizole were selected to combine with **26f** to evaluate the CI and DRI in a broad range of concentrations.

The combination of **26f** and Simeprevir resulted in greater inhibition than either compound alone at almost all tested concentrations (**Table S2**), while no obvious cytotoxicity was observed at all these concentrations. Synergistic interactions were observed in a broad range of concentrations, and the synergistic interactions strengthened gradually as the concentration of **26f** increasing (**Figure 3a**), while no obvious trend was observed as the concentration of Simeprevir increasing. Similar synergistic interactions and safety profiles were also observed in the Daclatasvir-**26f** and Sofosbuvir-**26f** combinations, In both assays the synergistic interactions were reinforced as the concentration of **26f**

increasing (**Figure 3b, c**). Because synergism at high effect levels is much more therapeutically relevant than at low effect levels for infectious diseases like hepatitis C,[41] our study had demonstrated the advantages of combination therapies based on 4BAH2 inhibitors like **26f**, especially when it combined with an inhibitor with high resistance barrier such as Sofosbuvir.

Recent study in cancer therapy have demonstrated that two drugs that hit the same biological target through different mechanism of actions can potently synergize to give enhanced anticancer effect.[43] We hypothesized that this combination effect could be applied to antiviral therapy. The potency and cytotoxicity of our 4BAH2 inhibitor **26f** in combination with Clemizole, a NS4B RNA binding inhibitor, were thus tested. The combination of **26f** and Clemizole resulted in additive to synergistic effects at low concentration combinations, while antagonistic interactions were observed at high concentrations of either compound (**Figure 3d**). Reasonable explanation may be that the NS4B is a membrane protein, and small molecular NS4B inhibitor like Clemizole or **26f** could disrupt the normal transmembrane structure and thus weaken the potency of another NS4B inhibitor, which in turn resulted in the antagonistic effects at high concentrations of either compound. Moreover, cytotoxicity was also observed under high concentrations of Clemizole, consistant with previous study which reported this compound had a narrow safety window for HCV therapy.[16] Anyway, our study had shown that two NS4B inhibitors with different mechanism of actions could, at least at low concentration combinations, synergistically inhibit the replication of HCV.

The DRI-effect (DRI-F_a) Plot (**Figure 4**) for above combination therapies showed that **26f** could reduce the dose of the other drugs from 1 to 7 -fold in almost all Fa range, including the NS4B RNA binding inhibitors Clemizole. And all of the four drugs could also reduce the dose of **26f** in almost all Fa range. This result further supported the advantages of combination 4BAH2 inhibitor **26f** with other DAAs for HCV therapy.

Over all, the combination therapy studies have provided us the first proof that 4BAH2 inhibitors

such as **26f** could combine with other DAAs in prevent HCV infection, which are worth developing to be new all-oral anti-HCV therapies.

Conclusion

In summary, we described the design, synthesis, SAR studies of a novel class of NS4B (4BAH2) inhibitors based on imidazo[2,1-b]thiazole scaffold. Extensive SAR studies were performed at C2- and C3-positions on imidazo[2,1-b]thiazole core to discover that a single substituent on the thiazole ring with a size of 3 carbon atoms (**26f)** was optimal. A series of tricyclic analogues which introduction of a saturated or an unsaturated ring fused to the imidazo[2,1-b]thiazole scaffold were also synthesized and evaluated to uncover that a simple benzene ring fused to the imidazo[2,1-b]thiazole scaffold (**28g**) was optimal. **26f (**EC_{50}=16 nM) and **28g** (EC_{50}=31 nM) outstanding as their good potency against HCV genotype 1b replicon, were selected for further resistance profile and combination therapy studies.

The resistance profile studies confirmed that **26f** and **28g** targeted at NS4B. And there was no cross-resistance between our 4BAH2 inhibitors and other DAAs targeting at NS3/4A, NS5A and NS5B. For the first time, the synergism of a series of combinations based on our 4BAH2 inhibitor **26f** was evaluated to discover that **26f** could be synergistic with NS3/4A inhibitor Simeprevir, NS5A inhibitor Daclatasvir and NS5B inhibitor Sofosbuvir to inhibit the replication of GT 1b replicon, and it could also reduce the dose of these drugs in almost all Fa range, suggesting favorable effects could be achieved by combination 4BAH2 inhibitors like **26f** with these approved drugs and new all-oral anti-HCV combinations based on 4BAH2 inhibitors are worth developing to supplement or even supplant current treatment strategies.

Combination **26f** with NS4B RNA binding inhibitor Clemizole aimed at determining whether two drugs that hit the same biological target through different mechanism of actions could potently synergize to inhibit viral replication revealed that **26f** could be additive/synergistic with Clemizole at low concentration combinations. Although no favorable effect was observed, our study implied that two

drugs acting on the same biological target through different mechanism of actions might potently synergize to inhibit the replication of HCV or other viruses.

Experimental Section

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE^{III} 400 spectrometer (Bruker Company, Germany) at 25 ℃ using DMSO-d_6 or $CDCl_3$ as the solvent. Chemical shifts (δ) were reported in ppm relative to Me_4Si (internal standard), and coupling constants (J) were reported in Hz, and peak multiplicity are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Mass Spectra (MS) were performed on a Waters Q-TOF Premier mass spectrometer (Micromass, Manchester, UK). Thin layer chromatography (TLC) was performed on 0.20 mm Silica Gel F-254 plates (Qingdao Haiyang Chemical, China), and column chromatography were performed using Silica gel 60 of 300-400 mesh (Qingdao Haiyang Chemical, China). The purity of all the title compounds was determined on an UltiMate 3000 (Dionex, USA) HPLC system, and were of >95% purity. HPLC conditions to assess the purity of the final compounds were as follows: column, Atlantis dC18, 4.6 mm × 150 mm, 5 μm; mobile phase, methanol(55%)/water(45%) or methanol(65%)/water(35%); flow rate, 1.0 mL/min; UV wavelength, 190 − 400 nm; temperature, 35 ℃; injection volume, 10 μ L.

5-Isopropylthiazol-2-amine **(8c)**.[26] A solution of bromine (1.0 mL, 20 mmol) in dichloromethane/ dioxane (V/V = 4/1, 12 mL) was slowly added to a cooled solution of isovaleraldehyde (1.721 g, 20 mmol) in dichloromethane/dioxane (V/V = 4/1, 36 mL). The mixture was stirred at 10 ℃ for 2 h and was slowly added to a suspension of thiourea (1.523 g, 20 mmol) in dichloromethane/dioxane (V/V = 4/1, 30 mL). Ethanol (6 mL) and triethylamine (2.424 g, 24 mmol) were added to the reaction mixture with vigorous stirring for 20 h at room temperature. The resulting mixture was diluted with water (100 mL), alkalized to pH = 12 with 12 N aqueous NaOH and stirred at room temperature for another 1 h.

Then it was extracted with dichloromethane, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to dryness. The residue was purified by column chromatography with ethyl acetate/ petroleum ether to afford 5-isopropylthiazol-2-amine **8c** (1.202 g, 42.26% yeild) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 (s, 1H), 4.84 (s, 2H), 2.99 (d, J = 6.8 Hz, 1H), 1.25 (t, J = 8.3 Hz, 6H).

4-Isobutylthiazol-2-amine **(8g)** and *4-isopropyl-5-methylthiazol-2-amine* **(8d)**. To a solution of 4-methylpentan-2-one (2.003 g, 20 mmol) in methanol at 0 ℃ was slowly added bromine (1.0 mL, 20 mmol) dropwise. The mixture was stirred at room temperature for 2 h, diluted with water and stirred for another 30 min. Then the mixture was partitioned between Et_2O/water and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford a mixture of 3-bromo-4-methylpentan-2-one (**5**) and 1-bromo-4-methylpentan-2-one (**6**), which was used in the next step without further purification.

The mixture of **5** and **6** from above step and thiourea (1.523 g, 20 mmol) were dissolved in ethanol and heated under reflux for 6 h. Then the reaction mixture was cooled to room temperature and concentrated under vacuum to dryness. The residue was dissolved in water, alkalized to pH = 12, and extracted with ethyl acetate, The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude intermediate was purified by column chromatography with ethyl acetate/ petroleum ether to afford **8d** and **8g**. 4-Isopropyl-5-methylthiazol-2-amine (**8d),** 0.384 g, 12.29% yeild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (br s, 2H), 3.07 (dt, J = 13.7, 6.8 Hz, 1H), 2.13 (s, 3H), 1.20 (d, J = 6.8 Hz, 6H). 4-Isobutylthiazol-2-amine (**8g**), 2.563g, 82.02% yeild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.07 (br s, 1H), 5.07 (s, 2H), 2.37 (d, J = 7.1 Hz, 2H), 1.98 (dd, J = 13.4, 6.7 Hz, 1H), 0.91 (d, J = 6.6 Hz, 6H).

4-Cyclopropylthiazol-2-amine **(8f).** Compound **8f** was prepared from 1-cyclopropylethanone (1.681 g, 20 mmol) by following the procedures described for the preparation of **8d** and **8g**. 1.900 g, 67.86% yield

as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.63 (br s, 2H), 5.99 (s, 1H), 1.78-1.69 (m, 1H), 0.80-0.69 (m, 4H).

Ethyl imidazo[2,1-b]thiazole-6-carboxylate **(9a).** To a solution of 2-amino thiazole (0.500 g, 5 mmol) in tetrahydrofuran was added ethyl bromopyruvate (90%, 1.625 g, 7.5 mmol) dropwise and the reaction was stirred at room temperature for 8 h. Then the precipitate was filtered and washed with THF. The filter cake was then dissolved in ethanol and heated under reflux for 8 h. Then the resulting reaction mixture was cooled to room temperature and concentrated under vacuum to dryness. The residue was diluted with water and the precipitate was filtered, washed with water, and dried in vacuum to afford intermediate **9a** (0.375 g, 38.27% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.95 (d, J = 4.5 Hz, 1H), 7.45 (d, J = 4.5 Hz, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H).

General Procedures of Method A for the Synthesis of 9b-9g. The intermediate 2-aminothiazoles (**8b-8g**) (1.0 equiv) and ethyl bromopyruvate (1.0 equiv) was dissolved in butanone and heated under reflux for 12 h, then another portion of ethyl bromopyruvate (1.0 equiv) was added to the reaction mixture and it was heated under reflux for another 12 h. The resulting reaction mixture was cooled to room temperature and concentrated under vacuum to dryness. The residue was extracted with ethyl acetate and water, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude intermediate was purified by column chromatography with dichloromethane/methanol to afford **9b-9g.**

Ethyl 2-methylimidazo[2,1-b]thiazole-6-carboxylate (**9b**), 16.86% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.51 (s, 1H), 4.23 (t, J = 8.0 Hz, 2H), 2.44 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H).

Ethyl 2-isopropylimidazo[2,1-b]thiazole-6-carboxylate (**9c**), 30.04% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.72 (s, 1H), 4.25 (d, J = 7.1 Hz, 2H), 3.18-3.12 (m, 1H), 1.30-1.25 (m, 9H).

Ethyl 2-isopropyl-3-methylimidazo[2,1-b]thiazole-6-carboxylate (**9d**), 22.09% yield, ^{1}H NMR (400

MHz, DMSO-d_6) δ 8.01 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 3.19-3.14 (m, 1H), 1.30-1.24 (m, 9H). MS(ESI) *m/z*: 251.0 $[M-H]^-$.

Ethyl 3-methylimidazo[2,1-b]thiazole-6-carboxylate (**9e**), 41.33% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 6.83 (s, 1H), 4.39 (q, J = 7.1 Hz, 2H), 2.48 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H). MS(ESI) *m/z*: 211.0 $[M+H]^+$.

Ethyl 3-cyclopropylimidazo[2,1-b]thiazole-6-carboxylate (**9f**), 37.89% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 6.49 (s, 1H), 4.42 (q, J = 6.9 Hz, 2H), 2.15 (s, 1H), 1.43 (t, J = 7.0 Hz, 3H), 1.11-1.01 (m, 2H), 0.85-0.79 (m, 2H). MS(ESI) *m/z*: 237.0 $[M+H]^+$.

Ethyl 3-isobutylimidazo[2,1-b]thiazole-6-carboxylate (**9g**), 17.74% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 6.77 (s, 1H), 4.40 (q, J = 6.9 Hz, 2H), 2.56 (d, J = 7.1 Hz, 2H), 2.05-1.95 (m, 1H), 1.40 (t, J = 7.0 Hz, 3H), 0.93 (d, J = 6.6 Hz, 6H). MS(ESI) *m/z*: 253.1 $[M+H]^+$.

General Procedures of Method B for the Synthesis of 10a - 10g. To a solution of **9a - 9g** (1.0 equiv, 0.2 mol/L) in N,N-dimethylformamide at room temperature was slowly added N-chlorosuccinimide (1.05 equiv), and the mixture was stirred for 3-6 h until the substrate had been completely consumed, then the reaction mixture was diluted with water and stirred for another 30 min. The precipitate was filtered, washed with cold water and dried in vacuum to afford intermediate **10a**-**10g.**

Ethyl 5-chloro-imidazo[2,1-b]thiazole-6-carboxylate (**10a**), 87.14% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 3.3 Hz, 1H), 7.21 (s, 1H), 4.46 (q, J = 7.0 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H).

Ethyl 5-chloro-2-methylimidazo[2,1-b]thiazole-6-carboxylate (**10b**), 84.45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 4.25 (t, J = 8.0 Hz, 2H), 2.45 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H).

Ethyl 5-chloro-2-isopropylimidazo[2,1-b]thiazole-6-carboxylate (**10c**), 78.01% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (s, 1H), 4.33 (d, J = 7.1 Hz, 2H), 3.24-3.17 (m, 1H), 1.45-1.35 (m, 9H).

Ethyl 5-chloro-2-isopropyl-3-methylimidazo[2,1-b]thiazole-6-carboxylate (**10d**), 100% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22 (q, J = 7.0 Hz, 2H), 3.18-3.12 (m, 1H), 1.33 (t, J = 7.1 Hz, 3H), 1.24 (d, J = 13.2 Hz, 6H).

Ethyl 5-chloro-3-methylimidazo[2,1-b]thiazole-6-carboxylate (**10e**), 52.11% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 1H), 4.35 (q, J = 7.0 Hz, 2H), 2.59 (s, 3H), 1.36 (t, J = 7.0 Hz, 3H).

Ethyl 5-chloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carboxylate (**10f**), 77.43% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (s, 1H), 4.43 (q, J = 6.9 Hz, 2H), 2.25 (s, 1H), 1.44 (t, J = 7.0 Hz, 3H), 1.13-1.03 (m, 2H), 0.89-0.81 (m, 2H).

Ethyl 5-chloro-3-isobutylimidazo[2,1-b]thiazole-6-carboxylate (**10g**), 58.58% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (s, 1H), 4.40 (q, J = 6.9 Hz, 2H), 2.81 (d, J = 7.1 Hz, 2H), 2.07-1.96 (m, 1H), 1.43 (t, J = 7.0 Hz, 3H), 0.95 (d, J = 6.6 Hz, 6H).

General Procedures for the Synthesis of 10h and 10i. To a solution of **9f** or **9g** (1.0 equiv, 0.2 mol/L) in DMF at room temperature was slowly added N-chlorosuccinimide (1.05 equiv), and the mixture was stirred for 3-6 h. Then another portion of N-chlorosuccinimide (1.05 equiv) was added and stirred for 12 h. The reaction mixture was then diluted with water and stirred for another 30 min. The precipitate was filtered, washed with cold water and dried in vacuum to afford dual-chlorinated intermediates **10h**-**10i**.

Ethyl 2,5-dichloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carboxylate (**10h**), 69.53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45 (q, J = 7.0 Hz, 2H), 2.05 (s, 1H), 1.45 (t, J = 7.0 Hz, 3H), 1.15-1.07 (m, 2H), 0.91-0.83 (m, 2H). MS(ESI) *m/z*: 305.0 $[M+H]^+$.

Ethyl 2,5-dichloro-3-isobutylimidazo[2,1-b]thiazole-6-carboxylate (**10i**), 43.11% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.43 (q, J = 7.0 Hz, 2H), 2.85 (d, J = 7.0 Hz, 2H), 2.09-1.98 (m, 1H), 1.45 (t, J = 7.0 Hz, 3H), 0.97 (d, J = 6.6 Hz, 6H) MS(ESI) *m/z*: 320.0 $[M+H]^+$.

General Procedures of Method C for the Synthesis of 11a-11i. 1N aqueous LiOH (5.0 equiv) was added to a solution of **10a-10i** in THF/methanol (v/v=1:1, 0.1 mol/L), the reaction mixture was stirred at room temperature for 3-12 h until the substrate had been completely consumed. Then the reaction mixture was concentrated under vacuum and the residue was dissolved in water and acidified to pH=3 with 4N aqueous HCl. The precipitate was filtered, washed with cold water, and dried in vacuum to afford **11a-11i**, which was used in the next step without further purification.

General Procedures of Method D for the Synthesis of 14a-14f.[27] NBS (1.05 equiv) was added slowly to a solution of **12a-12f** (1.0 equiv, 0.2 mol/L) in DMSO at room temperature and the mixture was stirred for 10-30 min. After completion of the reaction as monitored by TLC, the reaction mixture was quenched with ammonium chloride solution and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford the α-brominated cyclic ketones **13a-13f** which were used in the next step without further purification.

A solution of α-brominated cyclic ketones **13a-13f** and thiourea (1.523g, 20mmol) in ethanol was heated under reflux for 3-6 h until the substrate was consumed as monitored by TLC. The reaction mixture was cooled to room temperature and concentrated under vacuum to dryness. The residue was dissolved in water and alkalized to pH = 12 to allow the product to deposit. The product was collected by filtration, washed with cold water and dried under vacuum to afford the desired thiazol-2-amines **14a-14f**.

5,6-Dihydro-4H-cyclopenta[d]thiazol-2-amine (**14a**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (br s, 2H), 2.75 – 2.63 (m, 2H), 2.58 (dd, J = 10.1, 4.4 Hz, 2H), 2.35 – 2.23 (m, 2H).

4,5,6,7-Tetrahydrobenzo[d]thiazol-2-amine (**14b**), 74.68% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.79 (br s, 2H), 2.59-2.52 (m, 4H), 1.89 – 1.66 (m, 4H).

5,6,7,8-Tetrahydro-4H-cyclohepta[d]thiazol-2-amine (**14c**), 39.43% yield, ^{1}H NMR (400 MHz,

$CDCl_3$) δ 4.98 (s, 2H), 2.72 – 2.63 (m, 2H), 2.63 – 2.53 (m, 2H), 1.77 (d, J = 3.7 Hz, 2H), 1.73 – 1.60 (m, 4H).

2-Amino-5,6-dihydrobenzo[d]thiazol-7(4H)-one (**14d**), 23.27% yield, ^{1}H NMR (400 MHz, DMSO) δ 8.08 (s, 2H), 2.67 (t, J = 6.1 Hz, 2H), 2.36 (t, J = 6.4 Hz, 2H), 2.03 – 1.93 (m, 2H).

6-Methyl-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (**14e**), 65.32% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.81 (br s, 2H), 2.75 – 2.63 (m, 2H), 2.38 – 2.27 (m, 1H), 2.08-2.01 (m, 2H), 1.69 – 1.57 (m, 2H), 1.10 (d, J = 9.2 Hz, 3H).

6,6-Dimethyl-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (**14f**), 58.93% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82 (br s, 2H), 2.92 (t, J = 7.6 Hz, 2H), 2.38 (s, 2H), 1.76 (t, J = 7.6 Hz, 2H), 1.02 (s, 6H).

General Procedure for the Synthesis of 15a-15f. The tricyclic imidazo[2,1-b]thiazole intermediates **15a-15f** were prepared from **14a-14f** and ethyl bromopyruvate by following the procedures described in Method A**.**

Ethyl *3-chloro-6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b]thiazole-2-carboxylate* (**15a**), 32.29% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94(s, 1H), 4.40 (q, J = 7.2 Hz, 2H), 2.93 – 2.80 (m, 2H), 2.61-2.55 (m, 2H), 2.35 – 2.28 (m, 2H), 1.40(t, J = 7.2 Hz, 3H). MS(ESI) *m/z*: 237.1 $[M+H]^+$.

Ethyl 3-chloro-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carboxylate (**15b**), 40.36% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91(s, 1H), 4.39 (q, J = 7.2 Hz, 2H), 2.69 (s, 2H), 2.61 (s, 2H), 1.95(s, 4H), 1.42(t, J = 7.2 Hz, 3H). MS(ESI) *m/z*: 251.1 $[M+H]^+$.

Ethyl 3-chloro-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole-2-carboxylate (**15c**), 18.35% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93(s, 1H), 4.41 (q, J = 7.2 Hz, 2H), 2.75 – 2.52 (m, 4H), 1.79-1.70 (m, 2H), 1.69 – 1.58 (m, 4H), 1.43(t, J = 7.2 Hz, 3H). MS(ESI) *m/z*: 265.2 $[M+H]^+$.

Ethyl 3-chloro-7-methyl-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carboxylate (**15d**),

35.38% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 4.40 (q, J = 7.1 Hz, 2H), 2.81 – 2.62 (m, 2H), 2.41 – 2.28 (m, 1H), 2.08-2.01 (m, 2H), 1.68 – 1.55 (m, 2H), 1.40 (t, J = 7.0 Hz, 3H), 1.12 (d, J = 9.2 Hz, 3H). MS(ESI) *m/z*: 265.1 $[M+H]^+$.

Ethyl 3-chloro-7,7-dimethyl-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carboxylate (**15e**), 37.77% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 4.42 (q, J = 7.1 Hz, 2H), 2.96 (t, J = 7.6 Hz, 2H), 2.41 (s, 2H), 1.77 (t, J = 7.6 Hz, 2H), 1.43 (t, J = 7.0 Hz, 3H), 1.07 (s, 6H). MS(ESI) *m/z*: 279.2 $[M+H]^+$.

Ethyl 3-chloro-8-oxo-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carboxylate (**15f**), 19.13% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 4.42 (d, J = 7.1 Hz, 2H), 3.03 (d, J = 6.1 Hz, 2H), 2.72 (d, J = 6.7 Hz, 2H), 2.38 (s, 2H), 1.42 (t, J = 7.0 Hz, 3H). MS(ESI) *m/z*: 265.1 $[M+H]^+$.

General Procedure for the Synthesis of 16a-16f. The intermediates **16a-16f** were prepared from **15a-15f** by following two successive steps as described in method B and Method C**.**

General Procedure for the Synthesis of 18a-18e. The intermediates **18a-18e** were prepared from **17a-17e** by following three successive steps as described in method A, B and C**.**

Trans-4-((triisopropylsilyl)oxy)cyclohexanamine trifluoroacetate **(20).** To a solution of *tert*-butyl (*trans*-4-hydroxycyclohexyl)carbamate (4.305 g, 20 mmol) in DMF was added triisopropylchlorosilane (5.794 g, 30 mmol) and imidazole (3.401 g, 50 mmol) at room temperature and stirred for 12h. Then the reaction mixture was extracted with ethyl acetate/water, the organic layer was washed successively with distilled water and brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to afford *tert*-butyl (*trans*-4-((triisopropylsilyl)oxy)cyclohexyl)carbamate (**19**) as a white solid (6.233 g, 83.86%), which was used in the next step without further purification.

Compound **19** (5.574 g, 15.0 mmol) was dissolved in trifluoroacetic acid/dichloromethane (V/V = 1/4), and the mixture was stirred under room temperature for 1h. Then the solvent was removed under

vacuum to afford **20** as a white solid. 5.945 g, 100% yield. MS(ESI) *m/z*: 272.2 $[M+H]^+$.

2-(4-Nitrophenylsulfonamido)acetic acid **(22)**. To a solution of Glycine (10.0 g, 133 mmol) in 1 N aqueous sodium hydroxide (140 mL, 140 mmol) at 0 ℃ was slowly added 4-nitrobenzenesulfonyl chloride (29.5 g, 133 mmol). The reaction mixture was warmed to room temperature after stirring for 30min, and was alkalized to pH> 9 by 1 N sodium hydroxide solution. 2 h later, another portion of 1 N aqueous sodium hydroxide (150 mL, 150 mmol) was added and the mixture was extracted with ethyl acetate (100 mL × 3), the insoluble material in the aqueous phase was filtered and the filtrate was adjust to pH=9 with 6 N aqueous HCl and stirred for 30 min. The precipitate was collected by filtration to obtain **22 (**19.73 g, 56.88 %**)** as a white powder. MS(ESI) *m/z*: 261.1 $[M+H]^+$.

General Procedure for the Synthesis of 23a-23c. To a solution of **22** (1.0 equiv) and various amines (1.0 equiv) in DMF was successively added ethyl diisopropylamine (3.0 equiv) and HATU (1.5 equiv). The mixture was stirred at room temperature for 12 h. Then water was added to the reaction mixture to allow the product to deposit. The precipitate was collected by filtration to obtain **23a-23c.**

N-cyclopentyl-2-(4-nitrophenylsulfonamido)acetamide (**23a**): 73.12% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (d, J = 8.8 Hz, 2H), 8.39 (s, 1H), 8.02 (d, J = 8.8 Hz, 2H), 7.80 (d, J = 6.8 Hz, 1H), 3.86 – 3.77 (m, 1H), 3.51 (s, 2H), 1.72 – 1.27 (m, 8H), MS(ESI) *m/z*: 350.1 $[M+Na]^+$.

N-(cyclopent-3-en-1-yl)-2-(4-nitrophenylsulfonamido)acetamide (**23b**): 78.51% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 – 8.24 (m, 4H), 8.03 (d, J = 8.4 Hz, 2H), 5.66 (s, 2H), 4.25 – 4.03 (m, 1H), 3.52 (d, J = 5.9 Hz, 2H), 2.55 – 2.45 (m, 2H), 2.01 (dd, J = 15.4, 3.5 Hz, 2H). MS(ESI) *m/z*: 326.1 $[M+H]^+$.

2-(4-Nitrophenylsulfonamido)-N-(4-trans-((triisopropylsilyl)oxy)cyclohexyl)acetamide (**23c**): 53.13% yield, ^{1}H NMR (400 MHz, DMSO) δ 8.45-8.35 (m, 3H), 8.02 (d, J = 8.9 Hz, 2H), 7.74 (d, J = 7.6 Hz, 1H), 3.50 (d, J = 5.0 Hz, 2H), 3.38-3.21 (m, 2H), 1.73 (d, J = 9.9 Hz, 2H), 1.59 (d, J = 10.3 Hz, 2H), 1.19 – 0.99 (m, 22H), MS(ESI) *m/z*: 514.1 $[M+H]^+$.

General Procedure for the Synthesis of 24a-24c. To a solution of **23a-23c** (1.0 equiv) in dry DMF was added potassium carbonate (10.0 equiv). The mixture was stirred at 60 ℃ for 30 min and 1,2-dibromoethane (10.0 equiv) was added. After 24h, the solvent was distilled off under vacuum and the residue was suspended in water and stirred for another 30 min. The precipitates were collected by filtration to obtain the crude intermediates, which were recrystallized from ethanol to get **24a-24c.**

1-Cyclopentyl-4-((4-nitrophenyl)sulfonyl)piperazin-2-one (**24a**): 86.68% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 8.4 Hz, 2H), 8.08 (d, J = 8.4 Hz, 2H), 4.66-4.60 (m, 1H), 3.67 (s, 2H), 3.37-3.26 (m, 4H), 1.59 – 1.39 (m, 8H). MS(ESI) *m/z*: 354.1 $[M+H]^+$.

1-(Cyclopent-3-en-1-yl)-4-((4-nitrophenyl)sulfonyl)piperazin-2-one (**24b**): 48.71% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 5.77 (s, 2H), 4.25 – 4.03 (m, 1H), 3.68 (s, 2H), 3.39 (d, J = 5.0 Hz, 2H), 3.28 (d, J = 5.0 Hz, 2H), 2.75-2.65 (m, 2H), 2.06 (dd, J = 15.4, 3.7 Hz, 2H). MS(ESI) *m/z*: 352.1 $[M+H]^+$.

4-((4-Nitrophenyl)sulfonyl)-1-trans-(4-((triisopropylsilyl)oxy)cyclohexyl)piperazin-2-one (**24c**): 42.80% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 8.4 Hz, 2H), 8.09 (d, J = 8.4 Hz, 2H), 4.14-4.04 (m, 1H), 3.65 (s, 2H), 3.65-3.55 (m, 1H), 3.32 (d, J = 5.0 Hz, 2H), 3.26 (d, J = 5.0 Hz, 2H), 1.86 (d, J = 11.3 Hz, 2H), 1.57 – 1.00 (m, 6H), 1.00 (s, 18H). MS(ESI) *m/z*: 540.3 $[M+H]^+$.

General Procedure for the Synthesis of 25a-25c. To a suspension of lithium hydroxide (3.0 equiv) in dry acetonitrile/DMSO (49:1 v/v) at 50 ℃ was added 3-mercaptopropionate (2.5 equiv) with vigorous stirring. After 30 min, a solution of compound **24a-24c** (1.0 equiv) in dry acetonitrile/dimethyl sulfoxide (49:1 v/v) was slowly added to the reaction mixture, which was left to stir for another 1.5-3.0 h. After completion of the reaction as monitored by TLC, the solvent was removed under vacuum and the residue was dissolved in dichloromethane. This organic layer was extracted with 1 N aqueous HCl, and the aqueous phase was alkalized to pH=12 with aqueous KOH (10% m/m). Then the aqueous phase

was extracted with dichloromethane and the combined organic phase was dried over anhydrous $MgSO_4$ and concentrated under vacuum to afford **25a-25c**.

1-Cyclopentylpiperazin-2-one (**25a**): 70.5 % yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.03-4.94 (m, 1H), 3.55 (s, 2H), 3.23 (s, 2H), 3.07 (s, 2H), 1.85-1.45 (m, 8H).

1-(Cyclopent-3-en-1-yl)piperazin-2-one (**25b**): 48.57% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (s, 2H), 5.34 - 5.20 (m, 1H), 3.59 (br s, 2H), 3.38 – 3.26 (m, 2H), 3.20 – 3.05 (m, 2H), 2.59 (dd, J = 16.0 Hz, 8.8Hz , 2H), 2.19 (dd, J = 16.1 Hz, 8.8Hz , 2H).

1-(Trans-4-((triisopropylsilyl)oxy)cyclohexyl)piperazin-2-one (**25c**): 42.19% yield, ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64-4.47 (m, 1H), 3.67-3.50 (m, 3H), 3.26 (s, 2H), 3.11-2.98 (m, 2H), 1.86 - 1.07 (m, 8H), 0.98 (s, 18H).

General Procedures of Method E for the Synthesis of 26a-26k, 27a-27f, 28a-28g. To a suspension of acids (1.0 equiv) and amines (1.2 equiv) in dichloromethane was added EDCI (1.5 equiv) and HOBt (1.2 equiv). The mixture was stirred at room temperature for 3-12 h until the starting materials were consumed. Then water was added to the reaction mixture and the organic phase was washed successively with 1 N aqueous HCl, saturated potassium carbonate solution and brine, dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by column chromatography to afford the title compound.

4-(5-Chloroimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26a**). **26a** was obtained from **11a** (50 mg, 0.25 mmol) and **25a** (50 mg, 0.30 mmol) by following the procedures described in Method E: 56 mg, 64% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 3.4 Hz, 1H), 7.04 (d, J = 4.6 Hz, 1H), 4.95 (d, J = 9.0 Hz, 1H), 4.74 (s, 1H), 4.39 (s, 1H), 4.28 (s, 1H), 3.95 (s, 1H), 3.40 (s, 2H), 1.96 – 1.40 (m, 8H).^{13}C NMR (100 MHz, $CDCl_3$) δ 165.35, 146.51, 134.98, 120.76, 116.54, 115.63, 54.13, 50.92, 47.26, 40.03, 28.04, 24.12. MS(ESI) *m/z*: 391.1 $[M + K]^+$.

4-(5-Chloro-2-methylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26b**). **26b** was obtained from **11b** (64 mg, 0.30 mmol) and **25a** (60 mg, 0.36 mmol) by following the procedures described in Method E: 71 mg, 65% yield as a white solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (s, 1H), 4.87 (s, 1H), 4.62 (s, 1H), 4.33 (s, 1H), 4.15 (s, 1H), 3.87 (s, 1H), 3.33 (s, 2H), 2.43 (s, 3H), 1.79 (s, 2H), 1.72 – 1.37 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.25, 161.33, 145.49, 131.23, 108.22, 54.21, 50.85, 47.11, 40.24, 28.11, 24.12, 14.30. MS(ESI) *m/z*: 389.2 $[M + Na]^+$.

4-(5-Chloro-2-isopropylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26c**). **26c** was obtained from **11c** (118 mg, 0.48 mmol) and **25a** (98 mg, 0.58 mmol) by following the procedures described in Method E: 136 mg, 72% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (s, 1H), 4.96 (s, 1H), 4.75 (s, 1H), 4.38 (s, 1H), 4.29 (s, 1H), 3.94 (s, 1H), 3.38 (s, 2H), 3.20 – 3.04 (m, 1H), 1.86 - 1.40 (m, 8H), 1.38 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.27, 161.09, 145.64, 132.84, 130.94, 128.85, 105.65, 54.11, 50.91, 47.25, 40.25, 29.31, 28.05, 24.11, 23.47. MS(ESI) *m/z*: 395.1 $[M + H]^+$.

4-(5-Chloro-2-isopropyl-3-methylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26d**). **26d** was obtained from **11d** (49 mg, 0.19 mmol) and **25a** (39 mg, 0.23 mmol) by following the procedures described in Method E: 49 mg, 63% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ4.87 (s, 1H), 4.51 (s, 1H), 4.30 (s, 1H), 4.14 – 3.94 (m, 1H), 3.85 (s, 1H), 3.34 (s, 2H), 3.18 (d, J = 5.3 Hz, 1H), 2.54 (s, 3H), 1.79 (s, 2H), 1.72-1.36 (m, 6H), 1.23 (d, J = 13.3 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.11, 160.47, 145.95, 136.25, 123.10, 54.15, 50.93, 47.19, 39.91, 30.03, 28.11, 24.11, 23.79, 11.95. MS(ESI) *m/z*: 431.2 $[M + Na]^+$.

4-(5-Chloro-3-methylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26e**). **26e** was obtained from **11e** (64 mg, 0.30 mmol) and **25a** (60 mg, 0.36 mmol) by following the procedures described in Method E: 74 mg, 67% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.52 (s, 1H), 5.32 (s, 1H), 4.89 (d, J = 8.0 Hz, 1H), 4.56 (s, 1H), 4.31 (s, 1H), 3.87 (s, 1H), 3.33 (s, 2H), 2.61 (s, 3H),

1.80 (s, 2H), 1.70-1.32 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.18, 160.50, 148.07, 134.01, 130.17, 54.19, 50.90, 47.18, 39.99, 28.08, 24.11, 14.51. MS(ESI) *m/z*: 389.2[M + Na] $^{+}$.

4-(5-Chloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26f**). **26f** was obtained from **11f** (243 mg, 1.00 mmol) and **25a** (201 mg, 1.20 mmol) by following the procedures described in Method E: 355 mg, 91% yield as a pale powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, J = 1.2 Hz, 1H), 4.87 (d, J = 7.9 Hz, 1H), 4.57 (s, 1H), 4.32 (s, 1H), 4.12 (s, 1H), 3.88 (s, 1H), 3.32 (s, 2H), 2.17 (s, 1H), 1.79 (s, 2H), 1.71-1.48 (m, 4H), 1.49 – 1.36 (m, 2H), 1.00 (q, J = 6.1 Hz, 2H), 0.81 – 0.72 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.40, 161.99, 146.63, 135.64, 130.92, 115.11, 109.16, 54.19, 50.99, 47.26, 40.09, 28.09, 24.17, 8.68, 7.28. MS(ESI) *m/z*: 415.2 [M + Na]$^{+}$. HRMS (ESI) m/z calcd for $C_{18}H_{21}ClN_4O_2S$, 393.1147; found, 393.1165.

4-(5-Chloro-3-isobutylimidazo[2,1-b]thiazole-6-carbonyl)-1-cyclopentylpiperazin-2-one (**26g**). **26g** was obtained from **11g** (30 mg, 0.12 mmol) and **25a** (25 mg, 0.15 mmol) by following the procedures described in Method E: 47 mg, 96% yield as a pale powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.52 (s, 1H), 4.86 (d, J = 7.5 Hz, 1H), 4.54 (s, 1H), 4.32 (s, 1H), 4.07 (s, 1H), 3.87 (s, 1H), 3.33 (s, 2H), 2.78 (d, J = 4.6 Hz, 2H), 2.12 – 1.40 (m, 9H), 0.96 (d, J = 6.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.15, 161.24, 145.67, 133.54, 129.78, 114.30, 110.78, 54.18, 50.88, 47.17, 39.92, 36.63, 28.08, 27.64, 24.11, 22.02. MS(ESI) *m/z*: 431.3 [M + Na]$^{+}$.

1-Cyclopentyl-4-(2,5-dichloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carbonyl)piperazin-2-one (**26h**). **26h** was obtained from **11h** (90 mg, 0.33 mmol) and **25a** (66 mg, 0.39 mmol) by following the procedures described in Method E: 54 mg, 38% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 – 4.72 (m, 1H), 4.53 (s, 1H), 4.31 (s, 1H), 4.07 (d, J = 10.0 Hz, 1H), 3.87 (s, 1H), 3.28 (s, 2H), 1.95 – 1.71 (m, 3H), 1.69-1.50 (m, 4H), 1.49 – 1.32 (m, 2H), 1.18-1.09 (m, 2H), 1.07-0.99 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.25, 161.37, 143.16, 134.09, 130.90, 118.59, 115.34, 54.19, 50.83, 47.19, 40.01, 28.05, 24.10, 8.72, 6.49..MS(ESI) *m/z*: 449.2 [M + Na]$^{+}$.

1-Cyclopentyl-4-(2,5-dichloro-3-isobutylimidazo[2,1-b]thiazole-6-carbonyl)piperazin-2-one (**26i**). **26i** was obtained from **11i** (65 mg, 0.22 mmol) and **25a** (42 mg, 0.25 mmol) by following the procedures described in Method E: 51 mg, 52% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96-4.83 (m, 1H), 4.54 (s, 1H), 4.32 (s, 1H), 4.08 (s, 1H), 3.87 (s, 1H), 3.32 (s, 2H), 2.84 (d, J = 4.6 Hz, 2H), 2.10 (s, 1H), 1.80 (s, 2H), 1.64 (s, 2H), 1.57 (s, 2H), 1.43 (s, 2H), 0.97 (d, J = 5.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.25, 161.21, 143.87, 134.15, 130.76, 117.21, 114.73, 54.23, 50.89, 47.23, 40.04, 33.34, 29.08, 28.07, 24.11, 21.91. MS(ESI) *m/z*: 465.1[M + Na] $^+$.

4-(5-Chloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carbonyl)-1-(trans-4-hydroxycyclohexyl)piperazin-2-one (**26j**). Compound **11f** (73 mg, 0.30 mmol) and **25c** (128 mg, 0.36 mmol) went through the procedures as described in method E affording the intermediate 4-(5-chloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carbonyl)-1-(trans-4-((triisopropylsilyl)oxy)cyclohexyl)piperazin-2-one (72 mg, 0.12 mmol), which was then dissolved in the methanol solution of hydrogen chloride and stirred for 1 h. The solvent was removed under vacuum and the residue was purified by column chromatography to afford **26j** (49 mg, 39% yield) as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.46 (s, 1H), 4.63 (s, 1H), 4.43 (s, 2H), 4.16 (s, 1H), 3.94 (s, 1H), 3.58 (s, 1H), 3.38 (s, 2H), 2.24 (s, 1H), 2.06 - 1.21 (m, 8H), 1.09 (s, 2H), 0.85 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.11, 161.93, 147.92, 135.59, 129.51, 114.49, 109.03, 69.78, 51.66, 47.45, 44.38, 40.27, 34.33, 27.19, 8.65, 7.27. MS(ESI) *m/z*: 445.2 [M + Na] $^+$.

4-(5-Chloro-3-cyclopropylimidazo[2,1-b]thiazole-6-carbonyl)-1-(cyclopent-3-en-1-yl)piperazin-2-one (**26k**). **26j** was obtained from **11f** (45 mg, 0.20 mmol) and **25b** (40 mg, 0.24 mmol) by following the procedures described in Method E: 26 mg, 33% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (s, 1H), 5.75 (s, 2H), 5.45 (s, 1H), 4.62 (d, J = 20.5 Hz, 1H), 4.39 (s, 1H), 4.17 (s, 1H), 3.93 (s, 1H), 3.33 (s, 2H), 2.78 – 2.63 (m, 2H), 2.35 – 2.16 (m, 3H), 1.14 – 1.01 (m, 2H), 0.94 – 0.77 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.86, 161.81, 147.87, 135.63, 129.43, 114.37, 109.27, 50.90, 50.51, 47.09,

40.02, 36.37, 8.63, 7.25. MS(ESI) *m/z*: 413.2[M + Na] $^{+}$.

4-(3-Chloro-6,7-dihydro-5H-cyclopenta[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**27a**). **27a** was obtained from **16a** (97 mg, 0.40 mmol) and **25a** (84 mg, 0.50 mmol) by following the procedures described in Method E: 106 mg, 68% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97 (s, 1H), 4.74 (s, 1H), 4.38 (s, 1H), 4.27 (s, 1H), 3.93 (s, 1H), 3.38 (s, 2H), 3.11 (s, 2H), 2.92 (t, J = 6.8 Hz, 2H), 2.63 – 2.51 (m, 2H), 1.95 – 1.45 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.31, 161.03, 145.79, 133.01, 127.49, 125.33, 54.10, 50.87, 47.20, 40.04, 33.09, 28.06, 26.34, 25.72, 24.13. MS(ESI) *m/z*: 415.2 [M + Na] $^{+}$.

4-(3-Chloro-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**27b**). **27b** was obtained from **16b** (128 mg, 0.50 mmol) and **25a** (102 mg, 0.60 mmol) by following the procedures described in Method E: 86 mg, 42% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (s, 1H), 4.66 (s, 1H), 4.38 (s, 1H), 4.20 (s, 1H), 3.94 (s, 1H), 3.40 (s, 2H), 3.06 (s, 2H), 2.72 (s, 2H), 2.03-1.77 (m, 6H), 1.77-1.56 (m, 4H), 1.56-1.42 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.29, 161.34, 145.14, 133.14, 127.20, 125.19, 117.78, 54.12, 50.94, 47.23, 40.00, 28.05, 24.71, 24.11, 23.34, 22.31, 21.61. MS(ESI) *m/z*: 429.1 [M + Na] $^{+}$.

4-(3-Chloro-6,7,8,9-tetrahydro-5H-cyclohepta[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**27c**). **27c** was obtained from **16c** (27 mg, 0.10 mmol) and **25a** (20 mg, 0.12 mmol) by following the procedures described in Method E: 21 mg, 50% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (s, 1H), 4.68 (s, 1H), 4.39 (s, 1H), 4.22 (s, 1H), 3.97 (s, 1H), 3.42 (s, 2H), 3.13 (t, J = 7.1 Hz, 2H), 2.79 (t, J =7.1 Hz, 2H), 2.03-1.57 (m, 12H), 1.55-1.40 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.31, 161.17, 145.43, 132.94, 127.27, 125.24, 54.11, 50.92, 47.25, 40.02, 29.33, 28.97, 28.43, 28.05, 26.51, 24.12, 19.79. MS(ESI) *m/z*: 421.2 [M + H] $^{+}$.

4-(3-Chloro-7-methyl-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**27d**). **27d** was obtained from **16d** (180 mg, 0.67 mmol) and **25a** (135 mg,

0.80 mmol) by following the procedures described in Method E: 160 mg, 57% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (s, 1H), 4.64 (s, 1H), 4.38 (s, 1H), 4.16 (s, 1H), 3.93 (s, 1H), 3.41 (s, 2H), 3.22 (d, J = 15.1 Hz, 1H), 2.98 (s, 1H), 2.77 (d, J = 13.9 Hz, 1H), 2.44 – 2.30 (m, 1H), 2.05 (d, J = 9.1 Hz, 2H), 1.86 (s, 2H), 1.78 – 1.41 (m, 7H), 1.14 (d, J = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.20, 160.91, 146.21, 132.62, 127.04, 125.16, 54.13, 50.90, 47.21, 39.90, 32.46, 29.57, 28.92, 28.06, 24.11, 22.99, 20.87. MS(ESI) *m/z*: 443.2 $[M + Na]^+$.

4-(3-Chloro-7,7-dimethyl-5,6,7,8-tetrahydrobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**27e**). **27e** was obtained from **16e** (45 mg, 0.16 mmol) and **25a** (34 mg, 0.20 mmol) by following the procedures described in Method E: 58 mg, 84% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (s, 1H), 4.58 (s, 1H), 4.30 (s, 1H), 4.09 (d, J = 17.9 Hz, 1H), 3.86 (s, 1H), 3.33 (s, 2H), 2.98 (s, 2H), 2.42 (s, 2H), 1.78 (s, 2H), 1.71 – 1.48 (m, 6H), 1.49 – 1.31 (m, 2H), 1.03 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.28, 160.89, 145.19, 133.28, 125.91, 124.51, 54.10, 50.89, 47.19, 40.23, 38.00, 34.44, 30.61, 28.05, 27.54, 24.11, 21.09. MS(ESI) *m/z*: 457.2 $[M + Na]^+$.

3-Chloro-2-(4-cyclopentyl-3-oxopiperazine-1-carbonyl)-6,7-dihydrobenzo[d]imidazo[2,1-b]thiazol-8(5H)-one (**27f**). **27f** was obtained from **16f** (181 mg, 0.67 mmol) and **25a** (135 mg, 0.80 mmol) by following the procedures described in Method E: 52 mg, 18% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.30 (s, 1H), 4.96 (s, 1H), 4.62-4.14 (m, 2H), 4.01 – 3.58 (m, 1H), 3.43 (s, 2H), 2.68 (t, J = 6.4 Hz, 2H), 2.29 (s, 2H), 1.97-1.40 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.42, 166.37, 161.57, 144.48, 125.38, 122.13, 54.10, 50.86, 47.27, 40.14, 37.16, 28.16, 24.12, 22.19, 21.96. MS(ESI) *m/z*: 419.5 $[M - H]^-$; MS(ESI) *m/z*: 421.3 $[M + H]^+$.

4-(3-Chlorobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**28a**). **28a** was obtained from **18a** (101 mg, 0.40 mmol) and **25a** (81 mg, 0.48 mmol) by following the procedures described in Method E: 70 mg, 43% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.54 – 7.40 (m, 2H), 5.09 – 4.86 (m, 1H), 4.75 (s, 1H), 4.41 (s, 1H),

4.26 (s, 1H), 3.96 (s, 1H), 3.39 (d, J = 15.4 Hz, 2H), 1.95 – 1.45 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.33, 161.34, 145.95, 135.02, 131.99, 130.41, 126.47, 126.17, 124.36, 114.26, 54.18, 50.93, 47.30, 40.15, 28.06, 24.13. MS(ESI) *m/z*: 403.1 $[M + H]^+$.

4-(3-Chloro-7-methoxybenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**28b**). **28b** was obtained from **18b** (57 mg, 0.20 mmol) and **25a** (40 mg, 0.24 mmol) by following the procedures described in Method E: 45 mg, 52% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.25 (s, 1H), 7.05 (d, J = 8.2 Hz, 1H), 4.98 (s, 1H), 4.77 (s, 1H), 4.42 (s, 1H), 4.28 (s, 1H), 3.97 (s, 1H), 3.91 (s, 3H), 3.42 (s, 2H), 1.89 (s, 2H), 1.72 – 1.44 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.37, 160.82, 158.07, 131.89, 126.59, 125.88, 119.66, 114.97, 113.74, 108.66, 55.99, 54.15, 50.91, 47.47, 40.05, 28.08, 24.13. MS(ESI) *m/z*: 455.2 $[M + Na]^+$.

1-Cyclopentyl-4-(3,7-dichlorobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)piperazin-2-one (**28c**). **28c** was obtained from **18c** (72 mg, 0.25 mmol) and **25a** (51 mg, 0.30 mmol) by following the procedures described in Method E: 78 mg, 71% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 7.6 Hz, 1H), 7.68 (s, 1H), 7.41 (d, J = 7.9 Hz, 1H), 4.87 (s, 1H), 4.66 (d, J = 14.6 Hz, 1H), 4.34 (s, 1H), 4.15 (d, J = 18.6 Hz, 1H), 3.89 (s, 1H), 3.35 (s, 2H), 1.80 (s, 2H), 1.72 – 1.50 (m, 4H), 1.46 (d, J = 14.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.34, 160.97, 145.09, 134.98, 132.10, 130.42, 127.02, 126.80, 124.19, 115.13, 114.98, 54.33, 50.82, 47.27, 40.07, 28.08, 24.12. MS(ESI) *m/z*: 437.3 $[M + H]^+$.

4-(7-Bromo-3-chlorobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**28d**). **28d** was obtained from **18d** (33 mg, 0.10 mmol) and **25a** (20 mg, 0.12 mmol) by following the procedures described in Method E: 28 mg, 58% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.4 Hz, 1H), 7.82 (s, 1H), 7.55 (d, J = 8.3 Hz, 1H), 4.88 (s, 1H), 4.67 (s, 1H), 4.34 (s, 1H), 4.18 (s, 1H), 3.89 (s, 1H), 3.34 (s, 2H), 1.81 (s, 2H), 1.71 – 1.50 (m, 4H), 1.44 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.29, 161.02, 144.89, 135.14, 130.87, 129.77, 127.00, 124.51, 119.32, 115.27, 54.25,

50.88, 47.29, 40.09, 28.09, 24.13. MS(ESI) *m/z*: 481.2 $[M + H]^+$.

4-(3-Chloro-7-methylbenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-cyclopentylpiperazin-2-one (**28e**). **28e** was obtained from **18e** (80 mg, 0.30 mmol) and **25a** (60 mg, 0.36 mmol) by following the procedures described in Method E: 92 mg, 74% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.24 (d, J = 8.3 Hz, 1H), 4.89 (s, 1H), 4.67 (s, 1H), 4.34 (s, 1H), 4.17 (s, 1H), 3.89 (s, 1H), 3.35 (s, 2H), 2.42 (s, 3H), 1.80 (s, 2H), 1.72 – 1.35 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.31, 160.93, 145.09, 136.84, 134.09, 130.4, 129.75, 127.64, 124.46, 113.96, 54.22, 50.85, 47.26, 40.08, 28.08, 24.13, 21.45. MS(ESI) *m/z*: 439.1 $[M + Na]^+$.

4-(3-Chlorobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-(trans-4-hydroxycyclohexyl)piperazin-2-one (**28f**). **28f** was obtained from **18a** (76 mg, 0.30 mmol) and **25c** (128 mg, 0.36 mmol) by following the procedures described in the synthesis of **26g**, 112mg, 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.53 (t, J = 7.5 Hz, 1H), 4.54 (s, 2H), 4.16 (s, 2H), 4.06 (s, 1H), 3.80 (s, 1H), 3.34 (s, 3H), 1.87 (d, J = 10.7 Hz, 2H), 1.54 (s, 4H), 1.24 (d, J = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.01, 160.06, 145.12, 134.99, 131.48, 129.71, 126.77, 126.19, 125.27, 113.76, 68.02, 51.33, 46.51, 43.88, 40.97, 34.34, 26.65. MS(ESI) *m/z*: 433.1 $[M + H]^+$.

4-(3-Chlorobenzo[d]imidazo[2,1-b]thiazole-2-carbonyl)-1-(cyclopent-3-en-1-yl)piperazin-2-one (**28g**). **28g** was obtained from **18a** (50 mg, 0.20 mmol) and **25b** (40 mg, 0.24 mmol) by following the procedures described in Method E: 29 mg, 36% yield as a pale solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 7.7 Hz, 1H), 7.56 – 7.42 (m, 2H), 5.77 (s, 2H), 5.49 (s, 1H), 4.78 (s, 1H), 4.43 (s, 1H), 4.26 (s, 1H), 3.96 (s, 1H), 3.36 (s, 2H), 2.84 – 2.65 (m, 2H), 2.31 (dd, J = 15.6, 3.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.84, 160.23, 145.05, 135.03, 131.96, 130.48, 129.45, 126.51, 126.23, 124.39, 114.30, 50.91, 50.52, 47.25, 40.09, 36.40. MS(ESI) *m/z*: 423.1 $[M + Na]^+$.

Biological Assays.

HCV Replicon Assay. Huh-7 cells harboring a genotype 1b HCV bicistronic replicon (Con1)[29] were plated at 8000 cells/well in 96-well plates. Compounds were added to the plates with a final concentration of 0.5% DMSO, and plates were incubated at 37°C for 72 h. Effect of test compounds on the proliferation of Huh-7 cells was determined using the cell proliferation reagent, CellTiter-Fluor (Promega, Madison, WI), according to the manufacturer's instructions. The relative levels of luciferase present were determined using the Bright-Glo luciferase substrate (Promega, Madison, WI) on an EnVision Multilabel Plate Readers. CC_{50} and EC_{50} values were calculated by GraphPad Prism software (San Diego, CA) and reported as the average of at least two independent determinations.

Mutation Replicon Assay. Huh-7 cells were transiently-transfected by electroporation with HCV genotype 1b replicon RNAs harboring various mutations, including A156T (NS3), Y93H (NS5A), S282T (NS5B), M423I (NS5B), P495A (NS5B), Y448H (NS5B), H3R (NS4B), Q26R (NS4B), H94N (NS4B), F98N (NS4B), V105M (NS4B), as described elsewhere.[15] Then the transiently-transfected Huh-7 cells were plated at 10000 cells/well in 96-well plates. Compounds were added to the plates with a final concentration of 0.5% DMSO, and plates were incubated at 37°C for 72 h. The relative levels of luciferase present were determined using the Bright-Glo luciferase substrate (Promega, Madison, WI) on a EnVision Multilabel Plate Readers. EC_{50} values were calculated by GraphPad Prism software (San Diego, CA) and reported as the average of two independent determinations with a maximum of 2-fold variation. For every mutation replicon, the EC_{50} value of a positive drug was determined parallel as well to validate the credibility of this replicon.

Combination Study. Huh-7 cells harboring a genotype 1b HCV bicistronic replicon (Con1) were plated at 8000 cells/well in 96-well plates. Simeprevir, Daclatasvir, Sofosbuvir and Clemizole were added separately or in combination with **26f** orthogonal at various concentrations to the plates with a final concentration of 0.5% DMSO, and plates were incubated at 37°C for 72 h. The relative levels of

luciferase present were determined using the Bright-Glo luciferase substrate (Promega, Madison, WI) on a EnVision Multilabel Plate Readers. The replicon inhibitory activity was expressed as a percentage relative to the untreated control. Cytotoxicity of these combinations was determined parallel as described above.

Analysis of Combination Data. Analysis of compound interactions was based on the median-effect principle and the combination index theorem as described by Chou and Talalay.[41] CIs and DRIs for these combinations were determined using CompuSyn software (Combosyn, Paramus, NJ). The DRI plots for every combination were generated by ploting DRI values with corresponding effect levels (Fa). The line of DRI=1 indicates no dose-reduction effect of a drug to the other drug, while favorable or unfavorable dose-reduction effects are indicated by values plotted to the above or below of this line, respectively.

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ABBREVIATIONS USED

CI, Combination Index; DAA, direct-acting antiviral; DRI, Dose-Reduction Index; DMSO, dimethylsuphoxide; DMF, dimethyl formamide; EDCI, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride; HATU, O-(7-Aza-1H-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; IFN, interferon; NCS, N-chlorosuccinimide; NBS, N-bromosuccinimide; SVR, sustained virologic response; THF,

tetrahydrofuran.

Supporting Information Available. EC_{50} of the positive controls against the mutation replicons and percent inhibitions of all combination studies.

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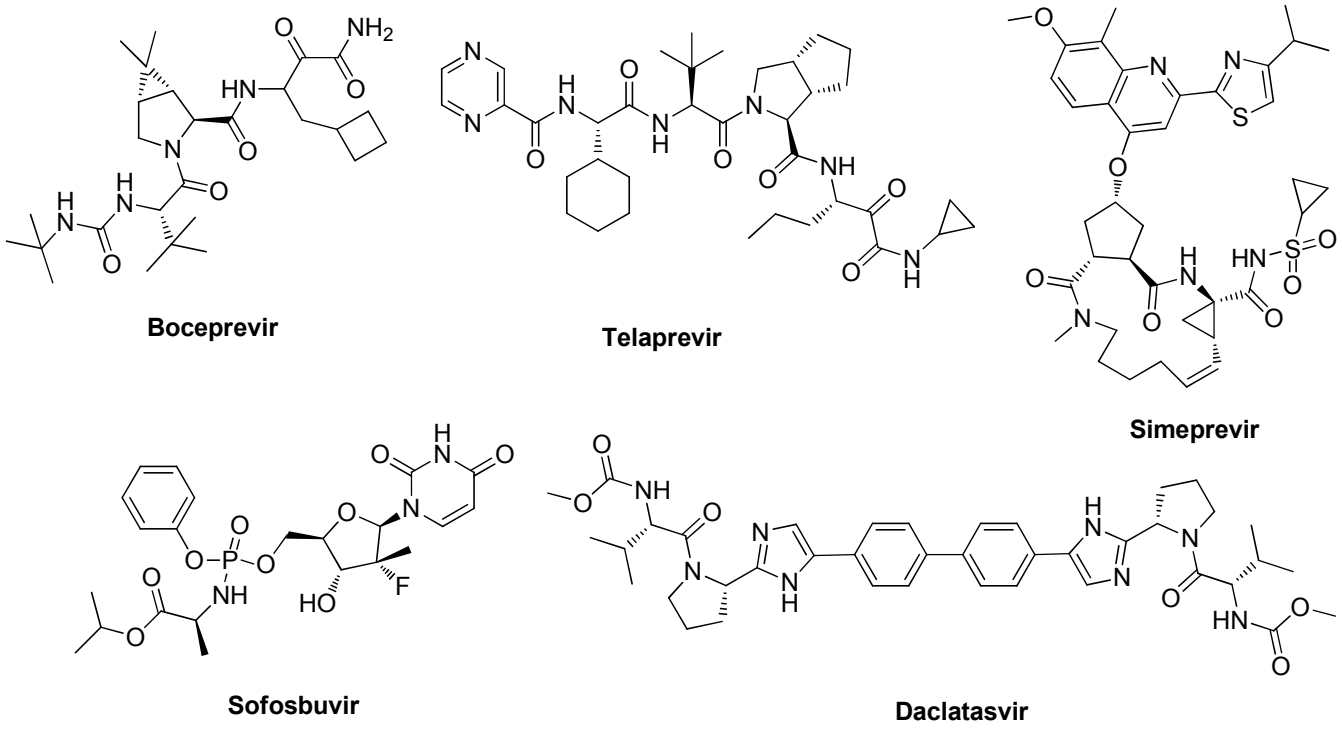


Figure 1. Structures of the HCV DAAs approved for clinical use.

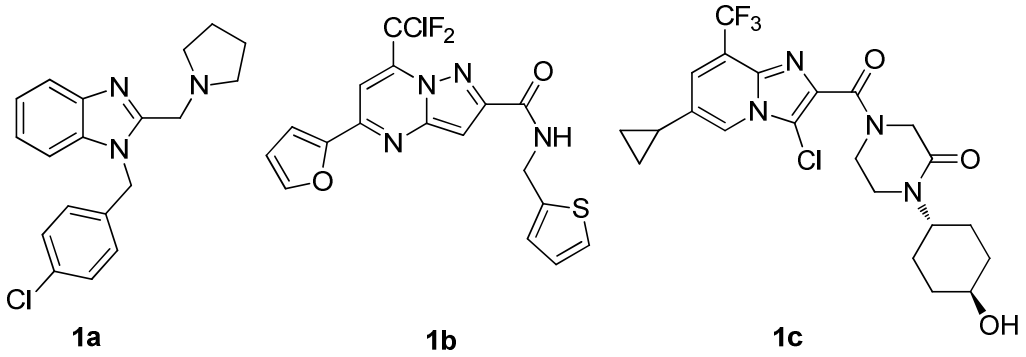


Figure 2. Representative NS4B inhibitors.

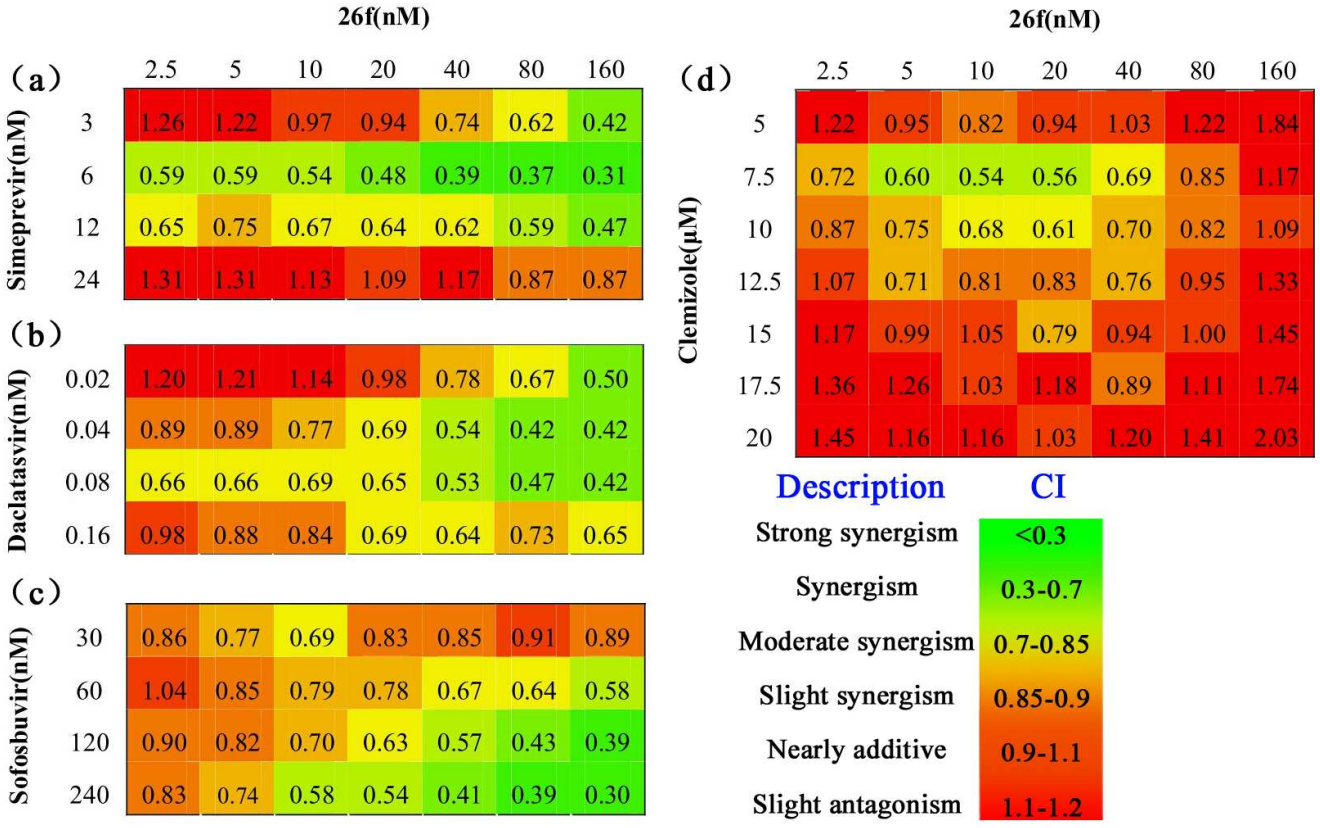


Figure 3. Synergistic interactions between **26f** and Simeprevir (a), Daclatasvir (b), Sofosbuvir (c) and Clemizole (d). CI values for every combination were calculated with Compusyn software, percent inhibitions of all these combinations were presented in Supporting Information. Values are heat mapped with highest values in red and lowest values in green.

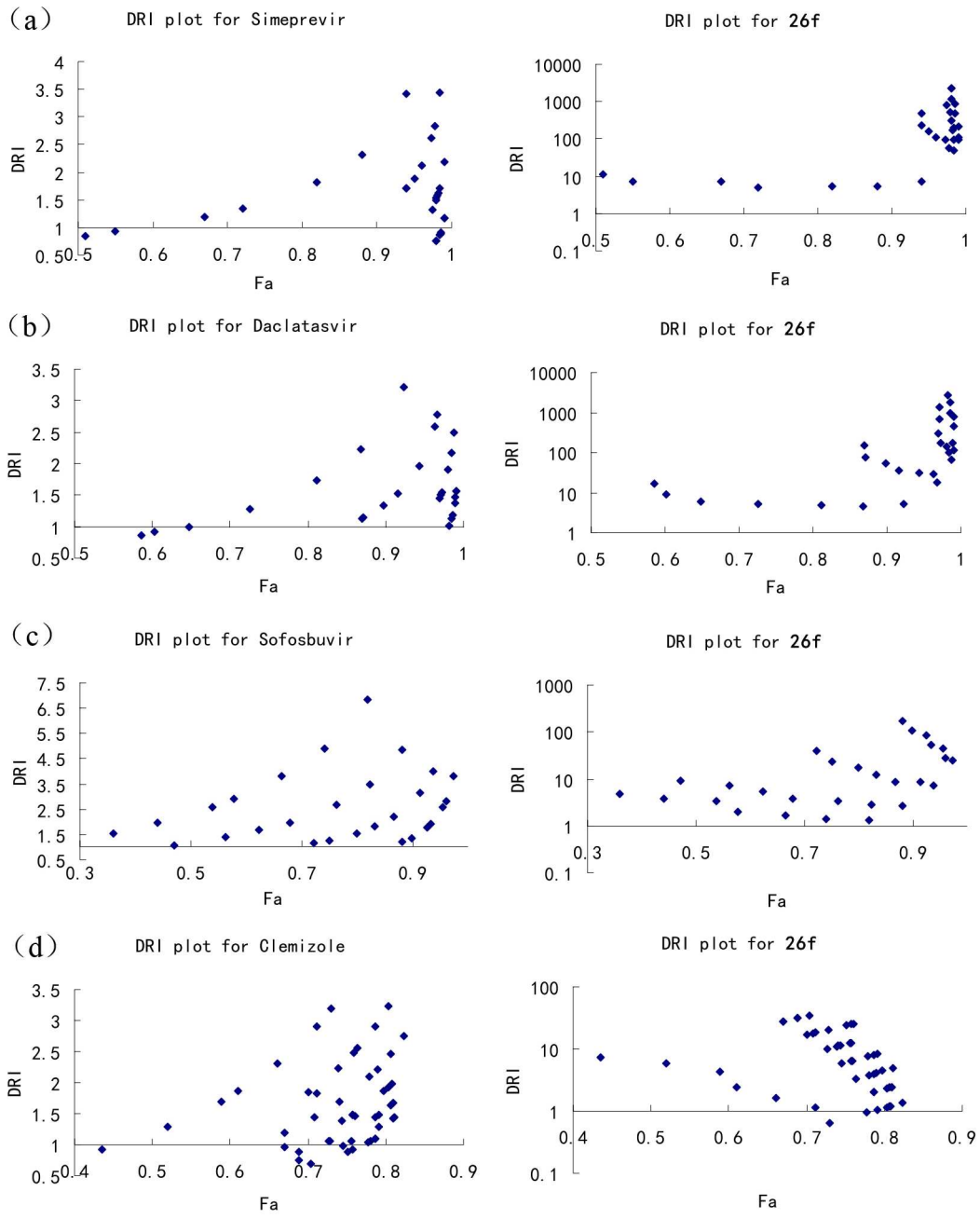
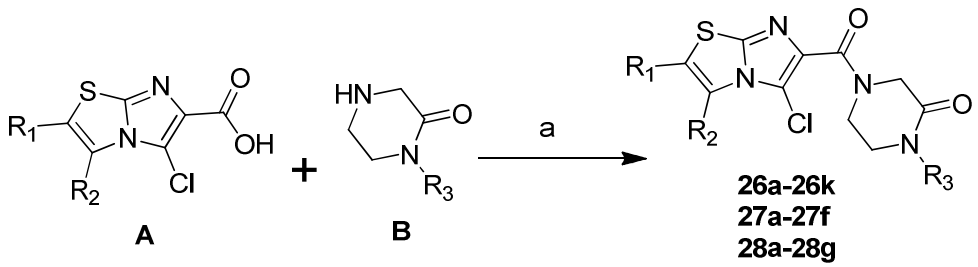


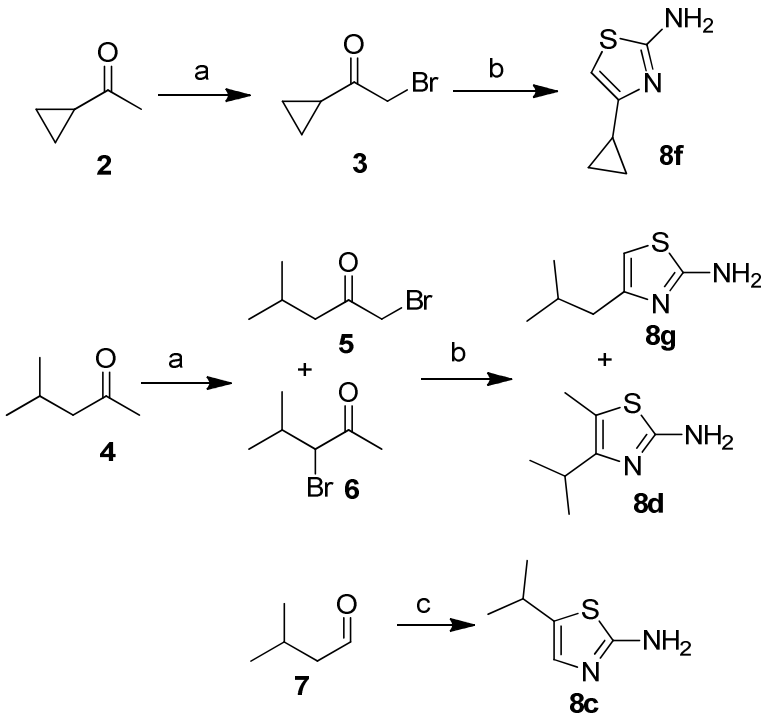
Figure 4. **26f** could reduce the doses of other DAAs (left) and all of these DAAs could also reduce the doses of **26f** (right), DRI values for every combination were calculated with Compusyn software. (a). Combination of **26f** with Semiprevir; (b). Combination of **26f** with Daclatasvir; (c). Combination of **26f** with Sofosbuvir; (d). Combination of **26f** with Clemizole.

Scheme 1. General method for the preparation of the title compounds 26a-26k, 27a-27f, 28a-28g by assembling the carboxylic acid segment A and amine segment B. [a]



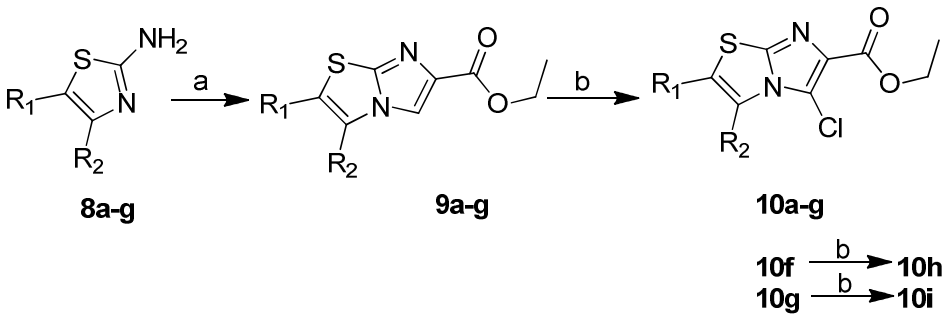
[a]Reagent and conditions: (a) EDCI, HOBt, dichloromethane, rt, 3-12 h.

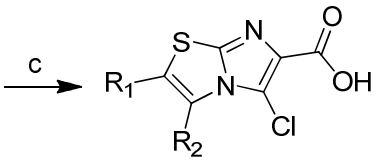
Scheme 2. Synthesis of intermediate 2-aminothiazoles.[a]



[a]Reagent and conditions: (a) Br_2, methanol, rt, 2h; (b) thiourea, ethanol, reflux, 6h; (c) Br_2, dichloromethane/dioxane, 10℃, 2h, then thiourea, ethanol, Et_3N, rt, 20h.

Scheme 3. Synthesis of carboxylic acid 11a-i. [a]



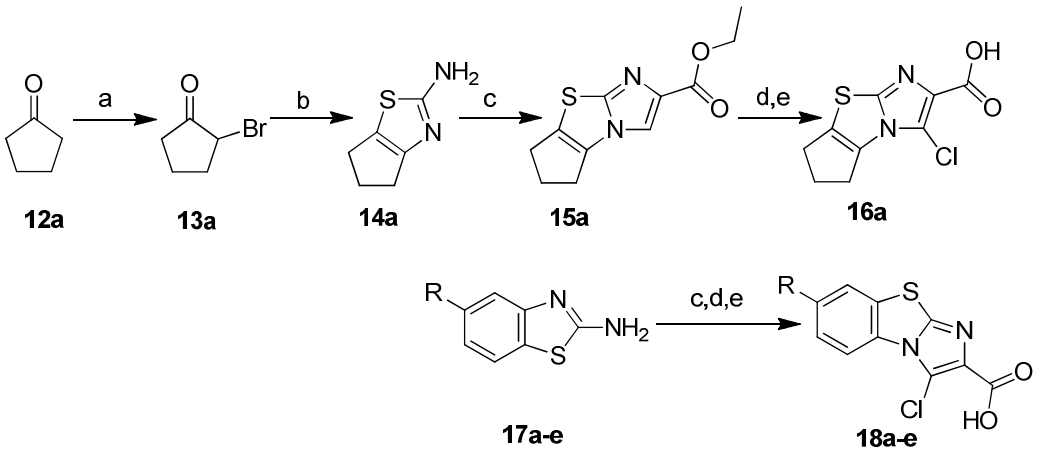


11a-i

	R_1	R_2
a.	H	H
b.	CH_3-	H
c.	$(CH_3)_2CH$-	H
d.	$(CH_3)_2CH$-	CH_3-
e.	H	CH_3-
f.	H	cyclopropyl
g.	H	$(CH_3)_2CHCH_2$-
h.	Cl	cyclopropyl
i.	Cl	$(CH_3)_2CHCH_2$-

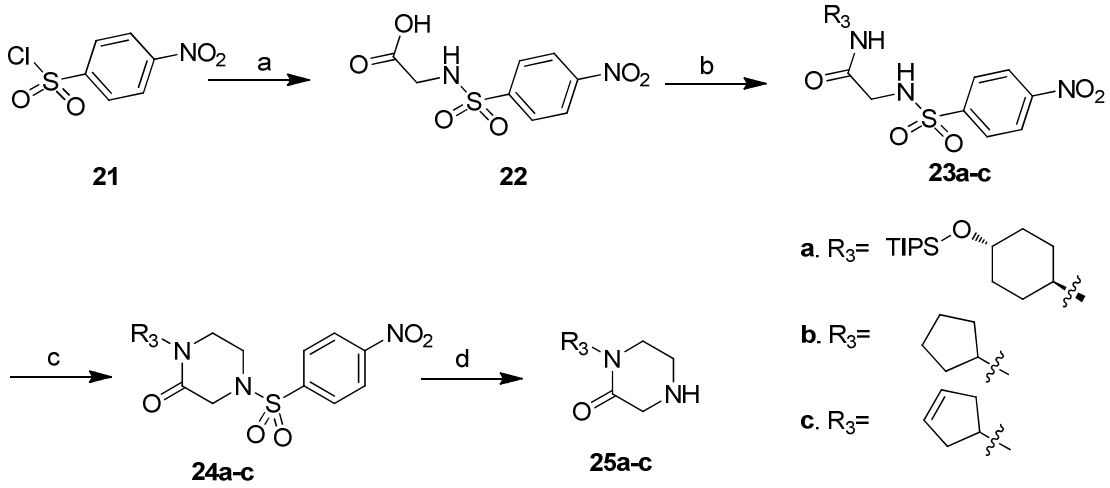
[a]Reagent and conditions: (a) ethyl bromopyruvate, butanone, reflux, 12h; (b) NCS, DMF, rt, 3-6h; (c) LiOH, methanol/THF/water, rt, 3h.

Scheme 4. Synthesis of tricyclic carboxylic acids. [a]



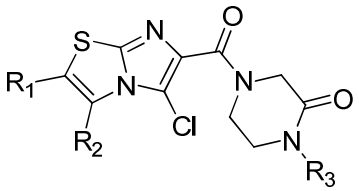
[a]Reagent and conditions: (a). NBS, DMSO, rt, 0.5 h; (b). thiourea, ethanol, reflux, 6 h; (c). ethyl bromopyruvate, butanone, reflux, 24h; (d). NCS, DMF, rt, 3-6h; (e). LiOH, methanol/THF/water, rt, 3h.

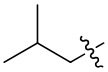
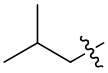
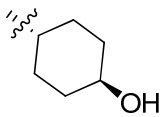
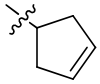
Scheme 5. Synthesis of amine building block B. [a]



[a]Reagent and conditions: (a). Glycine, NaOH, H_2O, 0℃, 30min; (b). R_3NH_2, DMF, DIEA, HATU, rt, 12h; (c). 1,2-dibromoethane, K_2CO_3, DMF, 60℃, 24h; (d). methyl 3-mercaptopropionate, LiOH, CH_3CN/DMSO, 50℃, 6h.

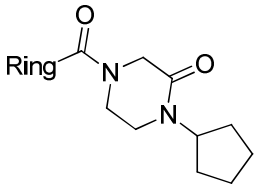
Table 1. Inhibitory Effects of Imidazo[2,1-b]thiazole Derivatives on HCV Replication in Huh 7 Cells (SAR Study on C2 and C3).

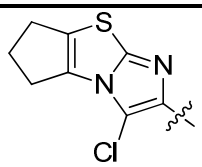
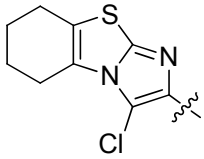
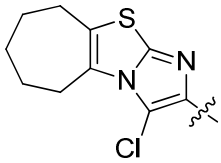
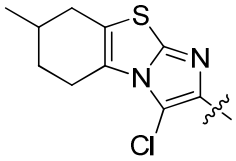
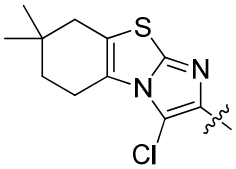
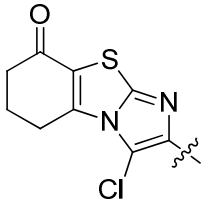
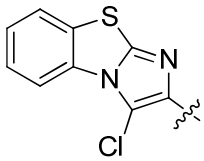


Compound	R_1	R_2	R_3	Cytotoxicity CC_{50} (μM)	HCV Replicon GT1b EC_{50} (μM)
26a	H	H		>10	>10
26b	Me	H		>10	46%@10μM[a]
26c		H		>10	1.32
26d		Me		>10	50%@10μM[a]
26e	H	Me		>10	53%@10μM[a]
26f	H			>10	0.016
26g	H			>10	0.74
26h	Cl			>10	8.6
26i	Cl			>10	6.7
26j	H			>10	0.89
26k	H			>10	0.079

[a] Percentage inhibition at 10 μM concentration

Table 2. Inhibitory Effects of Tricyclic Imidazo[2,1-b]thiazole Derivatives on HCV Replication in Huh 7 Cells.



Compound	Ring	Cytotoxicity CC_{50} (μM)	HCV Replicon GT1b EC_{50} (μM)
27a		>10	0.13
27b		>10	0.19
27c		>10	0.94
27d		>10	0.26
27e		>10	1.12
27f		>10	>10
28a		>10	0.092

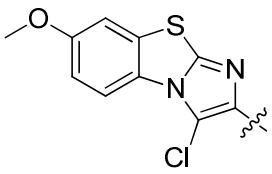
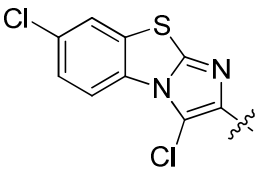
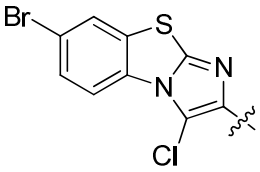
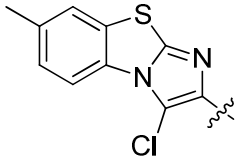
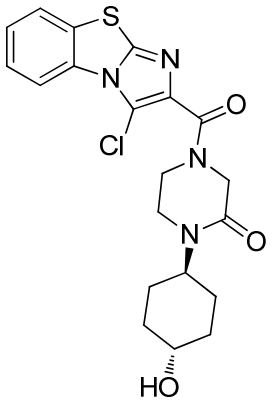
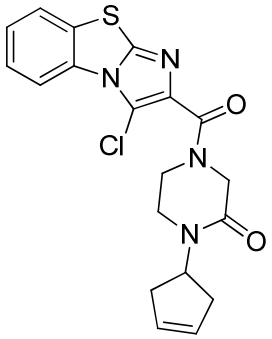
28b		>10	8.63
28c		>10	1.85
28d		>10	1.79
28e		>10	0.30
28f		>10	0.33
28g		>10	0.031

Table 3. Resistance profile studies of **26f** and **28g**[a]

Mutant Replicon	Gene	EC_{50}（nM）		
		26f	**28g**	**1c**
WT（1b）		16	31	1.2
A156T	NS3/4A	4	77	/
H3R	NS4B	1105	4853	33
Q26R		350	4965	20
H94R		3976	6402	35
F98C		27075	3270	304
V105M		27246	12465	116
Y93H	NS5A	14	19	/
S282T	NS5B (Active Site)	15	25	/
M423I	NS5B (Thumb 2)	9	32	/
P495A	NS5B (Thumb 1)	10	22	/
Y448H	NS5B (Palm)	8	33	/

[a]. All the mutation replicons were transiently transfected replicons, and the potency of the positive controls on Y93H, S282T, M423I, P495A, Y448H, A156T were presented in Supporting Information.

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