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PII: S0223-5234(20)30467-0

DOI: https://doi.org/10.1016/j.ejmech.2020.112495

Reference: EJMECH 112495

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 27 March 2020

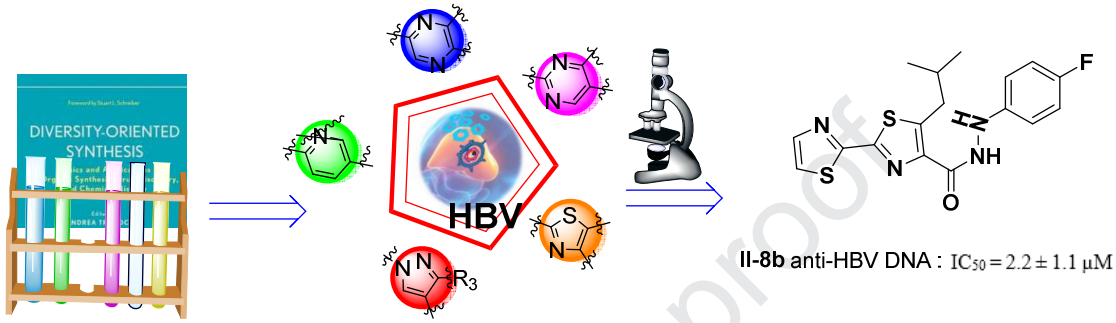
Revised Date: 16 May 2020

Accepted Date: 18 May 2020

Please cite this article as: H. Jia, J. Yu, X. Du, S. Cherukupalli, P. Zhan, X. Liu, Design, Diversity-Oriented Synthesis and Biological Evaluation of Novel Heterocycle Derivatives as Non-nucleoside HBV Capsid Protein Inhibitors, *European Journal of Medicinal Chemistry*, https://doi.org/10.1016/j.ejmech.2020.112495.

## Graphical abstract

Five skeletons of heterocycle derivatives (pyrazole, thiazole, pyrazine, pyrimidine and pyridine analogs) were designed as potential HBV non-nucleoside inhibitors. And seven synthetic routes were employed to acquire target compounds by diversity-oriented synthesis.



# Design, Diversity-Oriented Synthesis and Biological Evaluation of Novel Heterocycle Derivatives as Non-nucleoside HBV Capsid Protein Inhibitors

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**Abstract:**

The capsid assembly is a significant phase for the hepatitis B virus (HBV) lifespan and is an essential target for anti-HBV drug discovery and development. Herein, we used scaffold hopping, bioisosterism, and pharmacophore hybrid-based strategies to design and synthesize six series of various heterocycle derivatives (pyrazole, thiazole, pyrazine, pyrimidine, and pyridine) and screened for *in vitro* anti-HBV non-nucleoside activity. Drug candidate NZ-4 and AT-130 were used as lead compounds. Several compounds exhibited prominent anti-HBV activity compared to lead compound NZ-4 and positive drug Lamivudine, especially compound **II-8b**, showed the most prominent anti-HBV DNA replication activity

($IC_{50}$ = 2.2 ± 1.1 μM). Also compounds **IV-8e** and **VII-5b** showed the best *in vitro* anti-HBsAg secretion ($IC_{50}$ = 3.8±0.7 μM, $CC_{50}$ > 100 μM) and anti-HBeAg secretion ($IC_{50}$ = 9.7±2.8 μM, $CC_{50}$ > 100 μM) respectively. Besides, **II-8b** can interact HBV capsid protein with good affinity constants ($K_D$ = 60.0 μM), which is equivalent to lead compound NZ-4 (($K_D$ = 50.6 μM). The preliminary structure-activity relationships (SARs) of the newly synthesized compounds were summarized, which may help researchers to discover more potent anti-HBV agents.

**Keywords:** HBV, Capsid protein, Diversity-oriented synthesis, Heterocycle derivatives.

## 1. Introduction

Hepatitis B is a serious infectious disease caused by the hepatitis B virus (HBV). Long-term development of hepatitis B frequently causes acute or chronic viral hepatitis, severe hepatitis, liver cirrhosis, and hepatocellular carcinoma. The recent World Health Organization (WHO) statistics indicated nearly 2 billion people worldwide had been infected with HBV, of which about 350 million people with chronic HBV infection. An average of about 60 million people die every year due to acute or chronic viral hepatitis and related concurrency disease. China is a high endemic area of hepatitis B with about 93 million hepatitis B virus (HBV) carriers;an average of about 300,000 patients die each year due to infection with the hepatitis B virus[1, 2]. Due to the high incidence, long course and difficulty in curing, hepatitis B has become a major disease that seriously affects people's health and social development. Therefore research on effective drugs of HBV has become the top priority[3].

HBV is a member of hepadnaviridae, consisting partially double-stranded circular genome. HBV replication process possesses adsorption and fusion to hepatocyte, DNA repair and transcription of HBV covalently closed circular DNA (cccDNA), translation and reverse transcription of progenome RNA (PgRNA). It also possesses capsid assembly and DNA replication, viral particle recirculation and

release, etc. [4]. Few drugs have been developed for the treatment of chronic hepatitis B (CHB) by an in-depth understanding of the HBV life cycle and molecular biology. At present, only interferon (interferon-$\alpha$ and pegylated interferon-$\alpha$) and six nucleoside or nucleotide drugs such as Lamivudine (3TC), Adefovir dipivoxil (ADV), Entecavir (ETV), Telbivudine (LdT), Tenofovirdisoproxil fumarate (TDF) and Tenofovir alafenamide (TAF) have been approved by the U.S. Food and Drug Administration (FDA) for HBV treatment. Though they profoundly suppress HBV replication, the disadvantage of high price, low cure rate, serious side effects, resistance and high recurrence is undoubtedly essential to combat the HBV infection[5, 6].

The capsid is a T=4 icosahedral complex assembled from 120 copies of core protein homodimer, which is the most populous species found in HBV virions. It consists of the N-terminal assembly domain (Cp149, residues 1-149) and the C-terminal binding domain (protamine domain, residues 150-183) [7]. As capsids provide the structural background for encasement of the RNA pregenome, assembly of a viral core protein and RNA pregenome reverse transcription, interfering assembly of capsid formation is assumed to be less prone to developing drug resistance [8]. The HBV capsid protein and its assembly process have no human analogs, making HBV assembly has become an attractive target for new antiviral therapies [1, 9-11].

NZ-4 inhibits HBV replication by interfering with the interaction between pgRNA and HBcAg in the capsid assembly process, thus increasing the replication-deficient HBV capsids. NZ-4 suppressed intracellular HBV replication in HepG2.2.15 cells with an $IC_{50}$ value of 1.33 μmol/L, whereas the compound inhibited the cell viability with a $CC_{50}$ value of 50.4 μmol/L [12]. AT-130, a phenylpropanamide derivative, has more potent anti-HBV DNA replication activity with an $IC_{50}$ value of 0.13 μmol/L and $CC_{50}$ value of more than 51 μmol/L[6, 13-15]. NZ-4 and AT-130 also have four various fragments within the structure (yellow, aqua, pink and spring green), as illustrated in Figure 1. Herein, six small series of heterocycle derivatives (pyrazole,

thiazole, pyrazine, pyrimidine, and pyridine analogs) were designed as potential HBV non-nucleoside inhibitors through scaffold hopping, bioisosterism, and pharmacophore hybrid-based strategies.

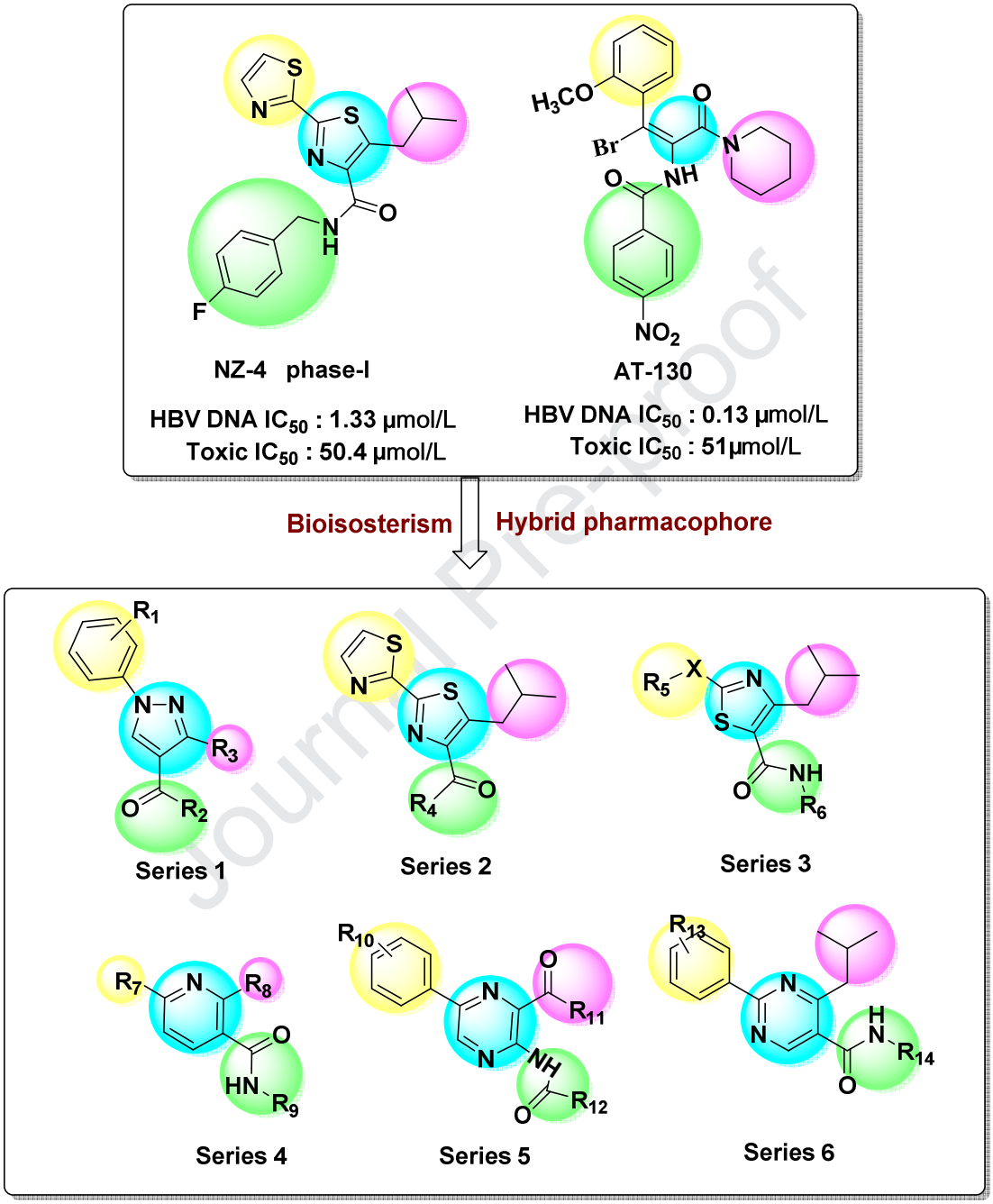



**Figure 1**. The structural modifications of six small series of heterocycle derivatives as potent non-nucleoside HBV inhibitors.

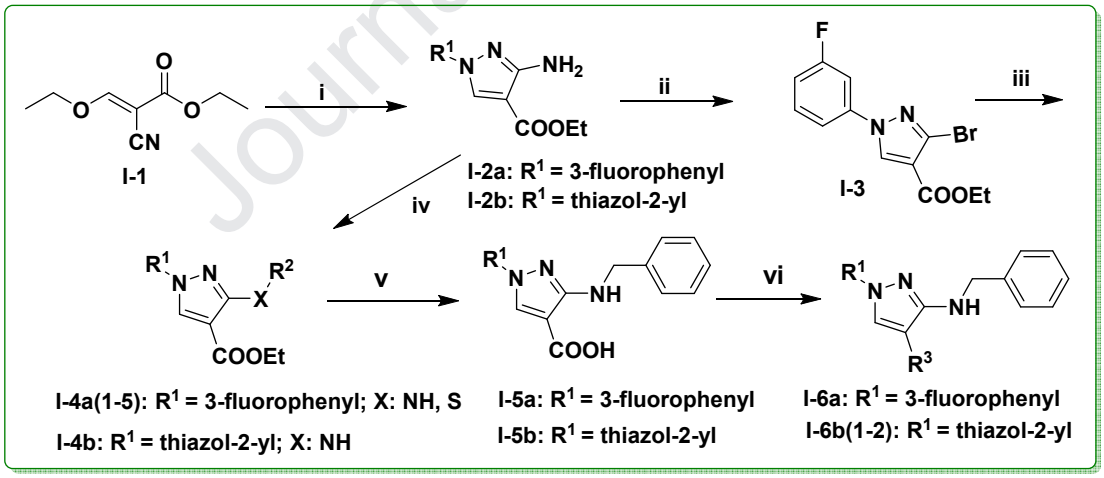
## 2. Results and discussion

### 2.1 Chemistry

The synthetic route for the target compounds **I-4a**(**1**-**5**), **I-4b**, **I-6a**, and **I-6b**(**1**-**2**)

is illustrated in **Scheme 1**. The key intermediates **I-2a** and **I-2b** were prepared from the commercially available starting material ethyl(*E*)-2-cyano-3-ethoxyacrylate (**I-1**) by condensation reaction with aromatic hydrazine at 120 □[16]. Further, the intermediate **I-3** was achieved from **I-2a** by allowing diazotization reaction. Intermediates **I-2a**, **I-2b** and **I-3** were converted to target compounds **I-4a(1-5)** and **I-4b** by treating with various substituted alcohol, amines, and halo hydrocarbon through nucleophilic substitution reaction. Further, **I-4a** and **I-4b** were hydrolyzed to obtain intermediates **I-5a** and **I-5b** respectively by hydrolysis reaction in the solution of sodium hydroxide, water, ethanol and tetrahydrofuran under 50 □. The intermediates I-5a and I-5b were allowed to react with corresponding substituted amines *via* an amide condensation reaction to achieve the final target compounds **I-6a** and **I-6b(1-2**)

[17]. Both analytical and spectral data of all the synthesized compounds are in full agreement with the proposed structures.

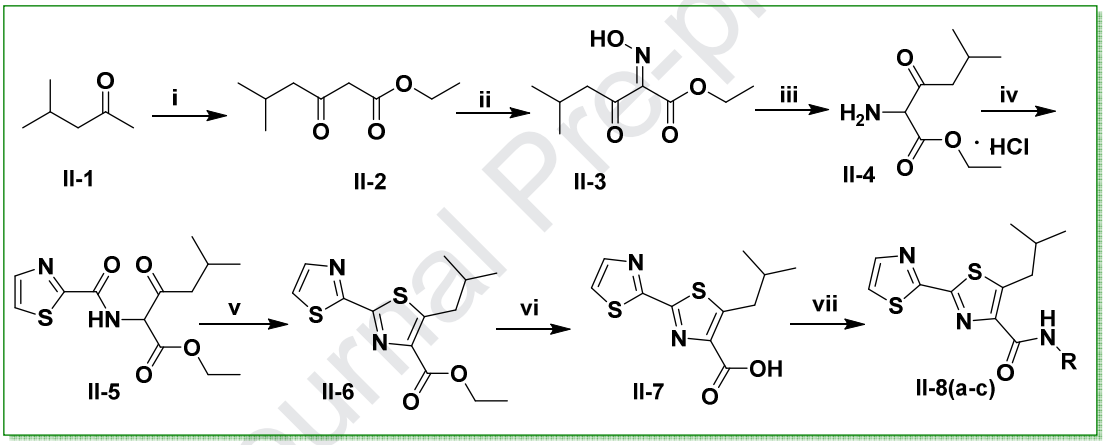



**Scheme 1.** Reagents and conditions: (i) $CH_3COONa$, $CH_3COOH$, $H_2O$, reflux; (ii) $CuBr_2$, $CH_3CN$, 50 □; (iii) DMF, NaH, $R^2$-X; (iv) DMF, NaH, $R^2OH$/ $R^2NH_2$; (v) NaOH, $C_2H_5OH$, $H_2O$, THF, 50 □; (vi) DCM, EDC, HOBt.

The synthetic route for the target compounds **II-8(a-c)** was conducted as illustrated in **Scheme 2**. The initial intermediate **II-2** was achieved by allowing the

substitution reaction between commercially available diethyl carbonate (**1**) and 4-methylpentan-2-one.

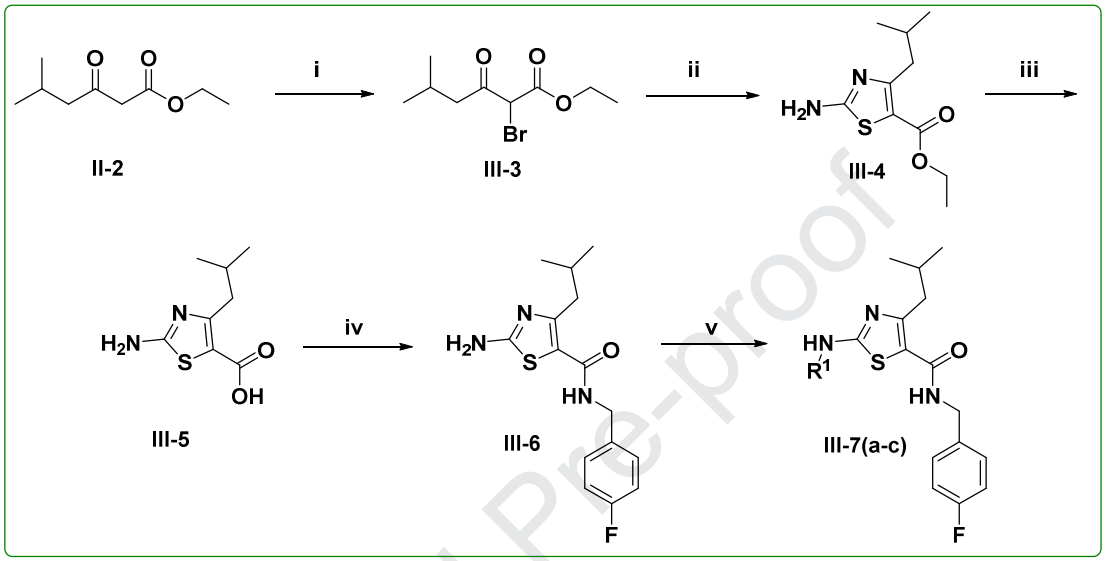
The key intermediate **II-5** was prepared from intermediate II-2 through oxime synthesis, reduction and amide condensation reaction, which was further converted to intermediate **II-6** by condensation cyclization reaction with Lawesson's reagent at 120 □. Then, II-6 was hydrolyzed to obtain intermediate **II-7** by hydrolysis reaction in the solution of sodium hydroxide, water, ethanol and tetrahydrofuran under 50 □. Treatment of intermediate II-7 with corresponding substituted amines *via* amide condensation reaction gave the target compounds **II-8(a-c)** [18].




**Scheme 2.** Reagents and conditions: (i) 4-methylpentan-2-one, NaH, AcOH, THF; (ii) $NaNO_2$, $CH_3COOH$, $H_2O$, -5 ℃; (iii) Pd/C, HCl/EtOH, RT; (iv) HATU, DMF, DIPEA, RT; (v) Lawesson's reagent, toluene, reflux; (vi) NaOH, THF, EtOH, $H_2O$, RT; (vii) HATU, DMF, DIPEA, RT.

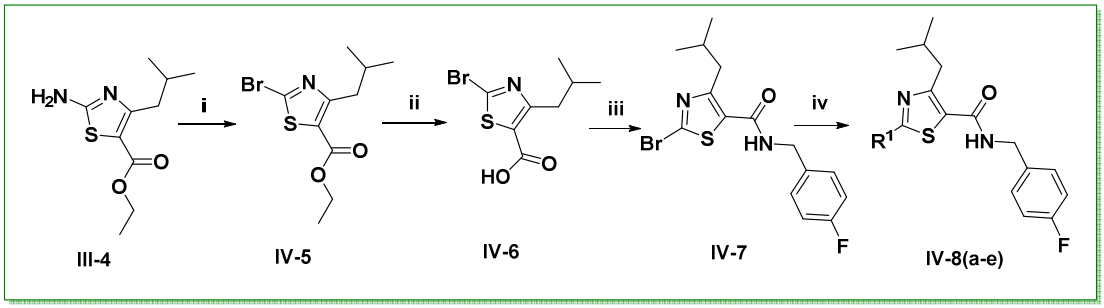
The synthetic route of the target compounds **III-7(a-c)** is demonstrated in **Scheme 3**. Intially, compound **III-3** was achieved by substitution reaction between intermediate (II-2) and *N*-bromo succinimide. The key intermediate ethyl 2-amino-4-isobutylthiazole-5-carboxylate (**III-4**) was obtained by treating ethyl 2-bromo-5-methyl-3-oxohexanoate (**III-3**) with thiourea by condensation reaction at

80 □. Then, intermediate III-4 was hydrolyzed to intermediate 2-amino-4-isobutylthiazole-5-carboxylic acid (**III-5**) by hydrolysis reaction as followed in Scheme 2. Treatment of intermediate III-5 with 4-fluorbenzylamine by the condensation reaction gave the intermediate **III-6**, which was further modified to obtain target compounds **III-7(a-c)**[19**]**.



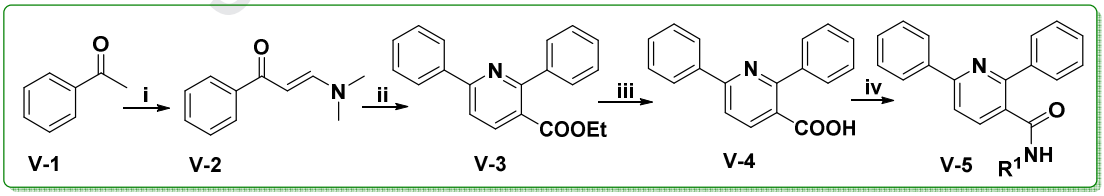

**Scheme 3.** Reagents and conditions: (i) DCM, NBS, RT; (ii) EtOH, thiourea, 80 ℃; (iii) NaOH, THF, EtOH, $H_2O$, 50 ℃; (iv) DMF, HATU, DIPEA, RT; (v) a: THF, DMAP, 80 ℃; b: HATU, DIPEA, RT.

The synthetic route of the target compounds **IV-8(a-e)** is displayed in **Scheme 4**. The key intermediate ethyl 2-bromo-4-isobutylthiazole-5-carboxylate (**IV-5**) was prepared from ethyl 2-amino-4-isobutylthiazole-5-carboxylate (III-4) through diazo substitution reaction. Then, intermediate IV-5 was hydrolyzed to obtain 2-bromo-4-isobutylthiazole-5-carboxylic acid (**IV-6**) *via* hydrolysis reaction in the solution of sodium hydroxide, water, ethanol and tetrahydrofuran at 50 ℃. Treatment of intermediate IV-6 with 4-fluorbenzylamine by the condensation reaction gave the intermediate **IV-7**, which was further modified by Suzuki reaction to obtain the target compounds **IV-8(a-e)**.



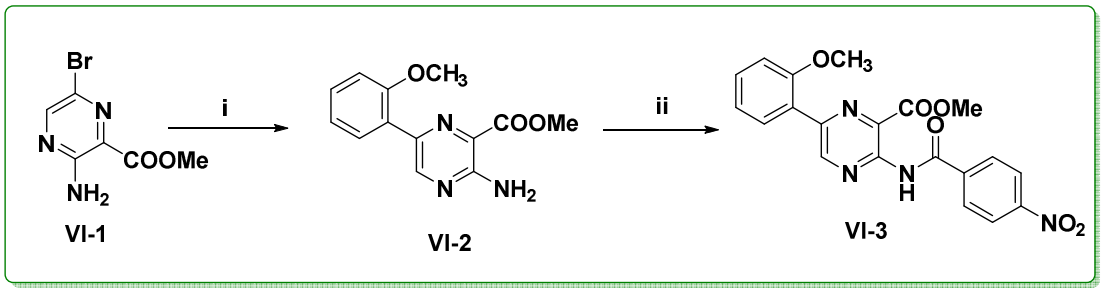

**Scheme 4** Reagents and conditions: (i) $CH_3CN$, CuBr, *tert*-Butyl nitrite, RT; (ii) NaOH, THF, EtOH, $H_2O$, 50 ℃; (iii) DMF, HATU, DIPEA, RT; (iv) $Pd(PPh_3)_4$, $K_2CO_3$, 1,4-Dioxane, $H_2O$, 100 ℃.

The synthetic route of the target compound **V-5** is illustrated in **Scheme 5**. Initially, compound **V-2** was achieved by allowing the reaction between acetophenone (**V-1**) and 1,1-dimethoxy-*N*,*N*-dimethylmethanamine 4-methylpentan-2-one. The key intermediate ethyl-2,6-diphenylnicotinate (**V-3**) was achieved by condensation reaction of (*E*)-3-(dimethylamino)-1-phenylprop-2-en-1-one (**V-2**) withethyl benzoylacetate at 120 ℃. Then, intermediate V-3 was hydrolyzed to obtain 2,6-diphenylnicotinic acid (**V-4**) by hydrolysis reaction in the solution of sodium hydroxide, water, ethanol and tetrahydrofuran under 80 ℃. Treatment of intermediate V-4 with different substituted amines leads to the target compound **V-5**[20].



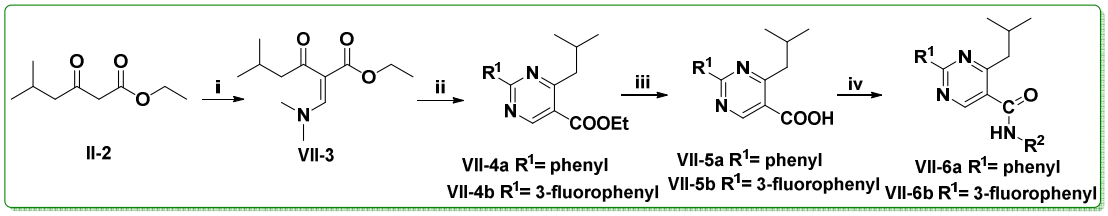

**Scheme 5.** Reagents and conditions: (i) 1,1-dimethoxy-*N*,*N*-dimethylmethanamine, 120 ℃; (ii) $CH_3COOH$, $CH_3COONH_4$, 120 ℃; (iii) NaOH, THF, EtOH, $H_2O$, 80 ℃; (iv) DMF, HATU, triethylamine, RT.

The synthetic route of the target compound **VI-3** is displayed in **Scheme 6**. The target compound **VI-3** was achieved from the commercially available methyl 3-amino-6-bromopyrazine-2-carboxylate and 2-methoxybenzeneboronic acid after Suzuki and acylation reactions[21, 22].




**Scheme 6.** Reagents and conditions: (i) $Pd(dppf)Cl_2$, $Cs_2CO_3$, 1,4-Dioxane, $H_2O$, 100 ℃; (ii) DMF, triethylamine, 100 ℃.

The synthetic route of the target compounds **VII-4(a-b)** and **VII-6(a-b)** is presented in **Scheme 7**. The key intermediate ethyl (*E*)-2-((dimethylamino)methylene)-5-methyl-3-oxohexanoate (**VII-3**) was prepared from ethyl 5-methyl-3-oxohexanoate (**II-2**) by condensation reaction with 1,1-dimethoxy-*N*,*N*-dimethylmethanamine at 100 □. And the target compounds **VII-4(a-b)** were achieved from compound VII-3 by allowing condensation reaction with benzamidine hydrochloride or 3-fluoro-benzamidine hydrochloride. Then, VII-4(a-b) were hydrolyzed to obtain the desired intermediates **VII-5a** and **VII-5b** correspondingly by hydrolysis reaction in the solution of sodium hydroxide, water, ethanol, and tetrahydrofuran under 50 □. Treatment of intermediates VII-5a or VII-5b with different substituted amines leads to the target compounds **VII-6a** and **VII-6b**, respectively[23].



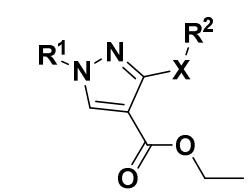

**Scheme 7.** Reagents and conditions: (i)1,1-dimethoxy-*N*,*N*-dimethylmethanamine, 100 ℃; (ii) $C_2H_5ONa$, $C_2H_5OH$, 80 ℃; (iii) NaOH, THF, EtOH, $H_2O$, 80 ℃; (iv) DMF, HATU, triethylamine, RT.

## 2.2. Biological activity and the SAR studies

All the synthesized compounds were preliminary evaluated for (*in vitro*) their anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cells (human HBV transgenic hepatocellular carcinoma cells) using standard PCR and cell counting kit-8(CCK-8) methods. Then the advanced target compounds were further evaluated for their cytotoxicity, inhibitory effect on HBV DNA replication and HBsAg, and HBeAg secretion in HepG2.2.15 cells *via* CCK-8, PCR and standard ELISA methods, respectively. The concentration of compound required for 50% inhibition of HBeAg, HBsAg secretion or DNA replication was defined as $IC_{50}$ and the concentration of compound that induced the death of the HepG2.2.15 cell cultures by 50% was defined as $CC_{50}$. Selectivity index (SI) was determined as the $CC_{50}/IC_{50}$ value. Lamivudine and lead compound NZ-4 was used as positive standards.

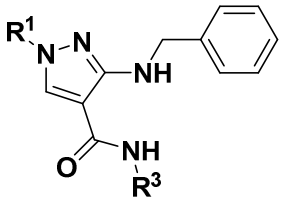
Primary anti-HBV DNA replication activity, cytotoxicity in HepG2.2.15 cell for **series I-II** target compounds and lead compound (NZ-4) and positive drug Lamivudine under 100 μM concentration were shown in **table 1**. Most of these pyrazole analogs exhibited low toxicity as compared to positive drug Lamivudine except compound **I-4a5**. It was observed that the electron-withdrawing group of $R^2$ might improve the toxicity of the inhibitors. However, most of these pyrazole analogs also exhibited low or none anti-HBV DNA replication activity. To our encouragement, compounds with amide substitution in pyrazole moiety (**I-6a**, **I-6b1**and **I-6b2**) exhibited better anti-HBV DNA replication activity than ester substitution. Besides, to our delight, series II target compounds with thiazole moiety exhibited advanced anti-HBV activity. Among thiazole series, compounds **II-8a** and **II-8b** showed potent anti-HBV DNA replication (83.8±3.5% and 89.8±3.3%, respectively) with 24.9±2.3% and none cytotoxicity under 100 μM, respectively. To the best, target compounds II -8a and II-8b have similar anti-HBV activity with positive drug Lamivudine (87.9±2.4 %) and they are better than the lead compound (NZ-4, II-8c), and II-8c possessed high cytotoxicity (98.8±0.2 %), which can be further evaluated.

**Table 1** Primary anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cell of **series I-II** target compounds, NZ-4, and Lamivudine under 100 μM concentration.



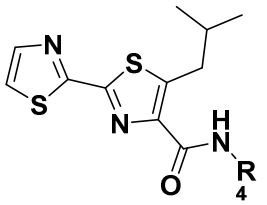
I-4a(1-5): $R_1$ = 3-fluoropheny

I-4b: R1 = thiazol-2-y



I-6a: $R^1$ = 3-fluorophenyl

I-6b(1-2): $R^1$ = thiazol-2-yl



II-(8a-8c)

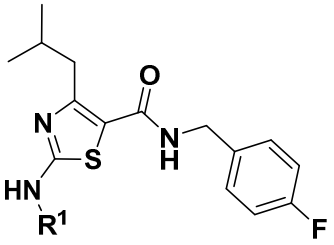
| Compounds | X | $R^2/R^3/R^4$ | Inhibition percentages (%) | |
|---|---|---|---|---|
| | | | DNA replication | HepG2.2.15 Cell |
| **I-4a1** | NH | benzyl | 14.6 | NI[a] |
| **I-4a2** | NH | ethyl | 39.9±8.7 | 10.9±9.7 |
| **I-4a3** | NH | methyl | NI | 5.7±1.7 |
| **I-4a4** | S | cyclohexane | NI | 18.1±7.5 |
| **I-4a5** | NH | 4-nitrobenzoyl | 49.5±2.0 | 51.3±9.2 |
| **I-4b** | NH | benzyl | NI | 30.4±2.2 |
| **I-6a** | | cyclopropyl | 38.8±7.1 | 40.8±4.6 |
| **I-6b1** | | 2,4-difluorobenzyl | 32.9±22.9 | 16.9±2.9 |
| **I-6b2** | | 2-chloro-4-fluorobenzyl | 41.6±1.3 | 29.2±6.1 |
| **II -8a** | | 2,4-difluorobenzyl | **83.8±3.5** | 24.9±2.3 |
| **II-8b** | | NH-(4-fluorophenyl) | **89.8±3.3** | NI |
| **II-8c(NZ-4)** | | 4-fluorobenzyl | **97.3±0.2** | **98.8±0.2** |
| **Lamivudine** | | | **87.9±2.4** | 12.7 ±3.7 |

[a] NI: None inhibition under 100 μM

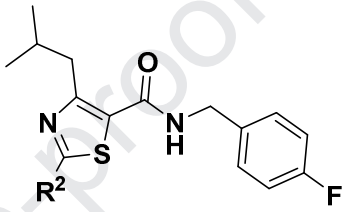
Primary anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cell for **series III-IV** target compounds under 20 μM concentration were shown in **table 2.**

To our surprise, most of these thiazole compounds showed low toxicity and anti-HBV DNA replication activity. Unfortunately, a significant reduction in anti-HBV DNA replication activity was observed by small changes in thiazole moiety and fragments of thiazole. However, we found that compound **IV-8e** exhibited moderate anti-HBV DNA replication activity with 57.8±13.2 %, which can be further evaluated.

**Table 2** Primary anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cell of **series III-IV** target compounds under 20 μM concentration.




III-7(a-c)




IV-8(a-e)

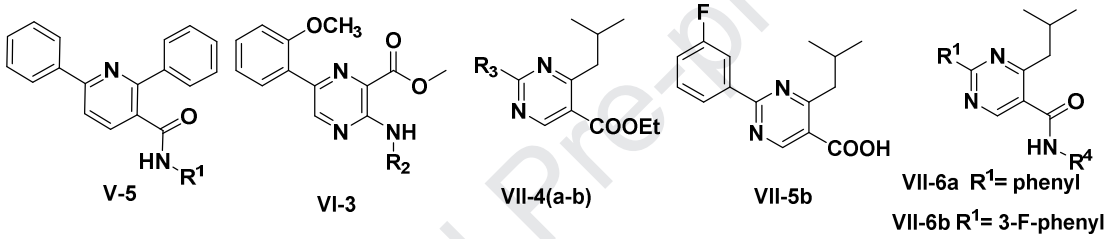
| Compouds | $R^1/R^2$ | Inhibition percentages (%) | |
|---|---|---|---|
| | | DNA replication | HepG2.2.15 Cell |
| **III-7a** | 2-thienylcarbonyl | NI[a] | 15.2 |
| **III-7b** | 4-fluorobenzenesulfonyl | NI | 17.5±20.2 |
| **III-7c** | 4-fluorobenzoyl | 29.2±5.0 | 20.7±18.6 |
| **IV-8a** | 2-thienyl | NI | 17.8±8.6 |
| **IV-8b** | 5-methylthiophen-2-yl | NI | NI |
| **IV-8c** | 3-thienyl | NI | 6.7±4.6 |
| **IV-8d** | benzo[b]thiophen-2-yl | 21.1±14.2 | NI |
| **IV-8e** | 4-pyridinyl | **57.8±13.2** | 35.8±5.9 |

[a] NI: None inhibition under 20 μM

Primary anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cell for **series V-VII** target compounds under 50 μM concentration were shown in **table 3.** From the results, it was noticed that pyridine compound **V-5** and pyrazine derivative **VI-3** showed low toxicity and none anti-HBV DNA replication activity. However,

most pyrimidine derivatives exhibited moderate anti-HBV DNA replication activity with none cytotoxicity in HepG2.2.15 cell. Among them, compound VII-5b presented the best anti-HBV DNA replication activity with 54.2±12.8 %. The preliminary structure-activity relationship (SAR) of pyrimidine derivatives was investigated and revealed that 3-fluorinated phenyl substitution is superior to the benzene group in the position of $R^1$ (**VII-4b > VII-4a, VII-6b > VII-6a)**. The obtained anti-HBV DNA replication activity results showed for 5 position derivatives showed as sequence: carboxyl group > ester group > amide group (**VII-5b > VII-4b > VII-6b)**.

**Table 3** Primary anti-HBV DNA replication activity and cytotoxicity in HepG2.2.15 cell of **Series V-VII** target compounds under 50 μM concentration.



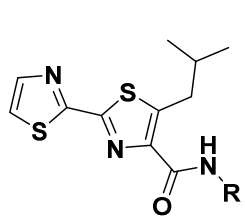

| Compounds | $R^1/R^2/R^3/R^4$ | Inhibition percentages (%) | |
|---|---|---|---|
| | | DNA replication | HepG2.2.15 Cell |
| **V-5** | 4-fluorobenzyl | NI[a] | 4.4±0.3 |
| **VI-3** | benzoyl | NI | 5.7±7.8 |
| **VII-4a** | phenyl | 37.3±0.9 | NI |
| **VII-4b** | 3-fluorophenyl | 46.3±4.1 | NI |
| **VII-5b** | | **54.2±12.8** | NI |
| **VII-6a** | benzyl | NI | NI |
| **VII-6b** | 4-fluorobenzyl | 35.6 | NI |

[a] NI: None inhibition under 50 μM

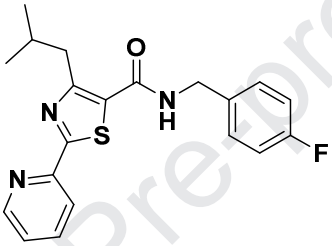
The activity of compounds II-8(a-c), IV-8e, VII-5b, and Lamivudine were further evaluated in HepG2.2.15 cells in a dose-dependent manner (100 μM, 20 μM, 4 μM, 0.8 μM, 0.16 μM) to analyze their $IC_{50}$ and $CC_{50}$ values. Among them,

compound **II-8b** showed the best *in vitro* anti-HBV DNA replication activity ($IC_{50}$ = 2.2 ± 1.1 μM, $CC_{50}$ = 80.8±14.4 μM), which was better than that of lead compound II-8c ($IC_{50}$ = 2.3 ± 0.5 μM, $CC_{50}$ = 59.2 ±2.4 μM). Compound **IV-8e** and **VII-5b** showed the best *in vitro* activity for anti-HBsAg secretion ($IC_{50}$ = 3.8±0.7 μM, $CC_{50}$ > 100 μM) and anti-HBeAg secretion ($IC_{50}$ = 9.7±2.8 μM, $CC_{50}$ > 100 μM), respectively, which were better than lead compound and positive drug Lamivudine.

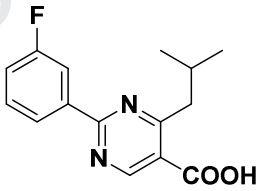
**Table 4.** Further evaluation for cytotoxicity, inhibitory effect on HBV DNA replication and HBsAg/HBeAg secretion of compounds II-8(a-c), IV-8e, VII-5b and Lamivudine.




II-(8a-8c)




IV-8e




VII-5b

| Compounds | R | $CC_{50}$[a] (μmol/L) | DNA $IC_{50}$[b] (μmol/L) | DNA SI[c] | HBsAg $IC_{50}$[b] (μmol/L) | HBsAg SI[c] | HBeAg $IC_{50}$[b] (μmol/L) | HBeAg SI[c] |
|---|---|---|---|---|---|---|---|---|
| **II-8a** | 2,4-difluorobenzyl | 45.4±2.2 | **2.7±3.0** | 16.8 | 21.0±1.7 | 2.2 | 30.1±8.3 | 1.5 |
| **II-8b** | NH-(4-fluorophenyl) | 80.8±14.4 | **2.2±1.1** | 36.7 | >100 | < 0.8 | >100 | < 0.8 |
| **IV-8e** | | >100 | 40.5±34.6 | >2.5 | **3.8±0.7** | >26.3 | 62.3±32.5 | >1.6 |
| **VII-5b** | | >100 | 9.9±3.7 | >10.1 | >100 | — | **9.7±2.8** | >10.3 |
| **II-8c**[d] | 4-fluorobenzyl | 59.2 ±2.4 | 2.3±0.5 | 25.7 | 31.7±7.8 | 1.9 | 31.7±4.1 | 1.9 |
| **Lamivudine** | | >100 | 0.6±0.4 | >167 | >100 | — | >100 | — |

[a] $CC_{50}$: Concentration required to reduce the viability of mock-infected cells by 50%.

[b] $IC_{50}$: Concentration of compound required for 50% inhibition of HBV DNA replication or HBsAg /HBeAg secretion.

[c] SI: Selectivity index, the ratio of $CC_{50}/IC_{50}$.

[d] IV-8c： The lead compound NZ-4.

## 2.3 Molecular modeling

II-8b, the best compound in cell-based HBV infection assay, was docked into the HBV capsid (PDB ID: 5gmz) using Sybyl-X 2.0 to study its binding mode. Default parameters were used as described in the Sybyl-X 2.0 manual unless otherwise specified, the result was displayed by PyMOL (**Figure 2)**. It was shown that the conformation of II-8b (green) can be well overlapped with the ligand (NVR10-001E2, yellow) in the core protein crystal structure. Thiazole ring region of II-8b occupied a hydrophobic pocket, surrounded by Pro-130 and Pro-129. And 2-fluorine phenyl region occupied another hydrophobic pocket, surrounded by Pro25, Asp29 and Leu30, while F atoms can form a hydrogen bond with Thr-33. Besides, the amide of II-8b form a hydrogen bond with Thr128, which might contribute to the anti-HBV activity.

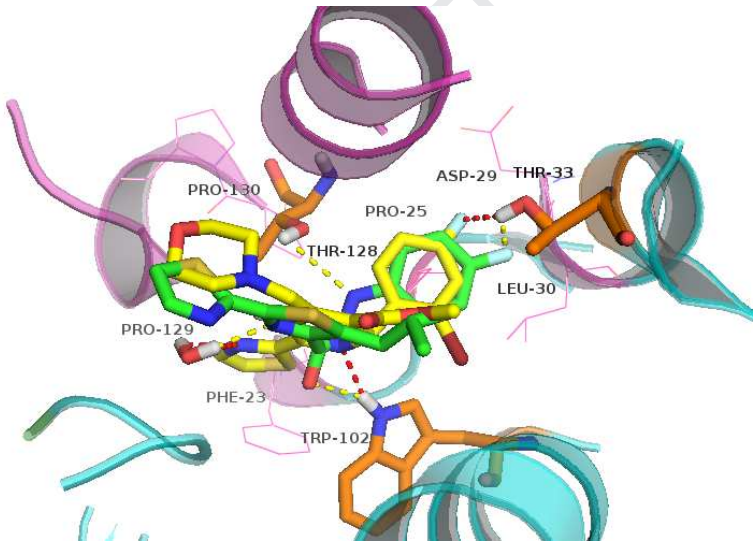



**Figure 2**. The molecular modeling of target compound II-8b with core protein crystal structure (PDB：5gmz)

## 2.4 Surface plasmon resonance (SPR)

To acquire better understand of the hydrogen bond interactions, we evaluated compound II-8b the binding affinity with HBV capsid protein by surface plasmon resonance (Biacore T200). The study was shown that compound II-8b can steady-state bind to HBcAg protein in a dose-dependent manner (22.2 μM, 14.8 μM, 14.8 μM, 9.9 μM, 6.6 μM and 0 μM) with response value (RU) of 2638, 21.33, 20.60, 18.83, 15.36 and 11.31, respectively. While the lead compound NZ-4 bound to HBcAg protein in a

dose-dependent manner (100 μM, 50 μM, 25 μM, 25 μM,12.5 μM, 6.25 μM and 0 μM) with response value (RU) of 63.55, 41.35, 38.93, 38.35, 23.85, 17.72 and 14.56), respectively. Therefore, fitted by 1:1 binding model the affinity constant $K_D$ value of compound II-8b and NZ-4 was 60.0 μM and 50.6 μM, respectively.

## 3. Conclusion

In conclusion, six small series of heterocycle derivatives (pyrazole, thiazole, pyrazine, pyrimidine and pyridine analogs) were designed as potential HBV non-nucleoside inhibitors through scaffold hopping, bioisosterism and pharmacophore hybrid-based strategies. All the designed compounds were evaluated for their anti-HBV activity. Among them, compound **II-8b** displayed the most potent anti-HBV DNA replication activity ($IC_{50}$ = 2.2 ± 1.1 μM). And compound **IV-8e** and **VII-5b** showed the best *in vitro* activity for anti-HBsAg secretion ($IC_{50}$ = 3.8±0.7 μM, $CC_{50}$ > 100 μM) and anti-HBeAg secretion ($IC_{50}$ = 9.7±2.8 μM, $CC_{50}$ > 100 μM), respectively. The surface plasmon resonance study revealed that the best anti-HBV DNA replication compound II-8b can interact HBV capsid protein with good affinity constants ($K_D$ = 60.0 μM), which was equivalent with lead compound NZ-4 (($K_D$ = 50.6 μM). The preliminary structure-activity relationships (SARs) of the new compounds were summarized, which may help in discovering more potent anti-HBV agents.

## 4. Experimental section

### 4.1. Chemistry

All melting points were determined on a micro melting point apparatus. $^1$H NMR spectra were obtained on a Brucker Avance-400/300 NMR spectrometer in the indicated solvents. Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with Silica Gel 60 (200–300 mesh). Solvents were

reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure. All solvents of 1,4-dioxane, tetrahydrofuran, DCM, $CH_3COOH$, DMF and ethanol were obtained from Sinopharm Chemical Reagent Co.,Ltd(SCRC), which were of AR grade. Chemicals and reagents of *N*-bromosuccinimide, $Pd(dppf)Cl_2$, $Cs_2CO_3$, Pd/C, HOBt, HATU, DIPEA, EDC etc. were obtained from beijing innochem science & technology Co.,Ltd, which were of CP grade.

**4.1.1. General procedure for preparation of compounds I-2a and I-2b**

To the mixture solution of water (1 mL) and sodium acetate (286 mg, 3.49 mmol) in acetic acid (3 mL) was added 3-fluorophenyl hydrazine hydrochloride (1.23 mmol) or thiazol-2-yl hydrazine hydrochloride (1.23 mmol). The reaction mixture was stirred at reflux temperature for 4 hours. Upon completion of the reaction, the solvent was cooled to room temperature and 20 mL cooled water was added and was extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (30 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compounds **I-2a** and **I-2b** in good yield.

Ethyl 3-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate (I-2a). Pale yellow solid, yield 91%, m.p.130-131 □; $^1$H NMR(400 MHz, $CDCl_3$): $\delta$ 7.79 (s, 1H), 7.51-7.45 (m, 1H), 7.37-7.30 (m, 2H ), 7.13-7.08 (m, 1H ), 5.36 (s, 1H), 4.31 (q, 2H, *J*=7.1 Hz), 1.37 (t, 3H, *J*=7.1 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.39, 163.20 (d, *J* = 255 Hz), 149.15, 140.97, 139.10 (d, *J* = 9 Hz), 131.05(d, *J* = 9 Hz), 118.87(d, *J* = 3 Hz), 115.0(d, *J* = 21 Hz), 111.29(d, *J* = 24 Hz), 96.57, 59.78, 14.49; ESI-MS: 250.4 $[M+H]^+$.

Ethyl 3-amino-1-(thiazol-2-yl)-1*H*-pyrazole-4-carboxylate (I-2b). white solid, yield 35%, m.p.99-101 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.75 (s, 1H), 7.53 (d, 1H, *J*=3.6 Hz), 7.14 (s, 2H), 7.05 (d, 2H, *J*=3.6 Hz), 4.30 (q, 2H, *J*=7.2 Hz), 1.36 (t, 3H, *J*=7.2 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.00, 162.38, 150.38, 142.70, 139.70,

114.54, 95.46, 77.35, 77.24, 77.04, 76.72, 59.82, 14.52; ESI-MS: 239.0 $[M+H]^+$.

**4.1.2. General procedure for preparation of compound Ethyl 3-bromo-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate (I-3)**

To the mixture of Ethyl 3-amino-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate (I-2a) (200 mg, 0.8 mmol) in acetonitrile (5 mL) was added cuprous bromide. Then the reaction mixture was cooled to 0 □ and the butyl nitrite (223 mg, 2.17 mmol) was added, which was stirred at 50 ℃ for overnight. Upon completion of the reaction, the solvent was cooled to room temperature and 50 mL water was added. It was extracted with ethyl acetate (25 mL × 3) and washed with saturated sodium chloride (50 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound I-3 as a yellow solid 195 mg, yield: 72%. m.p.76-79 ℃; $^1$H NMR(400 MHz, $CDCl_3$): $\delta$ 8.14 (s, 1H), 7.52-7.4 (m, 1H), 7.36 (dd, 1H, $J_1$=2.0 Hz, $J_2$=7.6 Hz), 7.32-7.28 (m, 1H ), 7.23-7.18 (m, 1H ), 4.37 (q, 2H, $J$=7.2 Hz), 1.37 (t, 3H, $J$=7.2 Hz ) , 1.40 (t, 3H, $J$=7.2 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.46 (d, $J$ = 248.7 Hz), 161.62, 143.43, 139.55 (d, $J$ = 10.1 Hz), 130.35 (d, $J$ = 8.9 Hz), 121.83 (d, $J$ = 3.4 Hz), 117.78, 116.39 (d, $J$ = 21.0 Hz), 115.55, 113.86 (d, $J$ = 24.8 Hz), 60.71, 14.33, ESI-MS: 313.3 $[M+H]^+$.

**4.1.3. General procedure for preparation of compounds I-4a (1-3) and I-4b**

To the solution of intermediate I-2a or I-2b (1.12 mmol) in DMF (5 mL) was added potassium carbonate, cesium carbonate or sodium hydride. The mixture solution was stirred for 5 min, added different substituent haloalkane (1.35 mmol) and then was further stirred under room temperature for overnight. Upon completion of the reaction, the solvent was added 50 mL water, extracted with ethyl acetate (25 mL × 3) and washed with saturated sodium chloride (50 mL × 3). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and

petroleum ether. Pure fractions were collected and concentrated, giving the desired compounds I-4a (1-3) and I-4b.

General procedure for preparation of compound Ethyl 3-(benzylamino)-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate(I-4a1). Light yellow oil, yield:73%; $^{1}$H NMR(400 MHz, $CDCl_3$): $\delta$ 7.81 (s, 1H), 7.43-7.4536 (m, 2H), 7.32-7.27 (m, 1H), 7.25-7.22 (m, 3H), 7.12-7.07 (m, 1H) 7.04 (dd, 2H, $J_1$=2.0 Hz, $J_2$=7.6 Hz) , 6.52 (s, 1H), 4.29 (q, 2H, *J*=7.2 Hz), 4.03 (s, 1H), 1.34 (t, 3H, *J*=7.2 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): 164.72, 162.76 (d, *J* = 248.3 Hz), 151.71, 141.21, 140.82 (d, *J* = 10.0 Hz), 137.81, 130.38 (d, *J* = 9.0 Hz), 128.66, 127.66, 127.19, 120.36 (d, *J* = 3.3 Hz), 115.18 (d, *J* = 21.1 Hz), 112.39 (d, *J* = 24.4 Hz), 99.11, 59.84, 49.79, 14.45; ESI-MS: 340.5 $[M+H]^+$.

General procedure for preparation of compound Ethyl 3-(ethylamino)-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate(I-4a2). Colorless oil, yield: 72%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.81 (s, 1H), 7.46-7.39 (m, 2H), 7.38-7.34 (m, 1H ), 7.11-7.06 (m, 1H ), 6.00 (s, 1H), 4.30 (q, 2H, *J*=7.2 Hz), 2.87 (q, 2H, *J*=7.2 Hz), 1.37 (t, 3H, *J*=7.1 Hz ) , 1.08 (t, 3H, *J*=7.1 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.87, 162.76 (d, *J* = 248.0 Hz), 152.33, 141.21, 141.06 (d, *J* = 10.0 Hz), 130.33 (d, *J* = 9.0 Hz), 120.06 (d, *J* = 3.3 Hz), 114.94 (d, *J* = 21.0 Hz), 112.04 (d, *J* = 24.5 Hz), 98.58, 59.76, 40.92, 15.55, 14.50; ESI-MS: 278.2 $[M+H]^+$.

General procedure for preparation of compound Ethyl 1-(3-fluorophenyl)-3-(methylamino)-1*H*-pyrazole-4-carboxylate(I-4a3). Colorless oil, yield: 38%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.80 (s, 1H), 7.47-7.32 (m, 3H), 7.12-7.07 (m, 1H ), 4.30 (q, 2H, *J*=7.2 Hz), 2.95 (s, 3H), 1.36 (t, 3H, *J*=7.2 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.88, 163.96, 153.23, 141.10, 140.85, 131(d, *J* = 9.0 Hz), 120.51(d, *J* = 3.0 Hz), 115.11(d, *J* = 21 Hz), 112.47(d, *J* = 24 Hz), 97.75, 59.78, 32.74, 14.49; ESI-MS: 264.3 $[M+H]^+$.

General procedure for preparation of compound Ethyl 3-(benzylamino)-1-(thiazol-2-yl)-1*H*-pyrazole-4-carboxylate(I-4b). White solid, yield: 35%, m.p.90-92 □; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.77 (s, 1H), 7.84 (s, 1H), 7.46 (d, 1H, *J*=3.6 Hz ), 7.35-7.24 (m, 5H), 7.03 (d, 1H, *J*=3.6 Hz ), 5.08 (d, 2H, *J*=6.0 Hz ), 4.25 (q, 2H, *J*=7.2 Hz), 1.32 (t, 3H, *J*=7.2 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$

163.10, 162.71, 150.23, 145.53, 139.32, 139.10, 128.63, 127.43, 127.33, 114.68, 97.00, 59.96, 49.40, 14.46; ESI-MS: 657.2 $[2M+H]^+$.

General procedure for preparation of compound Ethyl 3-(cyclohexylthio)-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylate(I-4a7). To the mixture solution of intermediate I-3 (200 mg, 0.64 mmol) in DMF (5 mL) was added with 60% NaH (31 mg, 0.77 mmol). The mixture solution was stirred for 5 min, added cyclohexane thiol and then was further stirred under room temperature for overnight. Upon completion of the reaction, to the solvent was added 50 mL water, extracted with ethyl acetate (25 mL × 3) and washed with saturated sodium chloride (50 mL × 3). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound I-4a7. Colorless oil, yield: 38%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.14 (s, 1H), 7.49-7.43 (m, 1H), 7.33-7.27 (m, 2H ), 7.19-7.15 (m, 1H ), 4.37 (q, 2H, *J*=7.2 Hz), 4.13-4.03 (m, 2H ), 3.30-3.28 (m, 1H ), 1.73-1.50 (m, 5H ), 1.40 (t, 3H, *J*=7.2 Hz ) , 1.21-1.15 (m, 5H ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.49, 162.30 (d, *J* = 247.8 Hz), 143.09, 140.33 (d, *J* = 10.0 Hz), 138.71, 129.90 (d, *J* = 8.9 Hz), 122.34 (d, *J* = 3.3 Hz), 118.63, 115.74 (d, *J* = 21.0 Hz), 114.18 (d, *J* = 24.6 Hz), 67.11, 60.45, 48.95, 32.99, 25.59, 25.44, 14.39; ESI-MS: 349.5 $[M+H]^+$.

General procedure for preparation of compound 1-(3-fluorophenyl)-3-(4-nitrobenzamido)-1*H*-pyrazole-4-carboxylate (I-4a8). To the mixture of intermediate I-2a (100 mg, 0.4 mmol) in DMF (5 mL) was added triethylamine (122 mg, 1.2 mmol). The mixture solution was cooled to 0 □, slowly added 4-nitrobenzoyl chloride and then was further stirred at 60 □ for overnight. Upon completion of the reaction, to the solvent was added 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound I-4a8.White solid, yield: 74%,

m.p.151-153 □; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.59 (s, 1H), 8.32 (d, 2H, *J*=8.4 Hz), 8.04 (d, 3H, *J*=6.8 Hz), 7.42-7.27(m, 3H), 7.07(t, 1H, *J*=8.0 Hz), 7.35 (q, 2H, *J*=7.2 Hz), 1.38 (t, 3H, *J*=7.2 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.03, 163.02, 162.75 (d, *J* = 248.0 Hz), 150.40, 141.24 (d, *J* = 10.2 Hz), 140.66, 139.71, 137.77, 130.47 (d, *J* = 9.0 Hz), 128.90, 124.15, 118.51 (d, *J* = 3.2 Hz), 115.33 (d, *J* = 21.0 Hz), 110.75 (d, *J* = 25.2 Hz), 105.62, 60.96, 14.33; ESI-MS: 399.3 $[M+H]^+$.

#### 4.1.4. General procedure for preparation of compounds I-5a and I-5b

To the mixture of intermediate I-4a (0.74 mmol) or I-4b (0.74 mmol) in ethanol (2 mL), water (2 mL) and THF (2 mL) was added along with NaOH (3.7 mmol). The mixture solution was stirred at 50 □ for overnight. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. Then the mixture was added to 30 mL ammonium chloride solution, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compounds I-5a and I-5b.

General procedure for preparation of compound 3-(benzylamino)-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxylic acid (I-5a). White solid, yield: 44%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.88 (s, 1H), 7.42 (td, J = 8.1, 6.1 Hz, 1H), 7.37 – 7.31 (m, 1H), 7.30 – 7.21 (m, 5H), 7.12 (tdd, *J* = 8.3, 2.5, 0.9 Hz, 1H), 7.02 (dd, *J* = 7.6, 1.8 Hz, 2H), 6.57 (s, 1H), 4.07 (s, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 169.57, 162.72 (d, *J* = 248.8 Hz), 152.18, 141.89, 140.54 (d, *J* = 10.0 Hz), 137.58, 130.42 (d, *J* = 9.0 Hz), 128.74, 127.77, 127.04, 120.74 (d, *J* = 3.3 Hz), 115.55 (d, *J* = 21.0 Hz), 112.78 (d, *J* = 24.3 Hz), 97.91, 49.50; ESI-MS: 312.4 $[M+H]^+$.

General procedure for preparation of compound 3-(benzylamino)-1-(thiazol-2-yl)-1*H*-pyrazole-4-carboxylic acid (I-5b). White solid, yield: 87%; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 12.19 (s, 1H), 8.67 (t, *J* = 6.5 Hz, 1H), 7.84 (s, 1H), 7.67 (d, *J* = 3.6 Hz, 1H), 7.54 (d, *J* = 3.6 Hz, 1H), 7.45 – 7.11 (m, 6H), 5.05 (d, *J* = 6.5 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 163.86, 162.81, 150.02, 146.01, 139.99, 139.87, 129.02, 127.66, 127.63, 117.11, 97.64, 48.39; ESI-MS: 299.5 $[M-H]^-$.

**4.1.5. General procedure for preparation of compounds I-6a and I-6b(1-2)**

To the mixture of intermediate I-5a (0.19 mmol) or I-5b (0.19 mmol) in DCM (5 mL) was slowly added EDCl (43 mg, 0.25 mmol) and HOBt (39 mg, 0.25 mmol) under low temperature. The mixture solution was stirred for 10 min, added different substituted amine and then was further stirred at room temperature for overnight. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compounds I-6a and I-6b(1-2).

General procedure for preparation of compound 3-(benzylamino)-*N*-cyclopropyl-1-(3-fluorophenyl)-1*H*-pyrazole-4-carboxamide (I-6a). White solid, yield: 32%, m.p.87-89 □; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.89 (s, 1H), 7.45-7.40 (m, 1H), 7.35-7.33 (m, 1H ), 7.33-7.24 (m, 4H ), 7.14-7.09 (m, 1H ), 7.02 (dd, 2H, $J_1$=2.0 Hz, $J_2$=7.6 Hz) , 6.57 (s, 1H), 4.07 (s, 1H); $^{13}$C NMR (100 MHz, CDCl3): $\delta$ 162.76 (d, $J$ = 248.2 Hz), 151.35, 140.87 (d, $J$ = 10.0 Hz), 137.97, 137.59, 130.35 (d, $J$ = 9.0 Hz), 129.17, 128.53, 127.46, 127.26, 120.22 (d, $J$ = 3.3 Hz), 115.09 (d, $J$ = 21.1 Hz), 112.25 (d, $J$ = 24.5 Hz), 100.82, 49.66, 29.70, 6.82; ESI-MS: 312.4 $[M+H]^+$.

General procedure for preparation of compound 3-(benzylamino)-*N*-(2,4-difluorobenzyl)-1-(thiazol-2-yl)-1*H*-pyrazole-4-carboxamide (I-6b1). White solid, yield: 70%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.37 (s, 1H), 7.61 (s, 1H), 7.49 (d, 1H, $J$=3.6 Hz ), 7.34-7.23 (m, 6H), 7.05 (d, 1H, $J$=3.2 Hz), 6.84-6.77 (m, 2H), 6.14 (s, 1H), 4.79 (s, 2H), 4.52 (d, 2H, $J$=6.4 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.83, 162.38 (d, $J$ = 236.7 Hz), 162.28, 148.76, 142.24, 139.47, 138.72, 131.25 (d, $J$ = 6.0 Hz), 131.17 (d, $J$ = 9.6 Hz), 128.56, 127.37, 127.31, 121.62, 114.90, 111.37 (dd, $J$ = 21.1, 3.7 Hz), 103.87 (t, $J$ = 25.4 Hz), 101.05, 49.36, 37.04; ESI-MS: 426.2

$[M+H]^+$.

General procedure for preparation of compound 3-(benzylamino)-N-(2-chloro-4-fluorobenzyl)-1-(thiazol-2-yl)-1H-pyrazole-4-carboxamide (I-6b2). White solid, yield: 45%, m.p.154-156 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.37 (s, 1H), 7.63 (s, 1H), 7.48 (d, 1H, $J$=3.6 Hz ), 7.36 (dd, 1H, $J_1$=6.0 Hz, $J_2$=8.4 Hz ), 7.21-7.20 (m, 5H), 7.11 (dd, 1H, $J_1$=2.4 Hz, $J_2$=8.4 Hz ), 7.04 (d, 1H, $J$=3.6 Hz ), 6.95 (dt, 1H, $J_1$=3.6 Hz, $J_2$=8.4 Hz ), 6.23 (t, 1H, $J$=5.6 Hz ), 4.78 (s, 2H), 4.56 (d, 2H, $J$=6.0 Hz ); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.83, 162.76, 161.87 (d, $J$ = 249.6 Hz), 148.76, 142.28, 139.47, 138.71, 134.20 (d, $J$ = 10.3 Hz), 131.94 (d, $J$ = 3.5 Hz), 131.37 (d, $J$ = 8.7 Hz), 128.57, 127.36, 127.31, 116.92 (d, $J$ = 24.8 Hz), 114.90, 114.21 (d, $J$ = 20.9 Hz), 101.07, 49.37, 40.97; ESI-MS: 442.1 $[M+H]^+$.

**4.1.6. General procedure for preparation of compound Ethyl 2-(hydroxyimino)-5-methyl-3-oxohexanoate (II-3)**

The commercially available 4-methylpentan-2-one was slowly added to the solution of 60% NaH (480 mg, 11.98 mmol) in anhydrous tetrahydrofuran (15 mL) at 0 □ with stirring. After 30 min. diethyl carbonate (II-1) (1.77 g, 14.98 mmol) was added to the mixture solution and was stirred at 60 □ for 4 h. The reaction mixture was added to the 50 mL ice cold water and neutralized with 1.5 mL $CH_3COOH$ at about 5 □. The mixture was extracted with ethyl acetate for three time and the combined organic layer was washed by 10% $Na_2CO_3$ and $H_2O$. The product ethyl 5-methyl-3-oxohexanoate (II-2) was obtained as brown oil by removing ethyl acetate solvent under reduced pressure. To the mixture solution of intermediate II-2 (1 g, 5.81 mmol) in acetic acid (5 mL) was slowly added sodium nitrite (1 g, 14.52 mmol) dissolved in water (4 mL) under -5 □. The mixture solution was stirred for 2 h and further stirred for 3 h at room temperature. Upon completion of the reaction, the mixture was added to 40 mL water, extracted with ethyl acetate (30 mL × 3), washed with sodium bicarbonate solution (50 mL), and washed with saturated sodium chloride (50 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum to afford oil with yield 54%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.24 (s, 1H), 4.39 (q, 2H, $J$=7.2 Hz), 2.67 (d, 1H, $J$=7.2 Hz), 2.27-2.18 (m,1H), 1.36 (t, 3H, $J$=7.2 Hz), 0.95 (d, 6H, $J$=6.0 Hz); ESI-MS: 202.3 $[M+H]^+$.

**4.1.7. General procedure for preparation of compound Ethyl 2-amino-5-methyl-3-oxohexanoate (II-4)**

To the mixture of intermediate II-3 (0.6 g, 2.98 mmol) in hydrochloric acid saturated solution of ethanol (10 mL) was added 10% Pd/C. The solution was stirred at room temperature under $H_2$ atmosphere for overnight. Upon completion of the reaction, the mixture was filtrated with diatomite and the residue was washed by ethanol (30 mL × 3). Then the solvent was removed under vacuum and the residue was recrystallized to obtain the desired compound II-4. White solid, yield: 65%; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 4.38 (q, 2H, $J$=7.2 Hz), 2.82-2.67 (m, 2H), 2.25-2.15 (m,1H), 1.36 (t, 3H, $J$=6.8 Hz), 0.99 (d, 3H, $J$=6.8 Hz), 0.94 (d,3H, $J$=6.4 Hz); $^{13}$C NMR (100 MHz, $CD_3OD$): 197.72, 163.28, 63.43, 48.78, 23.93, 21.31, 21.12, 12.89; ESI-MS: 375.4 $[2M+H]^+$.

**4.1.8. General procedure for preparation of compound Ethyl 5-methyl-3-oxo-2-(thiazole-2-carboxamido) hexanoate (II-5)**

To the solution of intermediate II-4 (346 mg, 1.55 mmol) in DMF (10 mL) was slowly added HATU (706 mg, 1.86 mmol) under lower temperature. The mixture solution was stirred for 10 mins, slowly added 2-carboxylthiazole (200 mg, 1.55 mmol) and DIPEA (600 mg, 4.64 mmol), then was further stirred at room temperature for overnight. Upon completion of the reaction, the solvent was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound II-5. White solid, yield: 59%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.33(d, 1H, $J$=6.4 Hz), 7.94(d, 1H, $J$=2.4 Hz), 7.62(d, 1H, $J$=2.8 Hz), 5.38(d, 1H, $J$=6.8 Hz), 4.31(q, 2H, $J$=7.2 Hz), 2.67(d, 1H, $J$=2.0 Hz), 2.65 (d, 1H, $J$=0.8 Hz), 2.29-2.22 (m,1H), 1.33 (t, 3H, $J$=7.2 Hz), 0.97 (d, 3H, $J$=6.8 Hz), 0.93 (d, 3H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 199.82, 165.61, 162.01, 159.20, 144.00, 125.05, 62.80, 49.56, 24.39, 22.51, 22.24, 14.09; ESI-MS: 299.3 $[M+H]^+$.

**4.1.9. General procedure for preparation of compound Ethyl-5-isobutyl-[2,2'-bithiazole]-4-carboxylate (II-6)**

To the solution of intermediate II-5 (360 mg, 1.21 mmol) in toluene (40 mL) was

slowly added Lawesson's reagent (732 mg, 1.81 mmol). The mixture was stirred under reflux temperature for 5 h. Upon completion of the reaction, toluene was removed under vacuum. The residue was added to ethyl acetate (60 mL), washed with sodium bicarbonate saturated solution (30 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound II-5. White solid, yield: 36%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.88(d, 1H, $J$=3.2 Hz), 7.47(d, 1H, $J$=3.2 Hz), 4.44(q, 2H, $J$=7.2 Hz),3.16(d, 2H, $J$=7.2 Hz), 2.06-1.96 (m,1H), 1.45 (t, 3H, $J$=7.2 Hz), 1.01 (d, 6H, $J$=6.4 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.18, 160.99, 157.66, 150.96, 143.80, 142.41, 121.46, 61.38, 36.25, 31.04, 22.31, 14.34; ESI-MS: 297.5 $[M+H]^+$.

**4.1.10. General procedure for preparation of compound 5-isobutyl-[2,2'-bithiazole]-4-carboxylic acid (II-7)**

To the mixture of intermediate II-6 (129 mg, 0.44 mmol) in ethanol (5 mL), water (5 mL) and THF (5 mL) was added along with NaOH (87 mg, 2.18 mmol). The solution was stirred under 30 □ for overnight. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. Then the mixture was added to 30 mL ammonium chloride solution, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compound II-7. White solid, yield: 85%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 13.22 (s, 1H), 7.99 (dd, 1H, $J$ = 9.6, 3.2 Hz), 3.15 (d, 2H, $J$=7.2 Hz), 1.98-1.91 (m,1H), 0.94 (d, 6H, $J$=6.4 Hz).

**4.1.11. General procedure for preparation of compounds II-8(a-c)**

To the mixture of intermediate II-7 (0.15 mmol) in DMF (5 mL) was slowly added HATU (68 mg, 0.16 mmol) under low temperature. The solution was stirred for 10 min, added different substituted amine, DIPEA (39 mg, 0.3 mmol), and then was further stirred under room temperature for overnight. Upon completion of the reaction, the mixture was added 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried

over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, gave the desired compounds II-8(a-c).

General procedure for preparation of compound *N*-(2,4-difluorobenzyl)-5-isobutyl-[2,2'-bithiazole]-4-carboxamide (II-8a). White solid, yield: 60%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.89(d, 1H, *J*=2.8 Hz), 7.80 (s, 1H), 7.46(d, 1H, *J*=2.8 Hz), 7.41 (dd, 1H, $J_1$=6.4 Hz, $J_2$=2.0 Hz), 6.89-6.81 (m,2H), 4.65(d, 2H, *J*=6.4 Hz), 3.30(d, 2H, *J*=7.2 Hz), 2.06-2.00 (m, 1H), 1.01 (d, 6H, *J*=6.8 Hz; $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.36 (dd, *J* = 248.2, 11.9 Hz), 162.01, 160.95 (dd, *J*= 248.8, 11.9 Hz), 160.83, 156.73, 148.32, 144.00, 143.45, 130.92 (dd, *J*= 9.7, 5.9 Hz), 121.44 (dd, *J*= 15.0, 3.7 Hz), 121.23, 111.36 (dd, *J*= 21.1, 3.7 Hz), 103.88 (t, *J*= 25.4 Hz), 36.48 (d, *J*= 3.7 Hz), 35.77, 31.07, 22.31; ESI-MS:394.3 $[M+H]^+$.

General procedure for preparation of compound N'-(4-fluorophenyl)-5-isobutyl-[2,2'-bithiazole]-4-carbohydrazide (II-8b). White solid, yield: 63%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.05 (s, 1H), 7.91(d, 1H, *J*=3.2 Hz), 7.49(d, 1H, *J*=3.2 Hz), 6.98-6.89 (m, 4H), 6.23(d, 1H, *J*=17.2 Hz), 3.25(d, 2H, *J*=7.2 Hz), 2.06-1.96 (m,1H), 0.99 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.07, 160.67, 158.04 (d, *J* = 238.7 Hz), 157.51, 149.40, 144.24 (d, *J* = 2.2 Hz), 144.12, 141.87, 121.43, 115.80 (d, *J* = 22.8 Hz), 115.05 (d, *J* = 7.8 Hz), 35.65, 31.06, 22.24; ESI-MS:377.3 $[M+H]^+$.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-5-isobutyl-[2,2'-bithiazole]-4-carboxamide (II-8c). White solid, yield: 54%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.88(d, 1H, *J*=3.2 Hz), 7.80 (s, 1H), 7.44(d, 1H, *J*=3.2 Hz), 7.35 (dd, 1H, $J_1$=5.6 Hz, $J_2$=3.2 Hz), 7.04(t, 2H, *J*=8.8 Hz), 4.62(d, 2H, *J*=6.0 Hz), 3.32(d, 2H, *J*=7.2 Hz), 2.08-2.01 (m, 1H), 1.02 (d, 6H, J=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 162.18 (d, *J* = 245.4 Hz), 161.97, 160.86, 156.70, 148.28, 144.01, 143.56, 134.26 (d, *J* = 3.2 Hz), 129.39 (d, *J* = 8.1 Hz), 121.18, 115.55 (d, *J* = 21.5 Hz), 42.37, 35.82, 31.10, 22.34; ESI-MS:376.3 $[M+H]^+$.

**4.1.12. General procedure for preparation of compound Ethyl-2-amino-4-isobutylthiazole-5-carboxylate (III-4)**

To the mixture of intermediate II-2 (4 g, 23.2 mmol) in DCM (150 mL) was

added NBS (6.74 g, 0.038 mol) under ice-bath. The solution was stirred at room temperature for 4 hours. Upon completion of the reaction, the mixture was washed with water (60 mL × 3) and washed with saturated sodium chloride (40 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum to obtain the desired compound III-3 as yellow oil. Intermediate III-3 (5.34 g, 21.3 mmol) and thiourea (1.62 g, 21.3 mmol) were dissolved in ethanol, and the mixture solution was stirred at 80 □ for 4 hours. Upon completion of the reaction, ethanol was removed under vacuum. The residue was added water (100 mL), extracted with ethyl acetate (45 mL × 3), washed with saturated sodium chloride (60 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound III-4. White solid, yield: 70%, m.p.148-149 □; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.68 (s, 2H), 4.28 (q, 2H, *J*=7.2 Hz), 4.23 (d, 2H, *J*=7.2 Hz), 2.09-1.98 (m,1H), 1.32 (t, 3H, *J*=7.2 Hz), 0.93 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 169.46, 162.67, 162.43, 111.83, 60.54, 39.32, 28.98, 22.47, 14.37; ESI-MS: 229.0 $[M+H]^+$, 457.7 $[2M+H]^+$.

**4.1.13. General procedure for preparation of compound 2-amino-4-isobutylthiazole-5-carboxylic acid (III-5)**

To the mixture of intermediate III-4 (500 mg, 2.19 mmol) in ethanol (5 mL) and THF (5 mL) was added sodium hydroxide solution (4 mL, 1mol/L). The solution was stirred under 50 □ for 3 hours. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. Then the mixture was adjusted the pH to less than 7 with HCl (1mol/L), added 60 mL water, extracted with ethyl acetate (30 mL × 3) and washed with saturated sodium chloride (50 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compound III-5. Light yellow solid, yield: 57%, m,p.148-149 ℃; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 12.20 (s, 1H), 7.58 (s, 2H), 2.72 (d, 2H, *J*=7.2 Hz), 2.03-1.97 (m,1H), 0.86 (d, 6H, *J*=6.4 Hz); $^{13}$C NMR

(100 MHz, DMSO): $\delta$ 169.26, 162.79, 161.41, 108.87, 37.96, 27.51, 21.77; ESI-MS: 199.2 $[M-H]^-$.

**4.1.14. General procedure for preparation of compound 2-amino-*N*-(4-fluorobenzyl)-4-isobutylthiazole-5-carboxamide (III-6)**

To the mixture of intermediate III-5 (102 mg, 0.51 mmol) in DMF (5 mL) was slowly added HATU (232 mg, 0.61 mmol) under low temperature. The mixture solution was stirred for 10 min, added 4-fluorobenzylamine (70 mg, 0.56 mmol), DIPEA (131 mg, 1.02 mmol), and then was further stirred at room temperature for 2h. Upon completion of the reaction, the mixture was added to 80 mL water, extracted with ethyl acetate (20 mL × 3) and washed with saturated sodium chloride (40 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound III-6. White solid, yield: 70%, m.p.123-124 ℃; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.30-7.27 (m,2H), 7.05-7.00 (m,2H), 5.78 (s, 1H), 5.47 (s, 2H), 4.51 (d, 2H, $J$=6.0 Hz), 2.75 (d, 2H, $J$=7.2 Hz), 2.08-2.01 (m,1H), 0.92 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): 166.58, 163.47, 162.08, 161.02, 157.78, 134.01 (d, $J$ = 3.2 Hz), 129.45 (d, $J$ = 8.1 Hz), 115.62 (d, $J$ = 21.6 Hz), 43.26, 39.60, 28.96, 22.49; ESI-MS: 308.0 $[M+H]^+$, 330.4 $[M+Na]^+$.

**4.1.15. General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-(thiophene-2-carboxamido) thiazole-5-carboxamide (III-7a)**

To the mixture of intermediate III-6 (100 mg, 0.33 mmol) in DMF (5 mL) was slowly added HATU (148 mg, 0.39 mmol) under low temperature. The solution was stirred for 5 min, added 2-thiazole carboxylic acid (42 mg, 0.33 mmol), DIPEA (84 mg, 0.65 mmol), and then was further stirred under room temperature for 2h. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (30 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was

removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound III-7a. White solid, yield: 44%, m.p.210-211 ℃; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 12.93 (s, 1H), 8.67 (t, 1H, $J$=5.6 Hz), 8.28 (s, 1H), 7.99 (s, 1H), 7.35 (dd,2H, $J_1$=5.6 Hz, $J_2$=8.4 Hz), 7.26 (t,2H, $J$=4.4 Hz), 7.16 (t,2H, $J$=8.8 Hz), 4.38 (d, 2H, $J$=6.0 Hz), 2.86 (d, 2H, $J$=7.2 Hz), 2.07-2.00 (m,1H), 0.85 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 162.82, 162.12, 160.41, 136.30 (d, J = 3.0 Hz), 134.43, 131.62, 129.72 (d, $J$ = 8.1 Hz), 129.10, 115.42 (d, $J$ = 21.2 Hz), 42.41, 28.92, 22.76; ESI-MS: 418.3 $[M+H]^+$, 440.3 $[M+Na]^+$.

**4.1.16. General procedure for preparation of compounds III-7(b-d).**

To the solution of intermediate III-6 (100 mg, 0.33 mmol) in THF (25 mL) was slowly added acyl chloride (0.39 mmol), DMAP (3.97 mg, 0.03 mmol) and DIPEA (126.14 mg, 0.98 mmol) under low temperature. The mixture solution was stirred for 4 hours under 80 ℃. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (30 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, gave the desired compounds III-7(b-d).

General procedure for preparation of compound *N*-(4-fluorobenzyl)-2-(4-fluorophenylsulfonamido)-4-isobutylthiazole-5-carboxamide (III-7b). White solid, yield: 39%, m.p.160-161 ℃; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 13.03 (s, 1H), 8.71 (t, 1H, $J$=6.0 Hz), 7.88 (dd,2H, $J_1$=5.2 Hz, $J_2$=8.8 Hz), 7.40 (t, 2H, $J$=8.8 Hz), 7.31 (dd,2H, $J_1$=6.0 Hz, $J_2$=8.8 Hz), 7.15 (t, 2H, $J$=8.8 Hz), 4.33 (d, 2H, $J$=6.0 Hz), 2.70 (d, 2H, $J$=7.6 Hz), 1.93-1.87 (m,1H), 0.80 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 166.21, 165.78, 163.29, 162.86, 160.40, 143.07, 138.91, 135.92 (d, $J$ = 3.0 Hz), 129.84 (d, $J$ = 8.1 Hz), 129.13 (d, $J$ = 9.4 Hz), 116.72 (d, $J$ = 22.6 Hz), 115.46 (d, $J$ = 21.3 Hz), 112.17, 42.43, 35.26, 28.35, 22.32; EI-MS: 466.4 $[M+H]^+$ , 488.4 $[M+Na]^+$.

General procedure for preparation of compound

2-(4-fluorobenzamido)-*N*-(4-fluorobenzyl)-4-isobutylthiazole-5-carboxamide (III-7c). White solid, yield: 57%, m.p.198-199 □; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 12.89 (s, 1H), 8.70 (t, 1H, *J*=6.0 Hz), 8.21-8.17 (m,2H), 7.41-8.34 (m,4H),7.17 (t, 2H, *J*=8.8 Hz), 4.38 (d, 2H, *J*=6.0 Hz), 2.87 (d, 2H, *J*=7.6 Hz), 2.07-1.99 (m,1H), 0.86 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 166.47, 163.97, 162.83, 162.18, 161.39, 160.42, 141.97, 136.31 (d, *J* = 3.0 Hz), 131.62 (d, *J* = 9.4 Hz), 129.72 (d, *J* = 8.1 Hz), 116.15 (d, *J* = 21.7 Hz), 115.43 (d, *J* = 21.2 Hz), 99.84, 55.36, 42.43, 28.92, 22.76; EI-MS: 452.4 $[M+Na]^+$ , 859.5 $[2M+H]^+$ , 881.6 $[2M+Na]^+$.

General procedure for preparation of compound 2-(4-acetamidophenylsulfonamido)-*N*-(4-fluorobenzyl)-4-isobutylthiazole-5-carboxa mide (III-7d). White solid, yield: 70%, m.p.123-124 □; $^{1}$H NMR (400 MHz, DMSO) $\delta$: 12.89 (s, 1H), 10.28 (s, 1H), 8.65 (t, 1H, *J*=5.6 Hz), 7.76-7.71 (m,4H), 7.33-7.29 (m,2H), 7.15 (t, 2H, *J*=8.8 Hz), 4.33 (d, 2H, *J*=6.0 Hz), 2.69 (d, 2H, *J*=7.6 Hz), 2.07 (s, 3H), 1.93-1.86 (m,1H), 0.80 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 169.38, 162.85, 160.51, 158.79, 143.21, 134.93, 129.82 (d, *J* = 8.1 Hz), 128.07, 127.36, 126.20, 118.99, 118.10, 115.45 (d, *J* = 21.2 Hz), 96.58, 42.43, 37.37, 35.28, 28.33, 24.58, 22.71, 22.33; EI-MS: 505.5 $[M+H]^+$, 527.4 $[M+Na]^+$.

**4.1.17. General procedure for preparation of compound Ethyl-2-bromo-4-isobutylthiazole-5-carboxylate (IV-5)**

To the mixture of intermediate III-4 (2.85 g, 12.5 mmol) in acetonitrile (150 mL) was slowly added cupric bromide (4.18 g, 18.7 mmol) and *tert*-butyl nitrite (1.38 g, 18.7 mmol) under ice-bath. The solution was stirred at room temperature for 3 hours. Upon completion of the reaction, the mixture was added to 100 mL water, extracted with ethyl acetate (50 mL × 3) and washed with saturated sodium chloride (60 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum to obtain the desired compound IV-5 as yellow oil. Yield :83%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 4.31(q, 2H, *J*=7.2 Hz), 3.00 (d, 2H, *J*=7.2 Hz), 2.15-2.09 (m,1H), 1.35 (t, 3H, *J*=7.2 Hz), 0.93 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.87, 160.77, 139.50, 126.43, 61.55, 39.08, 29.08, 22.30, 14.21;

ESI-MS: 292.2, 294.1 $[M+H]^+$.

**4.1.18. General procedure for preparation of compound 2-bromo-4-isobutylthiazole-5-carboxylic acid (IV-6)**

To the mixture of intermediate IV-5 (0.4 g, 1.37 mmol) in ethanol (10 mL), water (10 mL) and THF (10 mL) was added with NaOH (109 mg, 2.74 mmol). The mixture solution was stirred under 50 □. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. The mixture was added to 30 mL ethyl acetate and 50 mL water. Then aqueous phase was neutralized with diluted hydrochloric acid, extracted with ethyl acetate (30 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compound IV-6. White solid, yield: 76%, m.p.132 □; $^1$H NMR (400 MHz, DMSO): $\delta$ 2.94 (d, 2H, *J*=7.2 Hz), 2.05-2.01 (m,1H), 0.88 (d, 6H, *J*=6.4 Hz); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 162.57, 162.07, 139.42, 38.51, 28.88, 22.60; ESI-MS: 264.2, 266.2 $[M+H]^+$.

**4.1.19. General procedure for preparation of compound 2-bromo-N-(4-fluorobenzyl)-4-isobutylthiazole-5-carboxamide (IV-7)**

To the mixture of intermediate IV-6 (2.23 g, 8.46 mmol) in DMF (65 mL) was slowly added HATU (3.86 g, 10.2 mmol) under low temperature. The mixture solution was stirred for 5 min, added 4-fluorobenzylamine (1.27g, 10.2 mmol), DIPEA (2.19 g, 16.9 mmol), and then was further stirred at room temperature for 2h. Upon completion of the reaction, the mixture was added to 100 mL water, extracted with ethyl acetate (50 mL × 3) and washed with saturated sodium chloride (60 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound IV-7. Light red solid, yield: 82.3%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.25-7.22 (m,2H), 7.02-6.97 (m,2H), 5.99 (s, 1H), 4.49 (d, 1H, *J*=5.6 Hz), 4.33(q, 2H, *J*=7.2 Hz), 2.82 (d, 2H, *J*=7.2 Hz), 2.11-2.04 (m,1H), 0.87 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.62, 161.17, 160.37, 159.04, 136.27, 133.26 (d, *J* = 3.2 Hz), 130.22, 129.59 (d, *J* = 8.2 Hz), 115.79 (d, *J* = 21.5 Hz), 43.56, 39.40,

29.07, 22.34; ESI-MS: 373.2 $[M+H]^+$.

**4.1.20. General procedure for preparation of compounds IV-8(a-e)**

To the mixture of intermediate IV-7 (200 mg, 0.54 mmol) in dioxane (8 mL) and water (8 mL) was slowly added thiophene-2-borate (124 mg, 0.59 mmol), potassium carbonate (148 mg, 1.08 mmol) and tetraphenyl phosphine palladium (33 mg, 0.026 mmol) under $N_2$ atmosphere. The mixture was stirred for 2h at 100 □. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (30 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compounds IV-8(a-e).

General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-(thiophen-2-yl)thiazole-5-carboxamide (IV-8a). White solid, yield: 43%, m.p.138-139 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.51 (d, 1H, *J*=3.6 Hz), 7.42 (d, 1H, *J*=5.2 Hz), 7.31 (dd,2H, $J_1$=5.2 Hz, $J_2$=8.4 Hz), 7.08-7.02 (m,3H), 6.06 (s, 1H), 5.47 (s, 2H), 4.56 (d, 2H, *J*=5.6 Hz), 2.95 (d, 2H, *J*=7.2 Hz), 2.22-2.16 (m,1H), 0.87 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.57, 161.60, 161.12, 160.58, 160.26, 136.74, 133.62 (d, *J* = 3.2 Hz), 129.59 (d, *J* = 8.2 Hz), 128.82, 128.07, 127.57, 124.68, 115.73 (d, *J* = 21.5 Hz), 43.50, 39.38, 29.05, 22.44; ESI-MS: 375.1 $[M+H]^+$, 749.4 $[2M+H]^+$.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-(5-methylthiophen-2-yl)thiazole-5-carboxamide (IV-8b). White solid, yield: 76%, m.p.134-135 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.33-7.29 (m,3H), 7.01-7.07 (m,2H), 6.73 (dd,1H, $J_1$=0.8 Hz, $J_2$=3.6 Hz), 6.03 (s, 1H), 4.56 (d, 2H, *J*=5.6 Hz), 2.93 (d, 2H, *J*=7.2 Hz), 2.51 (s, 3H), 2.21-2.15 (m,1H), 0.94 (d, 6H, *J*=6.4 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.55, 161.70, 161.10, 160.82, 160.25, 144.23, 134.32, 133.69 (d, *J* = 3.2 Hz), 129.57 (d, *J* = 8.1 Hz), 127.76, 126.45, 123.96, 115.71 (d, *J* = 21.5 Hz), 43.46, 39.37, 29.03, 22.44, 15.60; ESI-MS: 388.9 $[M+H]^+$.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-(thiophen-3-yl)thiazole-5-carboxamide (IV-8c). White solid, yield: 58%, m.p.132-133 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.87 (dd,1H, $J_1$=0.12 Hz, $J_2$=2.8 Hz), 7.51 (dd,1H, $J_1$=0.12 Hz, $J_2$=4.8 Hz), 7.38 (dd,2H, $J_1$=0.32 Hz, $J_2$=5.2 Hz), 7.05 (t, 2H, $J$=8.4 Hz), 6.06 (s, 1H), 4.57 (d, 2H, $J$=5.6 Hz), 2.97 (d, 2H, $J$=7.2 Hz), 2.24-2.17 (m,1H), 0.95 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.57, 161.76, 161.12, 160.39, 135.23, 133.67 (d, $J$ = 3.2 Hz), 129.58 (d, $J$ = 8.1 Hz), 126.98, 126.23, 125.12, 124.64, 115.73 (d, $J$ = 21.5 Hz), 43.49, 39.48, 29.10, 22.46; ESI-MS: 375.3 $[M+H]^+$.

General procedure for preparation of compound 2-(benzo[b]thiophen-2-yl)-*N*-(4-fluorobenzyl)-4-isobutylthiazole-5-carboxamide (IV-8d). White solid, yield: 70%, m.p.154-155 □; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.83-7.78 (m,2H), 7.50 (s, 1H), 7.40-7.37 (m,2H), 7.35-7.31 (m,2H), 7.05 (t, 2H, $J$=8.4 Hz), 6.12 (t, 1H, $J$=4.8 Hz), 4.58 (d, 2H, $J$=6.0 Hz), 2.97 (d, 2H, $J$=7.2 Hz), 2.25-2.19 (m,1H), 0.97 (d, 6H, $J$=6.4 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.58, 161.49, 161.13, 160.60, 160.30, 140.58, 139.55, 136.42, 133.56 (d, $J$ = 3.2 Hz), 129.60 (d, $J$ = 8.2 Hz), 126.09, 125.80, 125.04, 124.51, 124.07, 122.52, 115.76 (d, $J$ = 21.5 Hz), 43.55, 39.41, 29.09, 22.48; EI-MS: 425.3 $[M+H]^+$.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-(pyridin-4-yl)thiazole-5-carboxamide (IV-8e). Yellow solid, yield: 30%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.70 (dd,2H, $J_1$=0.16 Hz, $J_2$=4.8 Hz), 7.77 (dd,2H, $J_1$=0.16 Hz, $J_2$=4.8 Hz), 7.33 (dd,2H, $J_1$=5.2 Hz, $J_2$=8.4 Hz), 7.06 (t, 2H, $J$=8.4 Hz), 6.21 (s, 1H), 4.59 (d, 2H, $J$=6.0 Hz), 2.99 (d, 2H, $J$=7.2 Hz), 2.26-2.16 (m,1H), 0.96 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 159.11, 158.87, 156.53, 156.42, 156.00, 146.01, 134.96, 128.68 (d, $J$ = 3.3 Hz), 124.87 (d, $J$ = 8.1 Hz), 122.75, 115.66, 111.04 (d, $J$ = 21.6 Hz), 71.95, 38.86, 34.76, 24.37, 17.71; EI-MS: 370.2 $[M+H]^+$.

**4.1.21. General procedure for preparation of compound Ethyl-2,6-diphenylnicotinate (V-3)**

Commercial starting material acetophenone (5 g, 41.6 mmol) was dissolved in *N*, *N*-dimethyl formamide dimethyl acetal (9.92 g, 83.2 mmol), and the mixture was stirred under reflux temperature. Upon completion of the reaction, the mixture was cooled to room temperature, and then the solvent was removed under vacuum to

afford the desired compound V-2 as white solid with 93% yield:. To the mixture of ethyl benzoylacetate (1.1 g, 5.71 mmol) in acetic acid (50 mL) was slowly added intermediate V-2 (1 g, 5.71 mmol) and ammonium acetate (880 mg, 11.4 mmol). The solution was stirred under reflux temperature. Upon completion of the reaction, the mixture was cooled to room temperature, added 100 mL water, extracted with ethyl acetate (40 mL × 3) and washed with saturated sodium chloride (60 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, to obtain the desired compound V-3. White solid, yield: 15%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.09 (d, 1H, $J$=1.6 Hz), 8.26 (dd, 1H, $J_1$=2.0 Hz, $J_2$=6.4 Hz), 8.11 (dd, 2H, $J_1$=2.0 Hz, $J_2$=6.4 Hz), 7.91 (d, 1H, $J$=8.0 Hz), 7.86 (d, 1H, $J$=7.2 Hz), 7.65 (t, 1H, $J$=7.2 Hz), 7.56-7.50 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 194.61, 160.28, 151.11, 138.34, 138.09, 137.04, 133.03, 131.33, 130.10, 129.97, 128.98, 128.61, 127.40, 119.99; ESI-MS: 304.4 $[M+H]^+$.

**4.1.22. General procedure for preparation of compound 2,6-diphenylnicotinic acid (V-4)**

To the mixture solution of intermediate V-3 (250 mg, 0.82 mmol) in ethanol (3 mL), water (3 mL) and THF (3 mL) was added with NaOH (197 mg, 4.94 mmol). The mixture solution was stirred under 80 □. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. The mixture was added to 30 mL ethyl acetate and 50 mL water. Then aqueous phase was neutralized with diluted hydrochloric acid, extracted with ethyl acetate (30 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compound V-4. White solid, yield: 88%; $^{1}$H NMR (400 MHz, DMSO): $\delta$ 13.20 (s, 1H), 8.20 (dd, $J$ = 11.3, 4.6 Hz, 3H), 8.04 (d, $J$ = 8.2 Hz, 1H), 7.67 (dd, $J$ = 7.7, 1.7 Hz, 2H), 7.59 – 7.42 (m, 6H); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 169.53, 157.40, 157.26, 140.48, 139.25, 138.13, 130.29, 129.36, 129.21, 129.05, 128.48, 127.45, 127.07, 118.58; ESI-MS: 276.4 $[M+H]^+$.

**4.1.23. General procedure for preparation of compound**

***N*-(4-fluorobenzyl)-2,6-diphenylnicotinamide (V-5)**

To the mixture solution of intermediate V-4 (107 mg, 0.39 mmol) in DMF (5 mL) was slowly added HATU (178 mg, 0.46 mmol) under low temperature. The mixture solution was stirred for 5 min, added 4-fluorobenzylamine (59 mg, 0.46 mmol), triethylamine (79 mg, 0.78 mmol), and then was further stirred under room temperature. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound V-5. White solid, yield: 81%, m.p.184-188 □; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.73 (dd, 1H, $J$=1.2 Hz, 4.4 Hz), 8.62 (d, 1H, $J$=8.4 Hz), 8.41 (dd, 1H, $J_1$=1.2 Hz, $J_2$=8.4 Hz), 8.22-8.20 (m, 2H), 7.93 (d, 1H, $J$=8.4 Hz), 7.89-7.87(m, 2H), 7.56-7.41 (m, 7H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 168.33, 162.20 (d, $J$ = 246.0 Hz), 157.94, 155.78, 139.34, 138.34, 138.26, 133.01 (d, $J$ = 3.2 Hz), 129.65, 129.57, 129.19, 129.15, 129.06, 128.81, 128.62, 127.20, 118.36, 115.44 (d, $J$ = 21.5 Hz), 43.53; ESI-MS: 383.4 $[M+H]^+$.

**4.1.24. General procedure for preparation of compound Methyl 3-amino-6-(2-methoxyphenyl)pyrazine-2-carboxylate (VI-2)**

To the mixture of 3-amino-6-bromopyrazine-2-methyl formate (1 g, 4.31 mmol) in dioxane (20 mL) and water (5 mL) was slowly added with 2-methoxybenzeneboronic acid (851 mg, 5.6 mmol), cesium carbonate (4.91 g, 15.08 mmol) and [1,1'-bis (diphenylphosphine) ferrocene] palladium dichloride (473 mg, 0.65 mmol) under $N_2$ atmosphere. The mixture solution was stirred under 100 □. Upon completion of the reaction, dioxane was removed under vacuum, and the residue was added to 60 mL water, extracted with ethyl acetate (25 mL × 3) and washed with saturated sodium chloride (50 mL × 3). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound VI-2. White solid, yield: 99%; $^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.79 (s, 1H), 7.79 (dd, 1H, $J_1$=2.0 Hz, $J_2$=6.0 Hz), 7.36 (dt, 1H, $J$=2.0, 8.4 Hz), 7.08 (t, 1H, $J$=7.6 Hz), 6.98 (d, 1H, $J$=8.4 Hz), 6.69 (s, 1H), 3.99 (s, 3H), 3.87 (s, 3H); $^{13}$C NMR (100 MHz, DMSO):

$\delta$ 167.08, 156.99, 154.72, 149.11, 139.24, 130.31, 130.25, 125.66, 122.58, 121.25, 112.28, 56.12, 52.64; ESI-MS: 260.3 $[M+H]^+$.

**4.1.25. General procedure for preparation of compound Methyl 6-(2-methoxyphenyl)-3-(4-nitrobenzamido)pyrazine-2-carboxylate (VI-3)**

To the mixture of intermediate VI-2 (100 mg, 0.39 mmol) in DMF (5 mL) was slowly added triethylamine (117 mg, 1.16 mmol) and 4-nitrobenzoyl chloride (72 mg, 0.39 mmol). The mixture solution was stirred under 80 □. Upon completion of the reaction, the solution was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, giving the desired compound VI-3. White solid, yield: 41%, m.p.198-200 □; $^1$H NMR (400 MHz, DMSO): $\delta$ 11.77 (s, 1H), 9.15 (s, 1H), 8.41 (d, 2H, $J$=8.8 Hz), 8.24 (d, 2H, $J$=8.8 Hz), 7.81 (dd, 1H, $J_1$=1.2 Hz, $J_2$=7.6 Hz),7.54-7.50 (m, 1H), 7.24 (d, 1H, $J$=8.4 Hz), 7.15 (t, 1H, $J$=7.6 Hz), 3.91 (s, 3H), 3.80 (s, 3H); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 165.36, 165.31, 157.47, 150.17, 146.97, 146.11, 143.81, 139.15, 138.30, 131.93, 131.13, 130.07, 124.43, 124.23, 121.51, 112.60, 56.34, 53.03; ESI-MS: 409.5 $[M+H]^+$.

**4.1.26. General procedure for preparation of compounds VII-4a and VII-4b**

To the mixture of different substituent benzamidine Hydrochloride in ethanol (60 mL) was slowly added intermediate II-2 (12.8 mmol mmol) and sodium acetate (1.74 g, 25.5 mmol). The resulting solution was stirred under 80 □. Upon completion of the reaction, ethanol was removed under vacuum, and the residue was added to 100 mL water, extracted with ethyl acetate (30 mL × 3) and washed with saturated sodium chloride (60 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, to obtain the desired compounds VII-4a and VII-4b.

General procedure for preparation of compound Ethyl 4-isobutyl-2-phenylpyrimidine-5-carboxylate (VII-4a). White solid, yield: 72%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.19 (s, 1H), 8.54-8.51 (m, 2H), 7.52-7.50 (m, 3H), 4.42

(q, 2H, *J*=7.2 Hz), 3.14 (d, 2H, *J*=7.2 Hz), 2.35-2.25 (m, 1H), 1.43 (t, 3H, *J*=7.2 Hz), 1.00 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 171.39, 165.34, 165.26, 159.34, 137.07, 131.40, 128.88, 128.62, 121.50, 77.23, 61.45, 44.77, 28.59, 22.57, 14.26; ESI-MS: 285.3 $[M+H]^+$.

General procedure for preparation of compound Ethyl 2-(3-fluorophenyl)-4-isobutylpyrimidine-5-carboxylate (VII-4b). White solid, yield: 66%; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.18 (s, 1H), 8.36 – 8.29 (m, 1H), 8.22 (ddd, *J* = 10.3, 2.6, 1.5 Hz, 1H), 7.46 (td, *J* = 8.0, 5.8 Hz, 1H), 7.23–7.15 (m, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.13 (d, *J* = 7.1 Hz, 2H), 2.29 (dt, *J* = 13.5, 6.8 Hz, 1H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.00 (d, *J* = 6.7 Hz, 7H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 171.54, 165.16, 164.06 (d, *J* = 3.2 Hz), 163.16 (d, *J* = 245.2 Hz), 159.36, 139.39 (d, *J* = 7.9 Hz), 130.10 (d, *J* = 7.9 Hz), 124.47 (d, *J* = 2.8 Hz), 121.92, 118.28 (d, *J* = 21.5 Hz), 115.62 (d, *J* = 23.3 Hz), 61.58, 44.71, 28.62, 22.55, 14.25; ESI-MS: 303.5 $[M+H]^+$.

**4.1.27. General procedure for preparation of compounds VII-5a and VII-5b**

To the mixture of intermediate VII-4a or VII-4b (0.7 mmol) in ethanol (2 mL), water (2 mL) and THF (2 mL) was added NaOH (167 mg, 4.2 mmol). The mixture solution was stirred under 80 □. Upon completion of the reaction, the solvent ethanol and THF were removed under vacuum. The mixture was added to 30 mL ethyl acetate and 50 mL water. Then aqueous phase was neutralized with diluted hydrochloric acid, extracted with ethyl acetate (30 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was recrystallized to obtain the desired compounds VII-5a and VII-5b.

General procedure for preparation of compound 4-isobutyl-2-phenylpyrimidine-5-carboxylic acid (VII-5a). White solid, yield: 89%, m.p.123-127 □; $^1$H NMR (400 MHz, $d_6$-DMSO): $\delta$ 9.10 (s, 1H), 8.46-8.44 (m, 2H), 7.56-7.55 (m, 3H), 3.11 (d, 2H, *J*=7.2 Hz), 2.30-2.23 (m, 1H), 0.94 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $d_6$-DMSO): $\delta$ 169.73, 167.88, 163.32, 159.01, 137.37, 131.53, 129.16, 128.50, 126.29, 44.26, 28.37, 22.87; ESI-MS: 257.4 $[M+H]^+$.

General procedure for preparation of compound 2-(3-fluorophenyl)-4-isobutylpyrimidine-5-carboxylic acid (VII-5b). White solid, yield: 79%, m.p.156-159 □;$^1$H NMR (400 MHz, DMSO): $\delta$ 13.67 (s, 1H), 9.17 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 10.0 Hz, 1H), 7.60 (dd, *J* = 14.0, 7.9 Hz, 1H), 7.42 (td, *J* = 8.4, 2.1 Hz, 1H), 3.08 (d, *J* = 7.0 Hz, 2H), 2.23 (dt, *J* = 13.4, 6.7 Hz,

1H), 0.93 (d, $J$ = 6.6 Hz, 6H); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 170.97, 166.63, 163.14 (d, $J$ = 3.2 Hz), 162.96 (d, $J$ = 243.5 Hz), 159.76, 139.42 (d, $J$ = 7.7 Hz), 131.43 (d, $J$ = 8.1 Hz), 124.77 (d, $J$ = 2.6 Hz), 123.34, 118.88 (d, $J$ = 21.3 Hz), 114.96 (d, $J$ = 23.2 Hz), 44.29, 28.42, 22.78; ESI-MS: 275.4 $[M+H]^+$.

**4.1.28. General procedure for preparation of compounds VII-6a(1-2) and VII-6b**

To the mixture of intermediate VII-5a or VII-5b (0.39 mmol) in DMF (5 mL) was slowly added HATU (178 mg, 0.46 mmol) under low temperature. The mixture solution was stirred for 5 min, added different substituent amine (0.46 mmol), triethylamine (79 mg, 0.78 mmol), and then was further stirred at room temperature. Upon completion of the reaction, the mixture was added to 30 mL water, extracted with ethyl acetate (15 mL × 3) and washed with saturated sodium chloride (20 mL). The organic layer was dried over anhydrous sodium sulfate, and then the solvent was removed under vacuum. The residue was chromatographed on silica gel using ethyl acetate and petroleum ether. Pure fractions were collected and concentrated, to obtain the desired compounds VII-6a(1-2) and VII-6b.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-4-isobutyl-2-phenylpyrimidine-5-carboxamide (VII-6a1). White solid, yield: 68%, m.p.109-111 □; $^1$H NMR(400 MHz, $CDCl_3$): $\delta$ 8.65 (s, 1H), 8.44-8.42 (m, 2H ), 7.51-7.45 (m, 3H ), 7.31 (dd, 2H, $J_1$=5.2 Hz, $J_2$=8.0 Hz), 7.03 (t, 2H, $J$=8.8 Hz), 6.47 (s, 1H), 4.55 (d, 2H, $J$=5.6 Hz), 2.83 (d, 2H, $J$=7.2 Hz), 2.30-2.23 (m, 1H ), 0.91 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 168.65, 166.49, 164.56, 162.38 (d, $J$ = 246.5 Hz), 154.88, 137.02, 133.46 (d, $J$ = 3.2 Hz), 131.21, 129.69 (d, $J$ = 8.1 Hz), 128.62, 128.54, 127.29, 115.77 (d, $J$ = 21.5 Hz), 43.94, 43.46, 28.39, 22.52; ESI-MS: 364.4 $[M+H]^+$.

General procedure for preparation of compound *N*-benzyl-5-isobutyl-2-phenylpyrimidine-4-carboxamide (VII-6a2). White solid, yield: 52%, m.p.107-109 □;$^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.67 (s, 1H), 8.44-8.42 (m, 2H), 7.49-7.46 (m, 3H), 7.36-7.30 (m, 5H ), 6.40 (s, 1H), 4.60 (d, 2H, $J$=5.6 Hz), 2.85 (d, 2H, $J$=7.2 Hz), 2.32-2.26 (m, 1H ), 0.92 (d, 6H, $J$=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 168.67, 166.44, 164.49, 154.89, 137.57, 137.07, 131.17, 128.93, 128.61, 128.54, 127.97, 127.92, 127.44, 44.24, 43.95, 28.39, 22.53; ESI-MS: 346.3 $[M+H]^+$.

General procedure for preparation of compound *N*-(4-fluorobenzyl)-2-(3-fluorophenyl)-4-isobutylpyrimidine-5-carboxamide (VII-6b).

White solid, yield: 63%, m.p.119-121 □;$^{1}$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.67 (s, 1H), 8.24 (d, 1H, *J*=8.0 Hz), 8.15-8.12 (m, 1H ), 7.46-7.41 (m, 1H ), 7.32 (dd, 2H, *J*=5.23, 2.0 Hz), 7.20-7.16 (m, 1H ), 7.04 (t, 2H, *J*=8.4 Hz), 6.36 (d, 1H, *J*=4.8 Hz), 4.58 (d, 2H, *J*=6.0 Hz), 2.85 (d, 2H, *J*=7.2 Hz), 2.32-2.24 (m, 1H ), 0.92 (d, 6H, *J*=6.8 Hz); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 167.52 (d, *J* = 247.8 Hz), 163.64, 163.45, 163.16 (d, *J* = 245.2 Hz), 161.19, 154.96, 139.43 (d, *J* = 7.3 Hz), 133.37 (d, *J* = 3.3 Hz), 130.07 (d, *J* = 7.9 Hz), 129.72 (d, *J* = 7.3 Hz), 127.70, 124.14 (d, *J* = 2.8 Hz), 118.03 (d, *J* = 21.4 Hz), 115.82 (d, *J* = 22.0 Hz), 115.34 (d, *J* = 23.3 Hz), 43.92, 43.52, 28.40, 22.50; ESI-MS: 382.4 $[M+H]^+$.

**4. 2 Biological Activity Evaluation**

***4.2.1 Cells and culture conditions****[24]*

HepG2.2.15 cell lines are HBV-transfected hepatoma cell lines, which were obtained from the cell bank of the Chinese Academy of Sciences. The cell lines were cultured in MEM medium supplemented with 10% fetal bovine serum, 100U • $mL^{-1}$ penicillin, 100 U • $mL^{-1}$ streptomycin, 0.38 mg/mL G418 and the mass fraction of 0.03% glutamine at 37 °C in a 5% $CO_2$ humidified atmosphere.

***4.2.2 Cytotoxicity measurements (CCK-8)***

Cytotoxicity of target compounds to HepG2.2.15 cells were measured by CCK-8 method. Three wells of tested compounds with single concentration were added to 96-well tissue culture plates with 4000 cells in every well for preliminary evaluation. And five different doses of tested compounds were added to 96-well tissue culture plates for further evaluation. Untreated cells with media alone were used as controls. The culture medium was replaced by fresh medium on day 4 and after 8-day 10% CCK-8 solution was added 0.5 h before the end of culture. OD absorbance values at 450 nm and 630 nm were collected by microplate reader (Bio-Rad), then the cell death percent was calculated.

***4.2.3 Inhibiting HBV DNA replication assay (real time fluorescent PCR)***

HepG2.2.15 cells were cultured in triplicate of 96-well tissue culture plates with single concentration for preliminary evaluation. And five different doses of tested compounds in triplicate were added in 96-well tissue culture plates for further

evaluation. The culture medium was replaced with fresh medium on day 4 during the 8-day experiment. Untreated cells with media alone were used as controls. On day 8, the supernatants of HepG2.2.15 cell were collected, which were quantified their HBV DNA using PCR-fluorescent probing (Quantitative diagnostic kit for HBV DNA).

***4.2.4 Inhibiting the secretion of HBeAg and HBsAg (ELISA)****[6]*

Five different doses of tested compounds in triplicate were added in 96-well tissue culture plates for further evaluation. The culture medium was replaced with fresh medium on day 4 during the 8-day experiment. Untreated cells with media alone were used as controls. On day 8, the supernatants of HepG2.2.15 cell were collected, which were detected content of HBsAg and HBeAg by using diagnostic kit (Autobio Diagnostics Co., Ltd, China). The absorbance of tested compounds was determined on microtiter plate ELISA reader.

**4.3 Molecular docking modeling**

The docking studies were performed with the Tripos molecular modeling package Sybyl-X 2.0. Default parameters were used as described in the Sybyl-X 2.0 manual unless otherwise specified. The published crystal structure of HBV capsid complex (PDB codes: 5GMZ) was retrieved from the Protein Data Bank. And the result was displayed by PyMOL.

**4.4 Surface plasmon resonance (SPR)**[1]

First, different concentration PBS, NaAc-HAc buffers and solvent calibration required solution were prepared. Then the recombinant HBV capsid protein was purified and immobilized on a CM5 sensor chip (carboxymethylated dextran covalently attached to a gold surface) with an amine coupling kit from GE Healthcare. At last, six different doses of tested compounds, eight calibration solvents, 50% DMSO solution and running buffer containing 5% DMSO were centrifuged for 15 minutes and then placed into the sample plate. The signals were recorded with a Biacore T200 instrument with the standard protocol.

**Acknowledgments**

The financial support from the National Natural Science Foundation of China (NSFC Nos. 81273354, 81573347, 81903468), Key Project of NSFC for International Cooperation (No. 81420108027), Young Scholars Program of Shandong University (YSPSDU, No. 2016WLJH32), the Fundamental Research Funds of Shandong University (No. 2017JC006), the Natural Science Foundation of Shandong Province (ZR2019BH068), the Science and Technology Development Project of Shandong Province (No. 2018WS061), Key research and development project of Shandong Province (No. 2017CXGC1401), Key Laboratory of Chemical Biology (Ministry of Education) Open Projects Fund (No. CB-201715) and Major Project of Science and Technology of Shandong Province (No. 2015ZDJS04001) is gratefully acknowledged.

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**Highlights**

1. Five skeletons of heterocycle derivatives (pyrazole, thiazole, pyrazine, pyrimidine and pyridine analogs) were designed as potential HBV non-nucleoside inhibitors.
2. Seven synthetic routes were employed to acquire target compounds by diversity-oriented synthesis.
3. Compound **II-8b** displayed the most potent anti-HBV DNA replication activity with $IC_{50}$ value of 2.2 ± 1.1 μM. And compound **IV-8e** and **VII-5b** showed the best inhibitory activity in vitro against HBsAg secretion ($IC_{50}$ = 3.8±0.7 μM, $CC_{50}$ > 100 μM) and HBeAg secretion ($IC_{50}$ = 9.7±2.8 μM, $CC_{50}$ > 100 μM), respectively.
4. Preliminary SARs of these new derivatives were detailed.

The authors declared that they have no conflicts of interest to this work.

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.