Bioorganic & Medicinal Chemistry 26 (2018) 2621–2631

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

# Design, synthesis, and structure-activity relationships of novel imidazo [4,5-c]pyridine derivatives as potent non-nucleoside inhibitors of hepatitis C virus NS5B

Moyi Liu, Qiaoling Xu, Su Guo, Ruixi Zuo, Yue Hong, Yong Luo, Yingxiu Li, Ping Gong, Yajing Liu *

*Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, 103 Wenhua Road, Shenhe District, Shenyang 110016, PR China*

ARTICLE INFO

*Article history:*
Received 9 March 2018
Revised 31 March 2018
Accepted 13 April 2018
Available online 16 April 2018

*Keywords:*
Hepatitis C virus
NS5B polymerase
Genotype 1b
Non-nucleoside inhibitor

ABSTRACT

The hepatitis C virus (HCV) NS5B polymerase is an attractive target for the development of novel and selective inhibitors of HCV replication. In this paper, the design, synthesis, and preliminary SAR studies of novel inhibitors of HCV NS5B polymerase based on the structure of tegobuvir have been described. The efforts to optimize the antiviral potency and reduce the treatment side effects with respect to genotype 1b resulted in the discovery of compound 3, which exhibited an $EC_{50}$ of 1.163 nM and a $CC_{50}$ >200 nM in a cell-based HCV replicon system assay. Additionally, testing for inhibition of the hERG channel showed a marked improvement over tegobuvir and the pharmacokinetic properties of compound 3 indicated that it was worthy of further investigation as a non-nucleoside inhibitor of HCV NS5B polymerase.


## 1. Introduction

Hepatitis C virus (HCV) is one of the most serious liver diseases worldwide.[1] Approximately 80 – 115 million people are infected with HCV and 75–85% of these individuals will develop chronic infection. In addition, an estimated 20–50% of these patients will develop liver fibrosis, cirrhosis, and hepatocellular carcinoma. Although vaccines exist for some hepatitis viruses, none are available for HCV.[2] In the past years, the standard of care (SOC) in antiviral therapy consisted of pegylated interferon (Peg-IFN) in combination with ribavirin.[3] However, this may cause patients to experience thyroid insufficiency, cognitive dysfunction, gastrointestinal symptoms, and other serious adverse reactions. In 2011, the HCV NS3 protease inhibitors telaprevir[4–6] and boceprevir[7,8] were approved successively as the first direct-acting antiviral agents (DAAs), which initiated a revolution in the field of HCV treatment. Compared with the traditional regimen, DAAs resulted in dramatically increased tolerability and efficacy in chronic HCV infection.[9]

The HCV NS5B RNA-dependent RNA polymerase (RdRp) plays an essential role in the life cycle of virus, especially in RNA replication; consequently, it has emerged as an attractive therapeutic target.[10] A variety of small molecule NS5B RdRp inhibitors have been developed in recent years, including Abbvie's dasabuvir, Gilead's tegobuvir, Pfizer's HCV-796, Abbott's ABT-072, and Roche's setrobuvir (Fig. 1). Among them, HCV-796 and tegobuvir progressed to clinical trials and exhibited excellent inhibitory activity. Unfortunately, as HCV-796 may cause severe hepatocellular injury and apoptosis, clinical research has been discontinued.[11] Tegobuvir exhibited a nanomolar $EC_{50}$ value (2.9 nM) and almost no cytotoxicity ($CC_{50}$ >100 nM) in the clinical trials. However, there have been no recent reports about tegobuvir owing to serious adverse events, such as the phenomenon of QT prolongation that may occur with a high dose.[12]

Although the binding site of tegobuvir is still unknown, compounds in this class have been shown to bind to an allosteric site in the NS5B polymerase. Interestingly, the compounds are selectively resistant for the NS5B polymerase; they do not show activity in the isolated polymerase enzyme assay. This class of compounds only shows activity when tested in the subgenomic replicon system with a hepatocyte as the host cell.[13] This is probably attributable to the special mechanistic action of tegobuvir. The structure-activity relationships (SARs) of 2,5-disubstituted imidazopyridine (moiety C) based NS5B RdRp inhibitors suggested that the imidazopyridine pharmacophore was responsible for insertion into the pocket,[14] with an aryl fragment (moiety D) probably involved in epoxidation with CYP-1A and reaction with glutathione, which

* Corresponding author.
*E-mail address:* lyjpharm@126.com (Y. Liu).

https://doi.org/10.1016/j.bmc.2018.04.029
0968-0896/

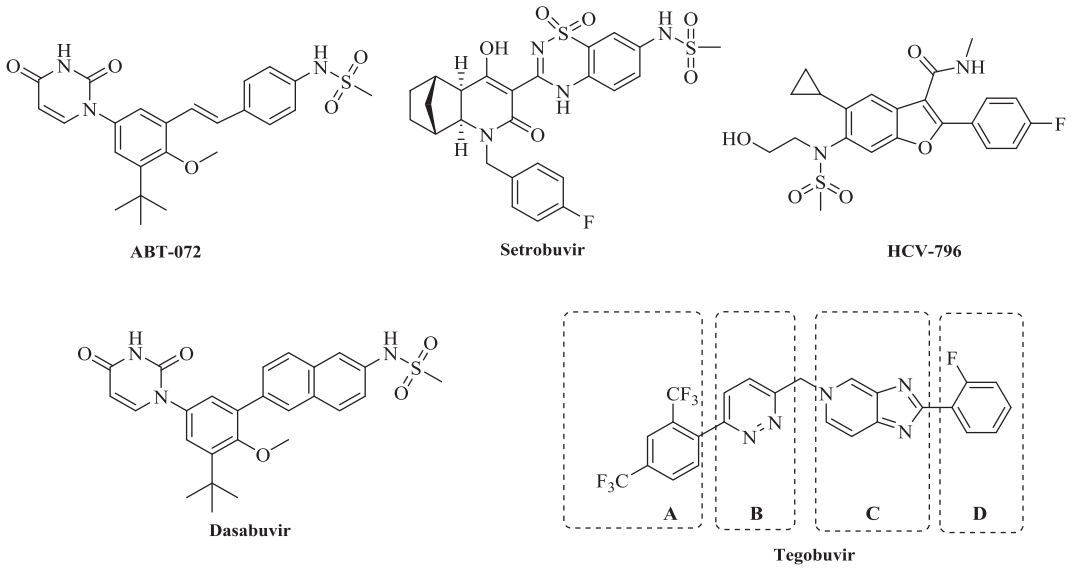



**Fig. 1.** Structures of small molecule NS5B RdRp inhibitors.

subsequently achieved inhibition of NS5B through covalent modification.[15] For the linker carbon between moieties B and C, monosubstitution and disubstitution of the methylene on the linker resulted in decreased activity and caused liver toxicity. According to these analyses, we decided to retain moieties C and D, and the linker of B and C.

It has been reported that substitution at the 2- and 4-positions of moiety A generated analogs with the best activities, with preference to alkyl and alkoxy groups; furthermore, moiety B can also be modified with nitrogen heterocycles, such as isoxazole, pyrimidine, and pyrazine.[13,16] Therefore, in order to maintain the highly optimized interactions between moiety C, moiety D, and the residues of the active sites, modifications specific to moiety A and moiety B were evaluated.

The initial phase I trials found that tegobuvir caused QT prolongation, which limited the dosing levels.[17] The inhibition of the hERG ion channel is known to result in QT prolongation, and several strategies have successfully reduced hERG inhibition, such as steric blocking, the introduction of a potassium channel activator, and the reduction of basicity.[18]

Compounds bearing 1,2,3-triazole scaffolds have been reported to exhibit a large range of biological activities, including antituberculosis, antitumor, and antiviral activities[19–21] (Fig. 2). Through the introduction of such a versatile moiety into organic molecules, compounds can be produced with moderate dipole character, hydrogen bonding capability, rigidity, and stability.[22,23] More importantly, 1,2,3-triazole was also reported as potassium channel activator [21,24,25].

On this basis, we expected that hERG inhibition could be reduced by the replacement of the pyridazine ring in moiety B with 1,2,3-triazole. In addition, we decided to retain the phenyl core in moiety A, while make changes on substituents on it to generate the compounds of series I (compounds 1 – 9) (Fig. 3). Furthermore,

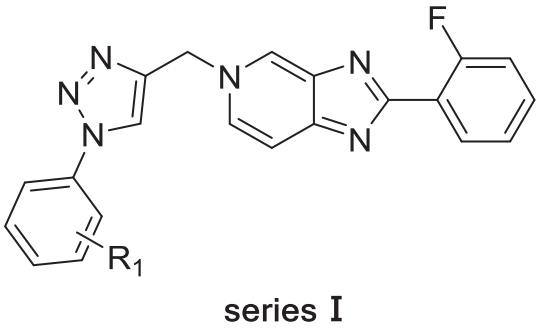



**Fig. 3.** General structure of series I.

based on the structure of series I, some nitrogen heterocycles with large scale were introduced into moiety A to generate series II (compounds 10 – 14) (Fig. 4). In addition, we expected to reduce the hERG inhibition by eliminating pyridazine ring in moiety B, which has relatively strong basicity, to generate series III (compounds 15 – 21) (Fig. 5).

## 2. Chemistry

### 2.1. Synthesis of target compounds of series I

The target compounds of series I were synthesized in accordance with the procedures outlined in Scheme 1. Intermediate E was achieved by using a convenient four-step procedure starting from 4-aminopyridine. The treatment of intermediate E with propargyl bromide afforded compound F; subsequently, the target compounds (compounds 1 – 9) of series I were obtained through

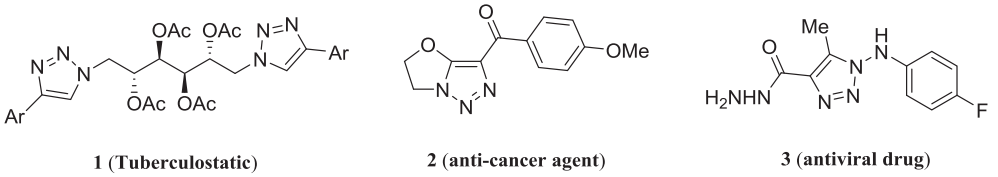



**Fig. 2.** Drugs bearing 1,2,3-triazole scaffolds.

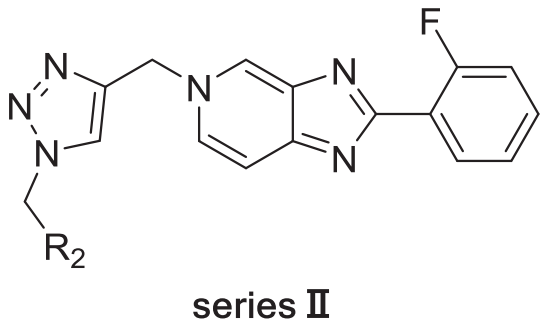



**Fig. 4.** General structure of series II.

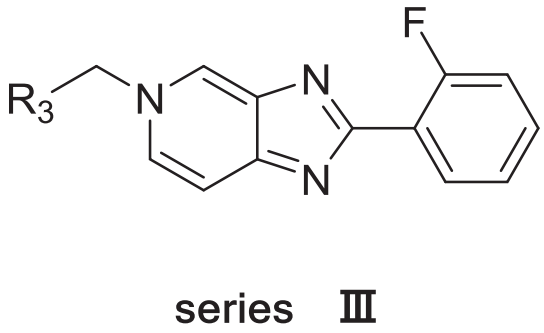



**Fig. 5.** General structure of series III.

the reaction of compound F with different azidobenzenes,[26] which were prepared from different substituted anilines via the Sandmeyer Reaction.

### 2.2. Synthesis of target compounds of series Ⅱ

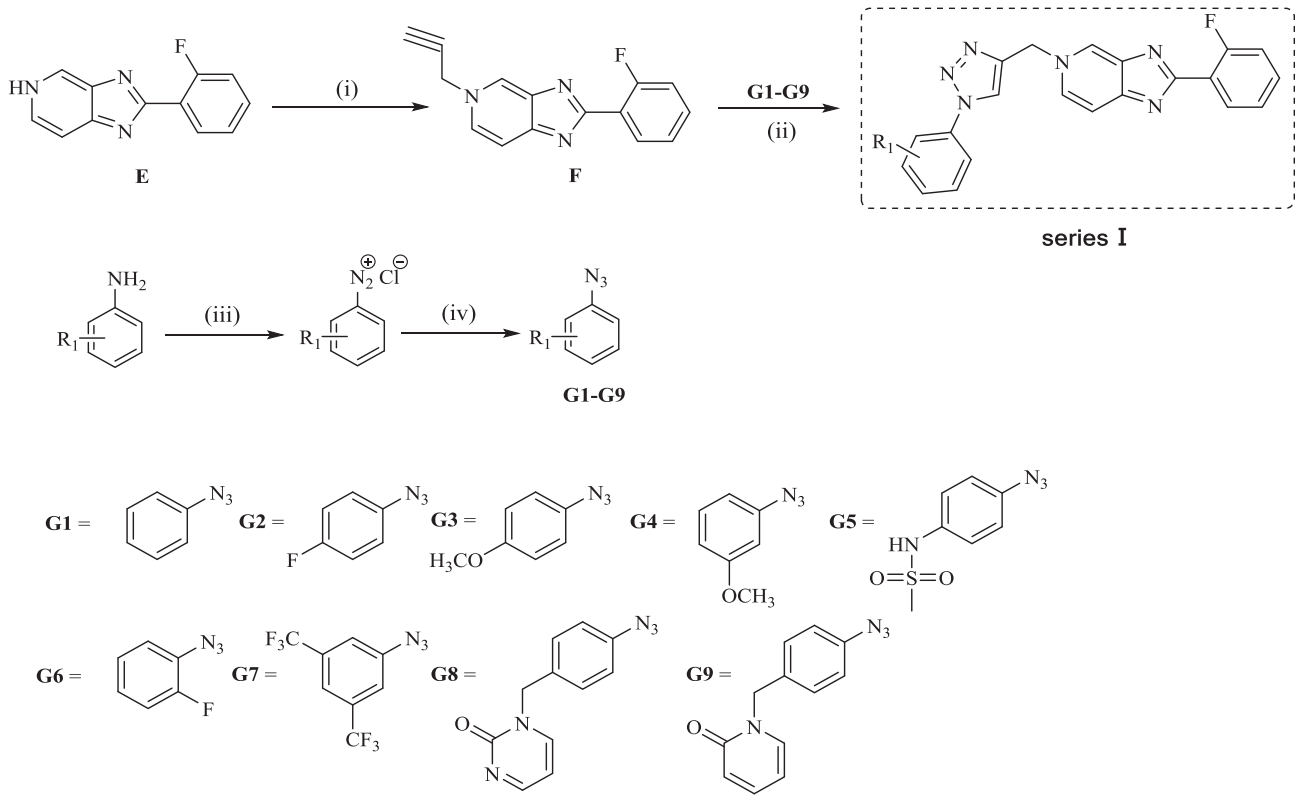
The target compounds of series Ⅱ were synthesized in accordance with the procedures outlined in Scheme 2. Compound F, prepared as reported in detail as above, was treated with different hydrazoates (b1, c2, d3, and d4)[27–29] to afford the target compounds (compounds 10 – 14). The hydrazoates were obtained via the direct substitution of different chlorides (a1, b2, c3, and c4) with sodium azide. Compound a1 was synthesized by the cycling of 2-aminopyrimidine and 1,3-dichloroacetone. Compound b2 was synthesized via cyclization of guanidine nitrate with 2,4-pentandione in the presence of $K_2CO_3$ followed by 1,3-dichloroacetone. Compound c3 was synthesized from aminoguanidine bicarbonate, which was cyclized with glycolic acid followed by 2,4-pentandione to provide intermediate b3. And then it was treated with thionyl chloride to afford compound c3. Compound c4 were obtained from differently substituted anilines, which were substituted with 1-fluoro-2-nitro-benzene followed by reduction and cyclization with chloroacetyl chloride to provide compound c4.

### 2.3. Synthesis of target compounds of series III

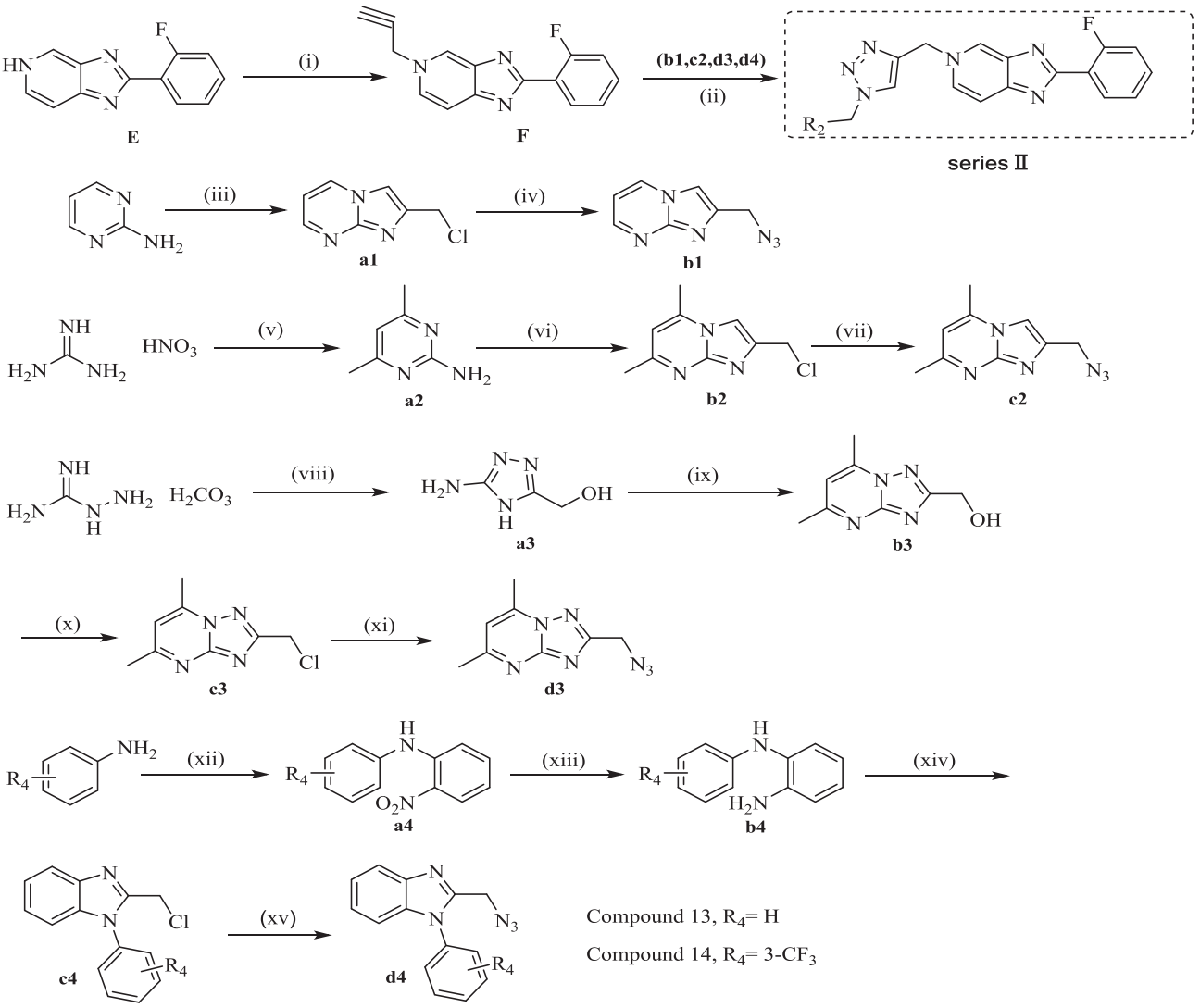
The target compounds of series III were synthesized in accordance with the procedures outlined in Scheme 3. As indicated, the target compounds (compounds 15 – 21) were prepared directly from intermediate E through *N*-alkylation reactions with the different chlorides a1, b2, c3, and c4, as shown in Scheme 3.

## 3. Results and discussion

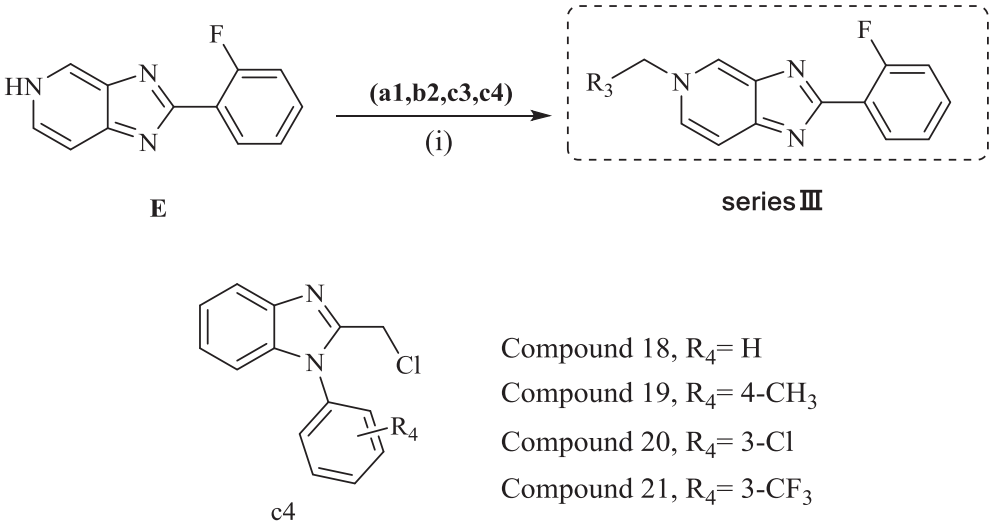
The inhibition of viral RNA replication was correlated with a decrease in HCV RNA and protein production. The reduction in HCV replication was measured indirectly through monitoring




**Scheme 1.** Reagents and conditions: (i) propargyl bromide, DIPEA, DMF, 25 °C, 8 h; (ii) CuI, DIPEA, ethanol, rt, 12 h; (iii) conc. HCl, $NaNO_2$, ethyl acetate, 0 °C–5 °C, 30 min; (iv) $NaN_3$, rt, 2 h.




**Scheme 2.** Reagents and conditions: (i) propargyl bromide, DIPEA, DMF, 25 °C, 8 h; (ii) CuI, DIPEA, ethanol, rt, 12 h; (iii) 1,3-dichloroacetone, dimethyl ether, rt, 2 h; (iv) $NaN_3$, DMF, rt, overnight; (v) 2,4-pentandione, $K_2CO_3$, $H_2O$, 80 °C, 7 h; (vi) 1,3-dichloroacetone, dimethyl ether, 45 °C, 10 h; (vii) $NaN_3$, DMF, rt, overnight; (viii) glycolic acid, $HNO_3$, $H_2O$, 108 °C, 22 h; (ix) 2,4-pentandione, acetic acid, reflux, 1 h; (x) thionyl chloride, $CH_2Cl_2$, rt, 3 h; (xi) $NaN_3$, DMF, rt, overnight; (xii) 1-fluoro-2-nitro-benzene, potassium fluoride, 140 °C, 24 h; (xiii) Fe, HCl, $NH_4Cl$, ethanol/$H_2O$, 70 °C, 30 min, reflux, 2 h; (xiv) chloroacetyl chloride, DMF, 80 °C, 2 h; (xv) $NaN_3$, DMF, rt, overnight.




**Scheme 3.** Reagents and conditions: (i) DIPEA, DMF, rt, 7 h.

HCV NS3 protease activity using an Indirect Immunofluorescence Assay (IFA). The cytotoxicity of the compounds in the replicon host cells was determined through the measurement of the fluorescence of Alamar blue dye, an indicator of cellular metabolism. The potency of the compounds described in this study was determined using a GT-1b subgenomic replicon assay. The $EC_{50}$, calculated from the dose-response curve, represents the concentration at which 50% inhibition of viral replication was achieved. The $CC_{50}$, calculated from the dose-response curve, represents the concentration at which the metabolic activity of the cells was reduced by 50% compared with untreated cells. The values are an average of a minimum of two determinations. Starting from series Ⅰ, the results were summarized in Table 1.

We found that most of the target compounds exhibited moderate to excellent anti-HCV activity, with the $EC_{50}$ values in the range from 1 nM to 100 nM. Furthermore, some were superior to sofosbuvir, which is approved for clinical use. Compound 3 exhibited more potent inhibition ($EC_{50}$ = 1.163 nM) than tegobuvir ($EC_{50}$ = 2.908 nM).

During the investigation of the C-4 phenyl position, the SAR at this position was found to be steep, with minor changes in the structure conferring large changes in potency, as illustrated in Table 1. Compound 1 ($EC_{50}$ = 22.83 nM), with an unsubstituted phenyl, showed acceptable anti-HCV potency that was nearly 3-fold higher than that of sofosbuvir ($EC_{50}$ = 64.26 nM). The introduction of a methoxyl group on the phenyl ring significantly increased replicon activity compared with compound 1. However, the comparison of the isomers 3 and 4 revealed marked differences between the anti-HCV activity of 3-methoxyl and 4-methoxyl in the structure. The 4-methoxyl derivative (3) ($EC_{50}$ = 1.163 nM) showed excellent activity, whereas the closely related 3-methoxyl analog (4) ($EC_{50}$ = 12.64 nM) resulted in an 11-fold decrease in this potency. The methanesulfonamide derivative (5) ($EC_{50}$ = 18.32 nM) was proven to be more potent than its unsubstituted counterpart (1), but did not provide any improvement in cellular potency compared with 4. The introduction of an additional fluorine to the C-4 phenyl position decreased the replicon assay potency to an $EC_{50}$ of 34.52 nM, probably owing to unfavorable interactions from the 4-fluoro group. Therefore, we speculated that the 4-methoxyl group was important for the anti-HCV activity of this series.

Compound 6, with a 2-fluoro substitution on the benzene ring, had an $EC_{50}$ of 52.34 nM, which was even less potent than that for the 4-fluoro derivative (2) ($EC_{50}$ = 34.52 nM). Furthermore, the introduction of 3,5-bis(trifluoromethyl) into the phenyl ring resulted in a complete loss of potency. Our preliminary SAR studies indicated that the introduction of electron-withdrawing groups on the phenyl ring could reduce the potency of the anti-HCV activity. In addition, the introduction of larger sized substituents at the 4-position of the phenyl ring also resulted in generally poor activity (compound 8, $EC_{50}$ = 96.35 nM; compound 9, $EC_{50}$ = 93.46 nM).

Series II, containing different nitrogenous heterocycles, was also examined, and the results were shown in Table 2. Compounds 10 ($EC_{50}$ = 21.36 nM) and 11 ($EC_{50}$ = 37.03 nM), with an imidazo[1,2-a]pyrimidine moiety, exhibited better anti-HCV potency than sofosbuvir, whereas compounds 13 and 14 resulted in a complete loss of activity in the GT-1b subgenomic replicon assay. A plausible explanation for this finding is that the steric hindrance produced by the introduction of large groups influenced the activity.

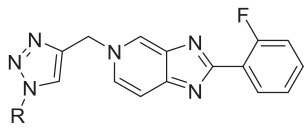
Further studies were performed to examine series III and these results were shown in Table 3. Unexpectedly, no compounds in this series exhibited inhibitory activity against the GT-1b replicon. Despite the negative results, when we focused on the differences between series Ⅱ and series III compounds, it is not difficult to find that compound 10, with 1,2,3-triazole, retained some replicon activity characteristics, whereas its counterpart 15 showed a complete loss of activity; this was supported by the relative potencies of compound 11 and 16. From the HCV replicon GT1b $EC_{50}$ data, we speculated that moiety B was crucial to the antiviral activity. Further studies about the influence of moiety B are currently underway.

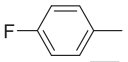
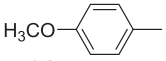
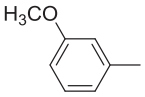
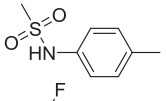
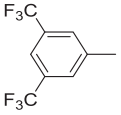
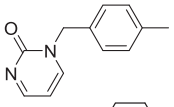
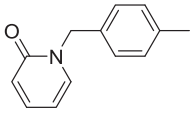
Because of its promising 1b replicon activity, the most potent compound 3 was examined in hERG determination. As initially hypothesized, testing for inhibition of the hERG channel showed a marked improvement over tegobuvir, and the results were shown in Table 4.

These impressive results suggested that the introduction of 1,2,3-triazole as moiety B would be a strategy that warranted further study. Furthermore, the phenyl ring in moiety A could be introduced into some substituents to improve the potency.

As compound 3 was discovered to be a potent inhibitor with superb potency, we evaluated the in vivo effects in Sprague–Dawley rats after oral administration at a dose of 35 mg/kg. The pharmacokinetic results were summarized in Table 5. We found that the rat in vivo pharmacokinetics after p.o. dosing of compound 3 was within a desirable range, with particularly good systemic absorption in terms of oral exposure (AUC) and high levels of $C_{max}$. In addition, the half-lives were moderate in this rodent species. Based on the strong antiviral activity and the encouraging PK profile, compound 3 was considered a potential candidate for further evaluation (See Fig. 6).

**Table 1**
HCV replicon activity of series I.

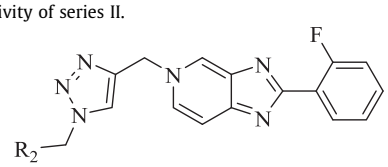


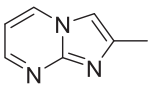
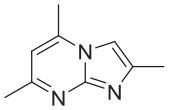
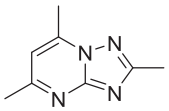
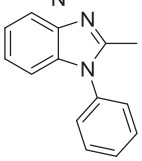
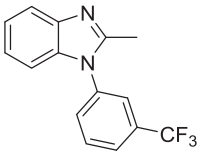
| Compound number | R | HCV replicon GT1b $EC_{50}$ (nM)[a] | Cytotoxicity $CC_{50}$ (nM)[a] |
|---|---|---|---|
| 1 |  | 22.83 | >200 |
| 2 |  | 34.52 | >200 |
| 3 |  | 1.163 | >200 |
| 4 |  | 12.64 | >200 |
| 5 |  | 18.32 | >200 |
| 6 |  | 52.34 | >200 |
| 7 |  | — | >200 |
| 8 |  | 96.35 | >200 |
| 9 |  | 93.46 | >200 |
| Tegobuvir | — | 2.908 | >200 |
| Sofosbuvir | — | 64.26 | >10000 |

All compounds were tested in duplicate. Each value is the mean of at least two determinations.

[a] $EC_{50}$ was measured by using GT 1b stable HCV subgenomic replicon.

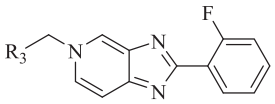
**Table 2**
HCV replicon activity of series II.

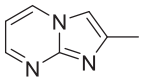
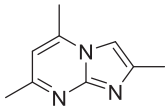
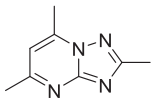
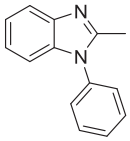
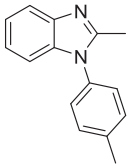
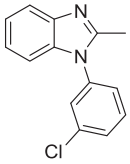
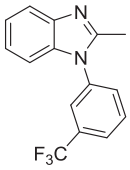


| Compound number | $R_2$ | HCV replicon GT1b $EC_{50}$ (nM)[a] | Cytotoxicity $CC_{50}$ (nM)[a] |
|---|---|---|---|
| 10 |  | 21.36 | >200 |
| 11 |  | 37.03 | >200 |
| 12 |  | 83.75 | >200 |
| 13 |  | – | >200 |
| 14 |  | – | >200 |
| Tegobuvir | – | 2.908 | >200 |
| Sofosbuvir | – | 64.26 | >10000 |

All compounds were tested in duplicate. Each value is the mean of at least two determinations.
[a] $EC_{50}$ was measured by using GT 1b stable HCV subgenomic replicon.

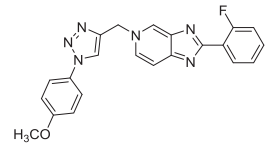
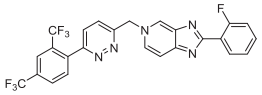
**Table 3**
HCV replicon activity of series III.



| Compound number | $R_3$ | HCV Replicon GT1b $EC_{50}$ (nM)[a] | Cytotoxicity $CC_{50}$ (nM)[a] |
|---|---|---|---|
| 15 |  | – | >200 |
| 16 |  | – | >200 |
| 17 |  | – | >200 |
| 18 |  | – | >200 |
| 19 |  | – | >200 |
| 20 |  | – | >200 |
| 21 |  | – | >200 |
| Tegobuvir | – | 2.908 | >200 |
| Sofosbuvir | – | 64.26 | >10000 |

All compounds were tested in duplicate. Each value is the mean of at least two determinations.
[a] $EC_{50}$ was measured by using GT 1b stable HCV subgenomic replicon.

**Table 4**
hERG determination of compound 3.

| Compound number | Structure | hERG $pIC_{50}$[a] |
|---|---|---|
| 3 |  | 5.2 |
| Tegobuvir |  | 6.1 |

[a] Compound testing was repeated on different cells.

## 4. Conclusions

To optimize the antiviral activity and reduce the side effects of tegobuvir treatment, three series of novel potent non-nucleoside inhibitors of the HCV NS5B polymerase were designed and synthesized. A preliminary investigation identified that several compounds (compounds 1, 3, 4, and 5) exhibited moderate to excellent anti-HCV potency in a cell-based HCV replicon system assay. The SARs, based on the $EC_{50}$ values shown in Table 1 along with the hERG determination confirmed the success of the introduction of 1,2,3-triazole. In particular, compound 3 showed desirable pharmacokinetics. The overall profile of compound 3 ($EC_{50}$ = 1.163 nM, $CC_{50}$ >200 nM), with respect to potency, pharmacokinetics, and safety, demonstrated that it was a good candidate for further study.

## 5. Experimental section

### 5.1. Chemistry

Unless otherwise noted, all commercially obtained solvents and reagents were used as received without further purification. Reactions' time and purity of the products were monitored by TLC on FLUKA silica gel aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm. All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). $^1H$

**Table 5**
Rat PO pharmacokinetic profiles of compound 3 (35 mg/kg PO; n = 3).

| Compound 3 | Rat number | | | Mean | SD |
|---|---|---|---|---|---|
| | 4 | 5 | 6 | | |
| $MRT_{0\rightarrow\infty}$(h) | 1.86 | 2.38 | 2.31 | 2.18 | 0.28 |
| $AUC_{0\rightarrow\infty}$(μg/L)·h | 81501.19 | 82274.55 | 53718.65 | 72498.13 | 16268.10 |
| $CL_z$/F(L/h/kg) | 0.43 | 0.42 | 0.65 | 0.50 | 0.13 |
| $V_z$/F(L/kg) | 1.04 | 2.33 | 2.99 | 2.12 | 1.00 |
| $T_{1/2z}$(h) | 1.67 | 3.80 | 3.18 | 2.88 | 1.10 |
| $C_{max}$(μg/L) | 28340.00 | 22049.00 | 16423.00 | 22270.67 | 5961.59 |
| $T_{max}$(h) | 1 | 2 | 1 | 1.33 | 0.58 |

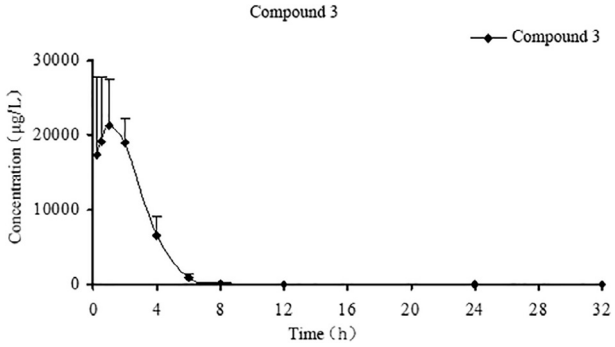



**Fig. 6.** Mean plasma concentration – time profile of compound 3 in male Sprague–Dawley rats.

NMR spectra were recorded on Bruker ARX-400, 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard.

### 5.2. Preparation of 2-(2-fluorophenyl)-1H-imidazo[4,5-c]pyridine (E)

#### 5.2.1. Preparation of 3-nitropyridin-4-amine (B)

Under ice bath, pyridin-4-amine (5.0 g, 50.0 mmol) was solubilized in concentrated sulfuric acid (20 mL), then fuming nitric acid (2.5 mL) was added drop-wise at 0–10 °C. After stirring for 5 h at rt, 90 °C for 3 h and continue to stir at rt overnight. Then the mixture was poured into ice-water, adjust the pH value to 7 with ammonia, The resulting precipitate was filtered, dried under reduced pressure to yield the title compound as a yellow solid (5.1 g, 70%). MS $[M+H]^+$ *m/z*: 140.04.

#### 5.2.2. Preparation of 2-fluoro-N-(3-nitropyridin-4-yl)benzamide (C)

Under ice bath, intermediate B (5.0 g, 40.0 mmol) and DIPEA (9.3 g, 80.0 mmol) were solubilized in DMF (50 mL), then 2-fluorobenzoyl chloride (6.3 g, 40.0 mmol) was added drop-wise. After stirring for 30 min at rt, the mixture was then heated to 60 °C for 2 h. The mixture was poured into ice-water, the resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a yellow solid (9.4 g, 90%). MS $[M+H]^+$ *m/z*: 262.05.

#### 5.2.3. Preparation of N-(3-aminopyridin-4-yl)-2-fluorobenzamide (D)

A mixture of iron powder (9.0 g, 16.0 mmol), hydrochloric acid (1.4 mL) and 90% ethanol (105 mL) was stirred at 30 °C for 10 min, the intermediate C (7.0 g, 27.0 mmol) was added at 60 °C, then the mixture was heated to reflux for 2 h. Immediate filter and the filtrate was concentrated under reduced pressure to yield the title compound as a yellow solid (5.3 g, 85%). MS $[M+H]^+$ *m/z*: 232.

#### 5.2.4. Preparation of 2-(2-fluorophenyl)-1H-imidazo[4,5-c]pyridine (E)

The intermediate D (5.0 g, 22.0 mmol) was solubilized in aceticacid (50 mL), heated to reflux for 3 h. The aceticacid was removed under reduced pressure and water was added, the precipitate was filtered and the filtrate was adjusted to pH 6–7 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a yellow solid (4.0 g, 86%). MS $[M+H]^+$ *m/z*: 214. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.10 (s, 1H), 9.05 (s, 1H), 8.40 (d, $J$ = 5.5 Hz, 1H), 8.32 (td, $J$ = 7.7, 1.7 Hz, 1H), 7.72–7.65 (m,2H), 7.58–7.45 (m, 2H).

#### 5.2.5. Preparation of 2-(2-fluorophenyl)-5-(prop-2-yn-1-yl)-5H-imidazo[4,5-c]pyridine (F)

A mixture of intermediate E (3.0 g, 14.0 mmol), DIPEA (3.6 g, 28.0 mmol) and DMF (30 mL) was stirred at rt for 30 min, then propargyl bromide (1.8 g, 15.0 mmol) was added drop-wise, the mixture was stirred at rt for 8 h. After that, water was added, the aqueous layer was extracted with $CH_2Cl_2$. The organic phase was washed with saturated aqueous lithium chloride solution. Then the organic layer was dried with $Na_2SO_4$, filtered and evaporated in vacuo to afford the title compound as a yellow solid (2.5 g, 71%). MS $[M+H]^+$ *m/z*: 252.

### 5.3. Preparation of target compounds of seriesⅠ (compound 1–9)

#### 5.3.1. General procedure for preparation of different azidobenzenes. (G1–G9)

Under 0 °C, different substituted anilines (5.0 mmol) was solubilized in ethyl acetate (5 mL), hydrochloric acid (1.5 mL) was added, then a solution of sodium nitrite (0.5 g, 6 mmol) was added drop-wise at 0–5 °C, the mixture was stirred at rt for 0.5 h. After that, a solution of sodium azide (0.4 g, 6.0 mmol) in water was added drop-wise at 0–5 °C, then stirred at rt for 2 h. The mixture was adjusted to pH 8–9 with saturated sodium carbonate solution, the aqueous layer was extracted with ethyl acetate. The organic phase was washed with brine. Then the organic layer was dried with $Na_2SO_4$, filtered and evaporated in vacuo to afford the title compounds (G1–G9) as yellow oil.

*5.3.1.1. Azidobenzene (G1).* Yellow oil; Yield: 72%; MS $[M+H]^+$ *m/z*: 120.

*5.3.1.2. 1-azido-4-fluorobenzene (G2).* Yellow oil; Yield: 70%; MS $[M+H]^+$ *m/z*: 138.

*5.3.1.3. 1-azido-4-methoxybenzene (G3).* Yellow oil; Yield: 77%; MS $[M+H]^+$ *m/z*: 150.

*5.3.1.4. 1-azido-3-methoxybenzene (G4).* Yellow oil; Yield: 75%; MS $[M+H]^+$ *m/z*: 150.

*5.3.1.5. N-(4-azidophenyl)methanesulfonamide (G5).* Yellow oil; Yield: 72%; MS $[M+H]^+$ *m*/*z*: 213.

*5.3.1.6. 1-azido-2-fluorobenzene (G6).* Yellow oil; Yield: 68%; MS $[M+H]^+$ *m*/*z*: 138.

*5.3.1.7. 1-azido-3,5-bis(trifluoromethyl)benzene (G7).* Yellow oil; Yield: 65%; MS $[M+H]^+$ *m*/*z*: 256.

*5.3.1.8. 1-(4-azidobenzyl)pyrimidin-2(1H)-one (G8).* Yellow oil; Yield: 72%; MS $[M+H]^+$ *m*/*z*: 228.

*5.3.1.9. 1-(4-azidobenzyl)pyridin-2(1H)-one (G9).* Yellow oil; Yield: 72%; MS $[M+H]^+$ *m*/*z*: 227.

*5.3.2. General procedure for preparation of target compounds (compound 1–9)*

Intermediate G (0.8 mmol) was solubilized in ethanol (2 mL), then intermediate F (0.2 g, 0.8 mmol), CuI (0.01 g, 0.5 mmol) and DIPEA (0.1 g, 0.8 mmol) were added, the mixture was stirred at rt for 12 h. After that, filtered, and the filtrate was concentrated under reduced pressure to yield the title compounds (compound 1–9) as white solid.

*5.3.2.1. Preparation of 2-(2-fluorophenyl)-5-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo [4,5-c]pyridine (compound 1).* White solid; Yield: 56%; Mp: 157–159 °C; Purity: 96.5%; MS $[M+H]^+$ *m*/*z*: 371. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.26 (s, 1H), 8.96 (s, 1H), 8.28 (s, 2H), 7.89 (d, *J* = 7.9 Hz, 2H),7.55 (m, 5H), 7.32 (s, 2H), 5.91 (s, 2H).

*5.3.2.2. Preparation of 2-(2-fluorophenyl)-5-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 2).* White solid; Yield: 58%; Mp: 155–157 °C; Purity: 97.3%; MS $[M+H]^+$ *m*/*z*: 389. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.21 (s, 1H), 8.93 (s, 1H), 8.33 (s, 1H), 8.28 (s, 1H), 7.96–7.90 (m, 2H), 7.86 (s, 1H), 7.52–7.40 (m, 3H), 7.32 (s, 2H), 5.92 (s, 2H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 162.98(2C), 161.35(2C), 143.55, 133.37, 133.35, 131.41(2C), 123.49(2C), 123.10(3C), 117.21(3C), 117.06(3C), 53.21.

*5.3.2.3. Preparation of 2-(2-fluorophenyl)-5-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 3).* White solid; Yield: 54%; Mp: 158–160 °C; Purity: 99.1%; MS $[M+H]^+$ *m*/*z*: 401. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.18 (s, 1H), 8.85 (s, 1H), 8.32 (s, 1H), 8.25 (d, *J* = 6.4 Hz, 1H), 7.83 (s, 1H), 7.79 (d, *J* = 9.0 Hz, 2H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.13 (d, *J* = 9.1 Hz, 2H), 5.89 (s, 2H), 3.82 (s, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 168.27, 161.67, 159.99, 159.81, 143.33, 132.06 (2C), 131.50, 131.24(2C), 130.19, 123.43(2C), 122.34(2C), 117.05, 116.91, 115.22(2C), 113.08, 55.90, 53.25.

*5.3.2.4. Preparation of 2-(2-fluorophenyl)-5-((1-(3-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)- 7. 5H-imidazo[4,5-c]pyridine (compound 4).* White solid; Yield: 54%; Mp: 156–157 °C; Purity: 98.6%; MS $[M+H]^+$ *m*/*z*: 401. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.32 (s, 1H), 8.98 (s, 1H), 8.40 (d, *J* = 6.5 Hz, 1H), 8.31 (t, *J* = 7.4 Hz, 1H), 7.92 (d, *J* = 6.5 Hz, 1H), 7.58–7.42 (m, 4H), 7.40–7.32 (m, 2H), 7.08 (d, *J* = 8.8 Hz, 1H), 5.97 (s, 2H), 3.84 (s, 3H).

*5.3.2.5. Preparation of N-(4-(4-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)-1H-1,2,3-triazol-1-yl)phenyl)methanesulfonamide (compound 5).* White solid; Yield: 57%; Mp: 164–166 °C; Purity: 96.0%; MS $[M+H]^+$ *m*/*z*: 464. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.12 (s, 1H), 9.43 (s, 1H), 8.89 (s, 1H), 8.51 (d, *J* = 6.4 Hz, 1H), 8.32 (t, *J* = 7.7 Hz, 1H), 8.00 (d, *J* = 6.6 Hz, 1H), 7.83 (d, *J* = 8.9 Hz, 2H), 7.63–7.56 (m, 1H), 7.44–7.36 (m, 4H), 6.01 (s, 2H), 3.07 (s, 3H). $^{13}$C NMR (150 MHz, DMSO-$d_6$) $\delta$ 161.47, 159.78, 143.01, 139.47, 134.53(2C), 133.04, 131.73, 121.95(2C), 119.91(3C), 119.86(3C), 117.26, 117.12(2C), 112.74, 53.87, 46.41.

*5.3.2.6. Preparation of 2-(2-fluorophenyl)-5-((1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 6).* White solid; Yield: 55%; Mp: 152–153 °C; Purity: 99.6%; MS $[M+H]^+$ *m*/*z*: 389. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.34 (s, 1H), 8.84 (s, 1H), 8.43 (d, *J* = 6.3 Hz, 1H), 8.31 (t, *J* = 7.0 Hz, 1H), 7.93 (d, *J* = 6.5 Hz, 1H), 7.86 (t, *J* = 7.3 Hz, 1H), 7.66–7.53 (m, 3H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.40–7.33 (m, 2H), 5.98 (s, 2H).

*5.3.2.7. Preparation of 5-((1-(3,5-bis(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(2-fl uorophenyl)-5H-imidazo[4,5-c]pyridine (compound 7).* White solid; Yield: 59%; Mp: 159–161 °C; Purity: 99.7%; MS $[M+H]^+$ *m*/*z*: 507. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.41 (s, 1H), 9.24 (s, 1H), 8.60 (s, 2H), 8.44 (s, 1H), 8.35 (s, 1H), 8.28 (s, 1H), 8.08–7.89 (m, 1H), 7.58–7.52 (m, 1H), 7.37 (s, 2H), 6.03 (s, 2H).

*5.3.2.8. Preparation of 1-(4-(4-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)-1H-1,2,3-triazol-1-yl)benzyl)pyrimidin-2(1H)-one (compound 8).* White solid; Yield: 54%; Mp: 173–175 °C; Purity: 94.5%; MS $[M+H]^+$ *m*/*z*: 479. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.82 (s, 1H), 8.96 (s, 1H), 8.87 (d, *J* = 7.1 Hz, 1H), 8.61–8.57 (m, 1H), 8.45–8.41 (m, 1H), 8.33–8.25 (m, 2H), 7.86 (d, *J* = 8.5 Hz, 2H), 7.79–7.72 (m, 1H), 7.60–7.49 (m, 4H), 6.53–6.49 (m, 1H), 6.15 (s, 2H), 5.13 (s, 2H).

*5.3.2.9. Preparation of 1-(4-(4-((2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridin-5-yl)methyl)-1H-1,2,3-triazol-1-yl)benzyl)pyridin-2(1H)-one (compound 9).* White solid; Yield: 55%; Mp: 167–169 °C; Purity: 97.0%; MS $[M+H]^+$ *m*/*z*: 478. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.21 (s, 1H), 8.91 (s, 1H), 8.31 (t, *J* = 7.7 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 4H), 7.49 (d, *J* = 8.4 Hz, 3H), 7.44 (m, 1H), 7.31 (t, *J* = 7.6 Hz, 2H), 6.43 (d, *J* = 9.1 Hz, 1H), 6.27 (t, *J* = 6.7 Hz, 1H), 5.92 (s, 2H), 5.16 (s, 2H).

*5.4. Preparation of target compounds of seriesⅡ (compound 10–14)*

*5.4.1. Preparation of 2-(chloromethyl)imidazo[1,2-a]pyrimidine (a1)*

A mixture of pyrimidin-2-amine (6.0 g, 64.0 mmol), 1,3-dichloroacetone (8.1 g, 64.0 mmol) and dimethyl ether (30 mL) was stirred at rt for 2 h. After that, The resulting precipitate was filtered, added to ethanol (50 mL) and the mixture was refluxed for 2 h . The solvent was removed under reduced pressure, water (30 mL) was added and the mixture was adjusted to pH 8–9 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a white solid (7.0 g, 65%). MS $[M+H]^+$ *m*/*z*: 168.

*5.4.2. Preparation of 2-(chloromethyl)-5,7-dimethylimidazo[1,2-a] pyrimidine (b2)*

*5.4.2.1. Preparation of 4,6-dimethylpyrimidin-2-amine(a2).* A mixture of guanidine nitrate (3.0 g, 25.0 mmol), 2,4-pentandione (3.7 g, 37.0 mmol), $K_2CO_3$ (3.4 g, 25.0 mmol) and distilled water (15 mL) was stirred at 80 °C for 7 h. After that, the mixture was cooled to rt and the resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a white solid (2.6 g, 96%). MS $[M+H]^+$ *m*/*z*: 123.

*5.4.2.2. Preparation of 2-(chloromethyl)-5,7-dimethylimidazo[1,2-a] pyrimidine (b2).* 1,3-dichloroacetone (5.6 g, 41.0 mmol) and intermediate a2 (5.0 g, 41.0 mmol) were solubilized in dimethyl ether (30 mL), the mixture was stirred at 45 °C for 10 h . The resulting

precipitate was filtered, added to ethanol (50 mL) and the mixture was refluxed for 2 h. The solvent was removed under reduced pressure, water (30 mL) was added and the mixture was adjusted to pH 8–9 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a white solid (5.1 g, 63%). MS $[M+H]^+$ *m*/*z*: 196.

*5.4.3. Preparation of 2-(chloromethyl)-5,7-dimethyl-[1,2,4]triazolo [1,5-a]pyrimidine (c3)*

*5.4.3.1. Preparation of (5-amino-4H-1,2,4-triazol-3-yl)methanol (a3).* Aminoguanidine bicarbonate (4.7 g, 34.0 mmol) was slowly added to a well-stirred solution of glycolic acid (5.3 g, 68.0 mmol) in water (8 mL) at rt, then nitric acid (0.2 mL) was added. The mixture was stirred at 108 °C for 22 h. After that, the mixture was cooled to below 10 °C and stirred for 2 h. The resulting precipitate was filtered, washed with ethanol and dried under reduced pressure to yield the title compound as a white solid (3.0 g, 76%). MS $[M+H]^+$ *m*/*z*: 115.

*5.4.3.2. Preparation of (5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)methanol (b3).* Intermediate a3 (3.0 g, 16.0 mmol) was solubilized in ethanol (36 mL), 2,4-pentandione (1.8 g, 17.0 mmol) and aceticacid (0.3 mL) were added, the mixture was heated to reflux for 1 h. After that, the mixture was cooled to rt and filterd, washed with ethanol and the filtrate was concentrated in vacuo, then cooled to 0 °C and stirred for 2 h, the resulting precipitate was filtered, washed with ethanol and dried under reduced pressure to yield the title compound as a white solid (2.3 g, 81%). MS $[M+H]^+$ *m*/*z*: 179.

*5.4.3.3. Preparation of 2-(chloromethyl)-5,7-dimethyl-[1,2,4]triazolo [1,5-a]pyrimidine (c3).* Under ice bath, intermediate b3 (2.0 g, 11.0 mmol) was solubilized in dichloromethane (10 mL). a solution of thionyl chloride (12.0 mmol) in dichloromethane (10 mL) was added drop-wise. The mixture was stirred at rt for 3 h. After that, the solvent was removed under reduced pressure and ethyl acetate was added, then the mixture was washed by saturated sodium bicarbonate solution followed by brine, and dried with $Na_2SO_4$, filtered and evaporated in vacuo to get yellow solid (1.9 g, 89%). MS $[M+H]^+$ *m*/*z*: 197.

*5.4.4. Preparation of 2-(chloromethyl)-1-phenyl-1H-benzo[d] imidazole (c4)*

*5.4.4.1. Preparation of 2-nitro-N-phenylaniline (a4).* A mixture of o-nitrofluorobenzene (5.0 g, 35.0 mmol), aniline (5.0 g, 35.0 mmol) and KF(3.4 g, 35.0 mmol) was stirred at 140 °C for 24 h. After that, the mixture was cooled to rt and ethyl acetate was added, the organic phase was washed by 2 M HCl followed by brine, and dried with $Na_2SO_4$, filtered and evaporated in vacuo to get red solid (5.8 g, 77%). MS $[M+H]^+$ *m*/*z*: 215.

*5.4.4.2. Preparation of $N^1$-phenylbenzene-1,2-diamine (b4).* A mixture of iron powder (5.2 g, 92.0 mmol), hydrochloric acid (1 mL), ammonium chloride (0.6 g, 11.0 mmol) and 60% ethanol (100 mL) was stirred at 70 °C for 30 min, then the intermediate a4 (5.0 g, 23.0 mmol) was added, the mixture was heated to reflux for 2 h. After that, filtered through a Celite pad, the filtrate was concentrated and adjusted to pH 8–9 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a red solid (3.5 g, 82%). MS $[M+H]^+$ *m*/*z*: 185.

*5.4.4.3. Preparation of 2-(chloromethyl)-1-phenyl-1H-benzo[d]imidazole (c4).* The intermediate b4 (3.0 g, 16.0 mmol) and chloroacetyl chloride (2.8 g, 25.0 mmol) were added to DMF (30 mL), the mixture was heated to 80 °C for 2 h. After that, the mixture was poured into ice-water after cooled to rt. The mixture was adjusted to pH 8–9 with saturated sodium carbonate solution. The resulting precipitate was filtered, washed with water and dried under reduced pressure to yield the title compound as a pink solid (2.6 g, 68%). MS $[M+H]^+$ *m*/*z*: 243.

*5.4.5. General procedure for preparation of different hydrazoates (b1, c2, d3, d4)*

A mixture of sodium azide (2.0 g, 30.0 mmol), intermediate (a1, b2, c3, c4. 30.0 mmol) and DMF (50 mL) was stirred at rt overnight. After that, the mixture was poured into water and the aqueous layer was extracted with $CH_2Cl_2$. The organic phase was washed with water, followed by brine. The organic layer was dried with $Na_2SO_4$, filtered and evaporated in vacuo to afford a white soild.

*5.4.5.1. 2-(azidomethyl)imidazo[1,2-a]pyrimidine (b1).* White solid; Yield: 77.6%; MS $[M+H]^+$ *m*/*z*: 175.

*5.4.5.2. 2-(azidomethyl)-5,7-dimethylimidazo[1,2-a]pyrimidine (c2).* White solid; Yield: 82.8%; MS $[M+H]^+$ *m*/*z*: 203.

*5.4.5.3. 2-(azidomethyl)-5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidine (d3).* White solid; Yield: 76.3%; MS $[M+H]^+$ *m*/*z*: 204.

*5.4.5.4. 2-(azidomethyl)-1-phenyl-1H-benzo[d]imidazole (d4).* White solid; Yield: 79.3%; MS $[M+H]^+$ *m*/*z*: 250.

*5.4.6. General procedure for preparation of target compounds (compound 10–14)*

A solution of intermediate (b1, c2, d3, d4. 0.8 mmol) in ethanol (2 mL) was stirred to dissolve, intermediate F (0.2 g, 0.8 mmol) and CuI (0.01 g, 0.5 mmol) was added, then DIPEA (0.1 g, 0.8 mmol) was added drop-wise. The mixture was stirred at rt for 12 h. After that, filterd, and the filtrate was concentrated in vacuo to yield the title compounds (compound 10–compound 14) as solid.

*5.4.6.1. Preparation of 2-(2-fluorophenyl)-5-((1-(imidazo[1,2-a] pyrimidin-2-ylmethyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo [4,5-c]pyridine (compound 10).* Yellow solid; Yield: 54%; Mp: 199–200 °C; Purity: 95.6%; MS $[M+H]^+$ *m*/*z*: 426. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.09 (s, 1H), 8.51 (d, *J* = 6.8 Hz, 1H), 8.34 (s, 1H), 8.03–7.96 (m, 2H), 7.72 (s, 1H), 7.57 (d, *J* = 9.1 Hz, 1H), 7.50–7.47 (m 2H), 7.33–7.29 (m, 2H), 6.89 (t, *J* = 6.6 Hz, 1H), 5.78 (s, 2H), 5.52 (s, 2H).

*5.4.6.2. Preparation of 5-((1-((5,7-dimethylimidazo[1,2-a]pyrimidin-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine (compound 11).* White solid; Yield: 54%; Mp: 187–188 °C; Purity: 99.6%; MS $[M+H]^+$ *m*/*z*: 454. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.09 (s, 1H), 8.35 (s,1H), 8.28 (t, *J* = 7.2 Hz, 1H), 8.18 (d, *J* = 6.8 Hz, 1H), 7.80 (s, 1H), 7.74 (d, *J* = 6.8 Hz, 1H), 7.46–7.41 (m, 1H), 7.27 (t, *J* = 8.1 Hz, 2H), 6.86 (s, 1H), 5.76 (s, 2H), 5.69 (s, 2H), 2.56 (s, 3H), 2.44 (s, 3H).

*5.4.6.3. Preparation of 2-((4-((2-(2-fluorophenyl)-5H-imidazo[4,5-c] pyridin-5-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)-5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidine (compound 12).* Yellow solid; Yield: 57%; Mp: 193–195 °C; Purity: 95.0%; MS $[M+H]^+$ *m*/*z*: 455. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.15 (s, 1H), 8.45 (s, 1H), 8.30 (t, *J* = 7.1 Hz, 1H), 8.24 (d, *J* = 6.7 Hz, 1H), 7.80 (d, *J* = 6.7 Hz, 1H), 7.50–7.44 (m, 1H), 7.33–7.27 (m, 2H), 7.19 (s, 1H), 5.91 (s, 2H), 5.83 (s, 2H), 2.68 (s, 3H), 2.56 (s, 3H).

*5.4.6.4. Preparation of 2-(2-fluorophenyl)-5-((1-((1-phenyl-1H-benzo [d]imidazol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo [4,5-c]pyridine (compound 13).* Pink solid; Yield: 58%; Mp: 217–

219 °C; Purity: 98.5%; MS $[M+H]^+$ *m/z*: 501. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.08 (s, 1H), 8.32 (t, *J* = 7.7 Hz, 1H), 8.20 (s, 1H), 8.17 (d, *J* = 6.5 Hz, 1H), 7.81 (d, *J* = 6.3 Hz, 1H), 7.73 (dd, *J* = 6.3, 2.5 Hz, 1H), 7.51–7.44 (m, 6H), 7.34–7.26 (m, 4H), 7.14 (dd, *J* = 6.4, 2.5 Hz, 1H), 5.88 (s, 2H), 5.75 (s, 2H).

*5.4.6.5. Preparation of 2-(2-fluorophenyl)-5-((1-((1-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imid azol-2-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 14).* Starting from 3-trifluoromethylaniline, 2-(azidomethyl)-1-(3-(trifluoromethyl)phenyl)-1H-be nzo[d]imidazolate was prepared by the method of compound c4, MS [M+H]+ *m/z*: 318. The title compound was obtained with the method mentioned above.

Gray soild; Yield: 56%; Mp: 212–214 °C; Purity: 98.7%; MS $[M+H]^+$ *m/z*: 569. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.21 (s, 1H), 8.30 (d, *J* = 6.7 Hz, 2H), 8.28 (s, 1H), 8.00 (s, 1H), 7.88 (d, *J* = 7.3 Hz, 2H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.73 (t, *J* = 7.6 Hz, 2H), 7.57–7.48 (m, 1H), 7.40–7.28 (m, 4H), 7.18 (d, *J* = 6.8 Hz, 1H), 5.92 (s, 2H), 5.80 (s, 2H).

### *5.5. General procedure for preparation of target compounds (compound 15–21)*

Intermediate E (0.17 g, 0.8 mmol) and DIPEA (0.2 g, 1.6 mmol) were added to DMF (1.5 mL), the mixture was stirred at rt for 30 min, then intermediate (a1, b2, c3, c4. 0.8 mmol) was added drop-wise, then the mixture was continue to stir at room temperature for 7 h. After that, the mixture was poured into water to stir for another 30 min. The resulting precipitate was filtered, washed with cold water and dried under reduced pressure to yield the title compounds (compound 15–21) as solid.

#### *5.5.1. Preparation of 2-(2-fluorophenyl)-5-(imidazo[1,2-a]pyrimidin-2-ylmethyl)-5H-Imidazo [4,5-c]pyridine (compound 15)*

Yellow solid; Yield: 65%; Mp: 145–147 °C; Purity: 97.2%; MS $[M+H]^+$ *m/z*: 345. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.16 (s, 1H), 8.56 (d, *J* = 6.7 Hz, 1H), 8.31 (t, *J* = 7.1 Hz, 1H), 8.23 (d, *J* = 6.6 Hz, 1H), 8.04 (s, 1H), 7.77 (d, *J* = 6.8 Hz, 1H), 7.52 (d, *J* = 9.1 Hz, 1H), 7.50–7.43 (m, 1H), 7.34–7.22(m, 2H), 6.90 (t, *J* = 6.7 Hz, 1H), 5.81 (s, 2H).

#### *5.5.2. Preparation of 5-((5,7-dimethylimidazo[1,2-a]pyrimidin-2-yl) methyl)-2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine (compound 16)*

White soild; Yield: 67%; Mp: 136–138 °C; Purity: 97.8%; MS $[M+H]^+$ *m/z*: 373. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.18 (s, 1H), 8.32 (td, *J* = 7.6, 1.5 Hz, 1H), 8.28 (dd, *J* = 6.8, 1.4 Hz, 1H), 7.92 (s, 1H), 7.82 (d, *J* = 6.8 Hz, 1H), 7.53–7.46 (m, 1H), 7.39–7.30 (m, 2H), 6.91 (s, 1H), 5.84 (s, 2H), 2.63 (s, 3H), 2.48 (s, 3H).

#### *5.5.3. Preparation of 2-((2-(2-fluorophenyl)-5H-imidazo[4,5-c] pyridin-5-yl)methyl)-5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidine (compound 17)*

Yellow soild; Yield: 65%; Mp: 149–151 °C; Purity: 98.9%; MS $[M+H]^+$ *m/z*: 374. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.13 (s, 1H), 8.32 (t, *J* = 7.0 Hz, 1H), 8.23 (d, *J* = 5.7 Hz, 1H), 7.81 (d, *J* = 6.8 Hz, 1H), 7.47 (d, *J* = 5.9 Hz, 1H), 7.30 (t, *J* = 8.1 Hz, 2H), 7.21 (s, 1H), 6.03 (s, 2H), 2.70 (s, 3H), 2.56 (s, 3H).

#### *5.5.4. Preparation of 2-(2-fluorophenyl)-5-((1-phenyl-1H-benzo[d] imidazol-2-yl)methyl)-5H-imid azo[4,5-c]pyridine (compound 18)*

Pink soild; Yield: 61%; Mp: 153–155 °C; Purity: 97.7%; MS $[M+H]^+$ *m/z*: 420. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.99 (s, 1H), 8.31 (t, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 6.7 Hz, 1H), 7.80 (d, *J* = 6.7 Hz, 1H), 7.70 (s, 4H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.53–7.45 (m, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.29–7.19 (m, 3H), 6.01 (s, 2H).

#### *5.5.5. Preparation of 2-(2-fluorophenyl)-5-((1-(p-tolyl)-1H-benzo[d] imidazol-2-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 19)*

Starting from p-toluidine, 2-(chloromethyl)-1-(p-tolyl)-1H-benzo[d]imidazole was prepared by the method of compound c4, MS [M+H]+ *m/z*: 257. The title compound was obtained with the method mentioned above.

Yellow soild; Yield: 63%; Mp: 157–158 °C; Purity: 93.4%; MS $[M+H]^+$ *m/z*: 434. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.96 (s, 1H), 8.32 (t, *J* = 7.6 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 7.78 (d, *J* = 6.8 Hz, 1H), 7.67–7.62 (m, 1H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 3H), 7.31 (t, *J* = 8.2 Hz, 2H), 7.28–7.17 (m, 3H), 5.98 (s, 2H), 2.46 (s, 3H).

#### *5.5.6. Preparation of 5-((1-(3-chlorophenyl)-1H-benzo[d]imidazol-2-yl)methyl)-2-(2-fluorophenyl)-5H-imidazo[4,5-c]pyridine (compound 20)*

Starting from 3-chloroaniline, 2-(chloromethyl)-1-(3-chlorophenyl)-1H-benzo[d]imidazole was prepared by the method of compound c4, MS [M+H]+ *m/z*: 277. The title compound was obtained with the method mentioned above.

Pink soild; Yield: 62%; Mp: 154–156 °C; Purity: 97.2%; MS $[M+H]^+$ *m/z*: 454. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.97 (s, 1H), 8.32 (td, *J* = 7.6, 1.6 Hz, 1H), 8.11 (dd, *J* = 6.8, 1.3 Hz, 1H), 7.89 (s, 1H), 7.79 (d, *J* = 6.8 Hz, 1H), 7.75–7.72 (m, 2H), 7.71–7.63 (m, 2H), 7.51–7.43 (m, 1H), 7.35–7.24 (m, 5H), 6.02 (s, 2H).

#### *5.5.7. Preparation of 2-(2-fluorophenyl)-5-((1-(3-(trifluoromethyl) phenyl)-1H-benzo[d]imidazol-2-yl)methyl)-5H-imidazo[4,5-c]pyridine (compound 21)*

Starting from 3-trifluoromethylaniline, 2-(chloromethyl)-1-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole was prepared by the method of compound c4, MS [M+H]+ *m/z*: 311. The title compound was obtained with the method mentioned above.

White soild; Yield: 62%; Mp: 150–151 °C; Purity: 98.2%; MS $[M+H]^+$ *m/z*: 488. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.95 (s, 1H), 8.31 (t, *J* = 7.5 Hz, 1H), 8.13 (s, 1H), 8.09 (d, *J* = 6.8 Hz, 1H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.98–7.94 (m, 1H), 7.78 (d, *J* = 6.8 Hz, 1H), 7.70–7.64 (m, 1H), 7.51–7.44 (m, 1H), 7.34–7.27 (m, 4H), 7.26–7.21 (m, 1H), 6.01 (s, 2H).

### *5.6. Antiviral assays*

The evaluation of the antiviral as well as antimetabolic effect of each compound was performed in parallel. The GT-1b HCV subgenomic stable cell lines was used to measure anti-HCV activity. Replicon-containing cells were subcultured in DMEM supplemented with 10% FBS and grown for 3–4 days in 75 $cm^2$ tissue culture flasks. At 1 day before addition of the compound, cells were harvested and seeded at a density of 8000 cells per well (100 μL/well) in white 96-well plates for evaluation of the antiviral effect. The microtiter plates were incubated overnight (37 °C, 5% CO2, 95–99% relative humidity), yielding a nonconfluent cell monolayer. Serial fourfold dilutions (8 concentrations) of compounds are performed in DMSO followed by further dilution in cell culture media and subsequent addition to cell plates. Compound-treated cells are incubated 72 h at 37 °C in a 5% $CO_2$ incubator. Reduction in HCV replication was measured indirectly by monitoring HCV NS3 protease activity using Indirect Immunofluorescence Assay (IFA). The cytotoxicity of compounds in the replicon host cells was determined by measurement of the fluorescence of Alamar blue dye, an indicator of cellular metabolism. Both $EC_{50}$ and $CC_{50}$ values were obtained using the GraphPad Prism 5.0 software.

### *5.7. Indirect Immunofluorescence assay (IFA)*

Cells seeded on 96-well plates were washed with PBS and fixed with 4% paraformaldehyde for 20 min at RT. After being washed

three times with PBS, cells were incubated in blocking buffer (PBS containing 3% BSA, 0.3% Triton X-100 and 10% FBS) for 60 min and then in binding buffer (PBS containing 3% BSA and 0.3% Triton X-100) with MAbs against NS3 overnight at 4 °C. After three washes with PBS, cells were incubation with TRITC-conjugated goat anti-mouse IgG (Thermo) with binding buffer for 1 h at RT. Stained samples were then examined with High-content system (PerkinElmer Operetta).

### 5.8. Pharmacokinetics

All studies were conducted under IACUC approved protocols, and animals were allowed free access to food and water. Male Sprague–Dawley rats used in these studies weighed between 180 and 220 g and were dosed orally at doses of 35 mg/kg by gavage using a dosing formulation consisting of CMC (0.5%) in water for pharmacokinetic evaluations. Blood was collected into heparin tubes at typically 0.083 (5 min), 0.17 (10 min), 0.25 (15 min), 0.33 (20 min), 0.5 (30 min), 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 h after dosing. Samples were extracted by protein precipitation and centrifugation and analyzed by UPLC-MS.

## A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.04.029.

## References


1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of Hepatitis C virus infection. *Lancet Infect Dis*. 2005;5:558–567.
2. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the Hepatitis C virus infection. *J Hepatol*. 2014;61:S45–S57.
3. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut*. 2006;55:1350–1359.
4. Lin K, Perni RB, Kwong AD, Lin C. VX-950, a novel hepatitis C Virus (HCV) NS3−4A protease inhibitor, exhibits potent antiviral activities in HCV replicon cells. *Antimicrob Agents Chemother*. 2006;50:1813–1822.
5. Perni RB, Almquist SJ, Byrn RA, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C Virus NS3−4A serine protease. *Antimicrob Agents Chemother*. 2006;50:899–909.
6. Kwong AD, Kauffman RS, Hurter P, Mueller P. Discovery and development of telaprevir: an NS3−4A protease inhibitor for treating genotype 1 chronic hepatitis C virus. *Nat Biotechnol*. 2011;29:993–1003.
7. Njoroge FG, Chen KX, Shih N-Y, Piwinski JJ. Challenges in modern drug discovery: a case study of boceprevir, an HCV protease inhibitor for the treatment of hepatitis C virus infection. *Acc Chem Res*. 2008;41:50–59.
8. Poordad F, McCone J, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*. 2011;364:1195–1206.
9. Bidell MR, McLaughlin M, Faragon J, Morse C, Patel N. Desirable characteristics of hepatitis c treatment regimens: a review of what we have and what we need. *Infect. Dis. Ther.*. 2016;5:299–312.
10. Ali S, Leveque V, Le Pogam S, et al. Selected replicon variants with low-level in vitro resistance to the hepatitis C virus NS5B polymerase inhibitor PSI-6130 lack cross-resistance with R1479. *Antimicrob Agents Chemother*. 2008;52:4356–4369.
11. Feldstein A, Kleiner D, Krave D, et al. Severe hepatocellular injury with apoptosis induced by a hepatitis C polymerase inhibitor. *J Clin Gastroenterol*. 2009;43:374–381.
12. Gilead Quarterly Report: CA. Foster City: Gilead Sciences, May 2, 2008.
13. Leivers Martin, Miller John F, Chan Stephanie A, et al. Imidazopyridazine Hepatitis C Virus polymerase inhibitors. structure-activity relationship studies and the discovery of a novel, traceless prodrug mechanism. *J Med Chem*. 2014;57:1964–1975.
14. Shih I-hung, Vliegen Inge, Peng Betty, et al. Mechanistic characterization of GS-9190 (Tegobuvir), a novel nonnucleoside inhibitor of hepatitis C Virus NS5B polymerase. *Antimicrob Agents Chemother*. 2011;55:4196.
15. Hebner C, Han B, Brendza K, et al. The HCV Non-nucleoside inhibitor tegobuvir utilizes a novel mechanism of action to inhibit NS5B polymerase function. *PLoS ONE*. 2012;7:e39163.
16. Grandberg I, Faizova G, Kost A. Comparative basicities of substituted pyridines and electronegativity series for substituents in the pyridine series. *Chem Heterocycl Compd*. 1967;2:421–425.
17. Sofia Michael J, Chang Wonsuk, Furman Phillip A, Mosley Ralph T, Ross Bruce S. Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C Virus NS5B RNA-dependent RNA-polymerase. *J Med Chem*. 2012;55:2481–2531.
18. Vaz R, Li Y, Rampe D. Human ether-a-go-go related gene (HERG): a chemist's perspective. *Prog Med Chem*. 2005;43:1–18.
19. Ferreira ML, Souza MVN, Wardell SMSV, et al. Synthesis and antitubercular evaluation of new bis-1,2,3-triazoles derived from D-mannitol. *J Carbohydr Chem*. 2010;29:265–274.
20. Yan S-J, Liu Y-J, Chen Y-L, Liu L, Lin J. An efficient one-pot synthesis of heterocycle-fused 1,2,3-triazole derivatives as anticancer agents. *Bioorg Med Chem Lett*. 2010;20:5225–5228.
21. Jordao Alessandro K, Afonso Priscila P, Ferreira Vitor F, et al. Antiviral evaluation of N-amino-1,2,3-triazoles against Cantagalo virus replication in cell culture. *Eur J Med Chem*. 2009;44:3777–3783.
22. Xiaobin TANG, Zhenghui LI, Yonghong LI, et al. Synthesis and biological evaluation of novel saccharin derivatives containing 1,2,3-triazole moiety. *Chem Res Chin Univ*. 2015;31:71–77.
23. Kolb HC, Sharpless KB. *Drug Discovery Today*. 2003;8:1128.
24. Biagi Giuliana, Calderone Vincenzo, Giorgi Irene, et al. 5-(4′-Substituted-2′-nitroanilino) -1,2,3-triazoles as new potential potassium channel activators I. *Eur. J. Med. Chem.*. 2000;35:715–720.
25. Calderone V, Giorgi I, Martinotti E, Mantuano E, Martelli A, Nardi A. *Eur J Med Chem*. 2005;40:521–528.
26. Mekonnen, Belew et al. Preparation of benzodiazepine derivatives useful for treating cognitive impairment: WO, 2015095783[P]. 2015-6-25.
27. Ritzen, Andreas et al. Novel phenylimidazole derivatives as PDE10A enzyme inhibitors for treating neurode generative disorder, psychiatric disorder, or drug addiction: WO, 2009152825[P], 2009–12-23.
28. Pueschl, Ask et al. Preparation of heteroaromatic phenylimidazole derivatives as PDE10A enzyme inhibitors for treating psychiatric disorders, neurode generative disorders, and drug addiction: WO, 2011072694[P], 2011-6-23.
29. Wang M-Yi, Guo Y-ming. Optimization of the synthesis of 2-amino-4,6-dimethyl pyrimidine with ortho gonal experiment. *Guangzhou Hua Gong*. 2013;41:86–87.