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Design, synthesis and lipid-lowering activities of penipyridone derivatives

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ABSTRACT

On the basis of our earlier discovered natural product penipyridone **G** with potential lipid-lowering utility, 35 penipyridone derivatives were designed, synthesized and characterized. Based on the oleic acid-induced HepG2 cell lipid accumulation model, compounds **12c**, **14**, **15f**, **15k**, **15o**, **15p** and **16f** showed potent lipid-lowering activities among the synthetic compounds at 10 μM. In particular, compounds **4**, **15k**, **15o** showed significant activities on inhibiting lipid accumulation in insulin resistant HepG2 cells, and these three compounds were safe and non-toxic within the concentration range of 400 μM. In comparison, **15o** possessed the best lipid-lowering activity. Compared with the vehicle group, the triglyceride inhibition rate of **15o** was about 30.2%, and the total cholesterol inhibition rate was about 14.8% at 20 μM, which was equipotent to Simvastatin. Our research indicates that **15o** may serve as a promising lead compound for the development of hypolipidemic drugs.

1. Introduction

In recent years, the population of hyperlipidemia has increased gradually around the world due to the unhealthy lifestyles, such as smoking, high fatty acid intake and inherited genetic disorder.[1,2] Hyperlipidemia is a common dyslipidemia characterized by increased blood levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and decreased levels of high-density lipoprotein cholesterol (HDL-C).[3] Numerous epidemiological studies have confirmed that hyperlipidemia can cause many complications, such as atherosclerosis,[4] cardiovascular diseases,[5,6] hypertension and diabetes mellitus,[7] which poses a great threat to human health. Statins, as HMG-CoA reductase inhibitors, are currently the first-line drugs for the treatment of hyperlipidemia.[8] In addition, fibrates, niacin, cholesterol absorption inhibitors and bile acid sequestrants are also clinically used to treat hyperlipidemia, because they can affect the metabolism of cholesterol and triglycerides. However, their potential toxicities cause great trouble to the patient, which limit their long-term applications.[9] Therefore, it is always a hot research topic for medicinal chemists to seek hypolipidemic drugs with improved efficacy, safety and reliability.

Classically, natural products play a crucial role in drug discovery and development. The contribution of natural products in the therapeutics of dyslipidemia is phenomenal. Some promising natural compounds with lipid-lowering activity are either in the clinical stage or in the developmental stage. Many other potential lead compounds deserve further attentions.[10] In 2016, we reported seven pyridone alkaloid natural products **A**-**G** (Fig. 1A) (originally numbered **1**–**7**), isolated from the fermentation broth of an Antarctic moss-derived fungus, *Penicillium funiculosum* GWT2-24. *In vitro* fat regulating activity test results showed that compounds **A**, **B**, **E** and **G** possessed desirable inhibitory effects on intracellular lipid accumulation at 10 μM, and had lowering effects on total cholesterol and triglycerides. Especially, compound **G** exhibited greater inhibitory activity on lipid accumulation in insulin resistant HepG2 cells than other tested compounds.[11] By comparing lipid-lowering effects of compounds **D** with **E** and **F** with **G**, it seemed that acetyl was an indispensable pharmacophore. In order to verify the role of hydroxyethoxy group, compounds **1**, **2**, **3** and isomer **4** of compound **E** were synthesized firstly (Fig.1B). Then according to the activity results

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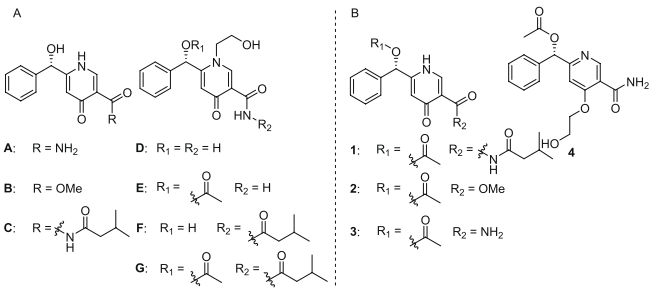


Fig. 1. (A) The structures of penipyridones **A**-**G**; (B) The structures of penipyridone analogues **1**–**4**.

of compounds **A**-**G** and **1**–**4**, compounds **G** and **4** were selected as the lead compounds. Consequently, thirty-one penipyridone derivatives were designed and synthesized based on the lead compounds. Meanwhile, the lipid-lowering activities of all synthetic compounds were evaluated and their structure–activity relationships (SARs) were also discussed.

2. Results and discussion

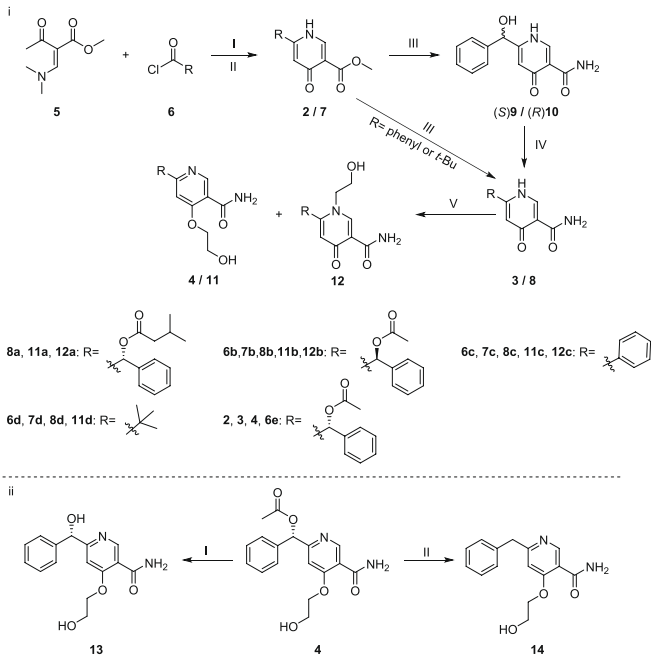
2.1. Chemistry

2.1.1. Lead compounds

The lipid-lowering activities of compounds **A**-**G** and **1**–**4** were evaluated by the model of HepG2 adipose accumulation. As a result, compound **4** showed comparable lipid-lowering activity with **G**, even slightly better than **G** (Fig. 2A). Comparing compound **G** with compound **1** and compounds **E** or **4** with compound **3**, the hydroxyethoxy group was beneficial to improve lipid-lowering activity, and the Unit B was a modifiable potential site. In addition, it seemed that the hydrogen of amide group was essential, which was likely to interact with protein and enhanced the lipid-lowering activity. Consequently, compounds **G** and **4** were selected as the lead compounds for further optimization. The Unit A, B and C of compounds **G** and **4** were modified as followed (Fig. 2B).

2.1.2. Synthetic procedures

The synthetic protocols for the penipyridone derivatives were outlined in Schemes 1, 2 and 3. Compound **5** was synthesized using commercially available methyl acetoacetate and N, *N*-dimethylformamide dimethyl acetal as the starting materials.[12] Compounds **6b** and **6e** were synthesized using different commercially available acids and oxalyl chloride as the starting materials.[13] Compounds **6c** and **6d** were purchased commercially.



Scheme 1. Structural optimization of Unit A. (i) Synthesis of compounds **11** and **12**. Reagents and conditions: (I) LiHMDS, THF, −78 °C, 30 min; (II) NH_4OAc, AcOH, 60 °C, 30 min; (III) saturated NH_3 in MeOH, 48 h, 80 °C; (IV) corresponding acid, EDCI, DMAP, CH_2Cl_2, 0 °C, 1 h. (V) 2-Bromoethanol, K_2CO_3, acetone, 48 h, 80 °C. (ii) Synthesis of compounds of **13** and **14**. Reagents and conditions: (I) CH_3ONa, MeOH, 4 h; (II) Pd/C, H_2, MeOH, 2 h.

In Scheme 1, compound **5** was treated with a series of different acyl chlorides **6b**–**6e** in the presence of lithium bis(trimethylsilyl) amide (LiHMDS) and then acetic acid and NH_4OAc were added to the reaction mixture to afford the compound **2** and intermediates **7b**-**7d**.[14] After that compounds **2** and **7b**-**7d** were deacylated in the presence of saturated

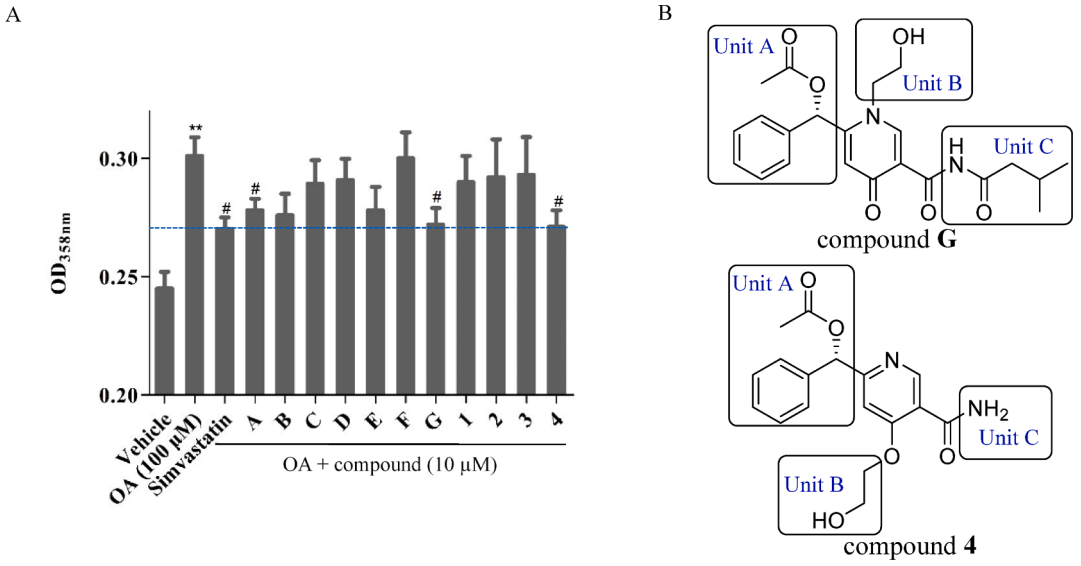
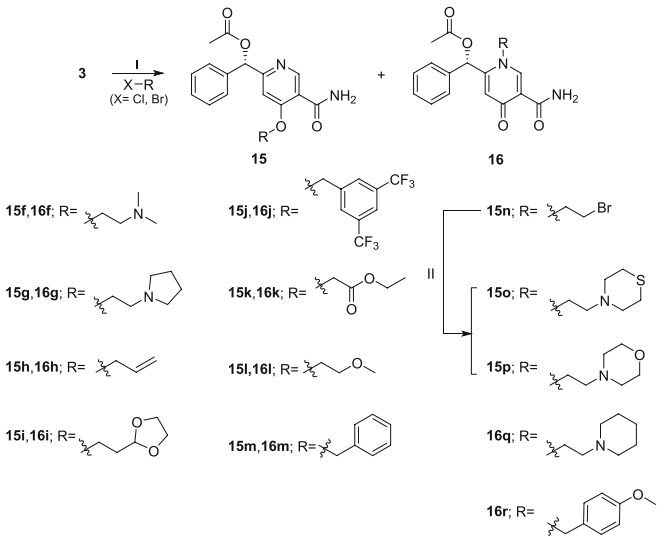
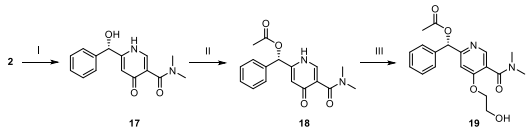


Fig. 2. (A) The inhibition activity of compounds **A**-**G** and **1**–**4** on lipid accumulation in HepG2 cells *in vitro* (Simvastatin and tested compounds at 10 μM). Data were expressed as the mean ± SD (n = 4) of each group of cells from at least three independent experiments; OA: Oleic Acid, $^{**}P < 0.01$, compared with DMSO-treated cells, $^{\#}P < 0.05$, compared with OA-treated cells. (B) Lead compounds with their structural units.



Scheme 2. Structural optimization of Unit B: Synthesis of compounds **15** and **16**. Reagents and conditions: (I) K_2CO_3, acetone, 80 °C, overnight. (II) morpholine or thiomorpholine, K_2CO_3, acetone, 80 °C, overnight.



Scheme 3. Structural optimization of Unit C: Synthesis of compound **19**. Reagents and conditions: (I) dimethylamine in MeOH, 48 h, 80 °C; (II) AcOH, EDCI, DMAP, CH_2Cl_2, 0 °C, 1 h; (III) 2-bromoethanol, K_2CO_3, acetone, 48 h, 80 °C.

NH_3 in MeOH to give the corresponding compounds **8c**, **8d**, (*S*)**9** and (*R*)**10**.[15] Subsequently, under the condition of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDCI) and 4-dimethylamino-pyridine (DMAP), the compounds (*S*)**9** and (*R*)**10** were condensed with corresponding carboxylic acid to obtained compounds **3**, **8a**, and **8b**.[16]

According to the synthetic protocols in the Scheme 1 (i), compounds **4**, **11a-11d** and **12a-12c** were synthesized in the presence of K_2CO_3 and 2-Bromoethanol.[17] Compound **13** was prepared by hydrolysis reaction of compound **4** in the presence of CH_3ONa.[18] Meanwhile, compound **4** was reduced by H_2 in a methanol solution using palladium on carbon as a catalyst to give compound **14** in Scheme 1 (ii).[16]

A series of compounds **15** and **16** were prepared from compound **3** which reacted with corresponding halogenated compounds in the presence of potassium carbonate (Scheme 2).[17]

The compound **2** was treated with 2 M dimethylamine in MeOH to give the compound **17**, then **18** was obtained by acylation of compound **17**. The compound **19** was prepared via similar procedures from compounds **15** and **16**, and the method was shown in Scheme 3.[17,19]

2.2. Structure-activity relationships of penipyridone derivatives

Taking compounds **G** and **4** as the lead compounds, we synthesized 31 penipyridone derivatives (Scheme 1, Scheme 2, Scheme 3). The lipid-lowering activities of 31 compounds and Simvastatin were assessed on oleic acid induced lipid accumulation in HepG2 cells *in vitro* at 10 μM. The activity data were divided into three groups, according to the Unit A, B and C of lead compounds (Fig.3).

The results demonstrated that compounds **12c**, **14**, **15f**, **15k**, **15o**, **15p** and **16f** exhibited potent lipid-lowering activities at 10 μM, among which, **15o** displayed the greatest potency and decreased lipid concentration. In addition, we also tested the cytotoxicity of the test

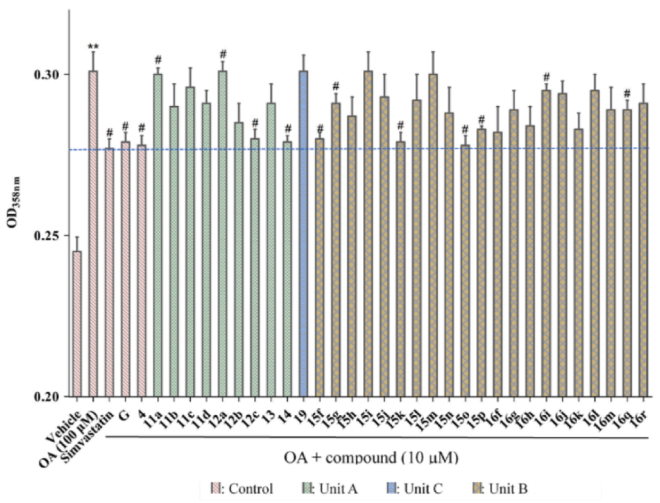


Fig. 3. The inhibition activity on lipid accumulation in HepG2 cells *in vitro* (Simvastatin and tested compounds at 10 μM). Data are expressed as the mean ± SD (n = 6); OA: Oleic Acid, $^{**}P < 0.01$, compared with DMSO-treated cells; # $P < 0.01$, compared with OA-treated cells. Control (pink), Unit A (green), Unit B (gold), Unit C(blue).

compounds in HepG2 cells by MTT assays (Table S1), none of them showed cytotoxic effect under tested concentrations (100 μM).

SARs study revealed that the activities of compounds with pyridine ring were better than that of pyridinone ring. In addition, Unit A was an essential pharmacophore and *S* configuration of C-7 was preferred. *R* configuration obviously impaired the lipid-lowering activity (**11b**). Removel of acetyl group at C-7 (**13**), deacetylation (**14**) or increased the length of C-16 (**11a** and **12a**) all resulted in decreased activities than those of compounds **G** and **4**. When Unit A was replaced by phenyl (**11c** and **12c**) or *tert*-butyl (**11d**), lipid-lowering activities were lower than that of compounds **G** and **4**. Compound **15o** with 4-ethylthiomorpholine group at Unit B displayed the best lipid-lowering activity among all compounds. The key factors affecting activities might be moderate size, flexibility and hydrophilicity of Unit B. These results suggested that either too large (bis(trifluoromethyl)benzyl) or too small (hydroxyethyl) or too rigid (phenyl) R substituted groups was unfavorable. Compared with compounds **15g**, **16g** and **16q**, **15f** and **16f** showed better lipid-lowering activities, possibly attributed to their better hydrophilicity. Compounds **15l** and **16l**, methylated derivatives of compounds **E** and **4** with reduced hydrophilicity of Unit B, showed significantly decreased activities. Bis (trifluoro-methyl) benzyl (**15j** and **16j**), benzyl (**15m** and **16m)** and methoxybenzyl(**16r**) at R-position reduced the activities, whereas, relatively moderate size and flexibility substituent groups (**15o** and **15p**) had a positive effect on the potency, possibly because their conformational effects enhanced the binding of compounds to the corresponding pockets of the active site. Methylation of the amino group (Unit C) in amide (**19**) leaded to completely loss of the activity, possibly that the hydrogen of amino group forms hydrogen bond with amino acid, which enhanced the affinity with protein and enhances the lipid-lowering activity (Fig. 4).

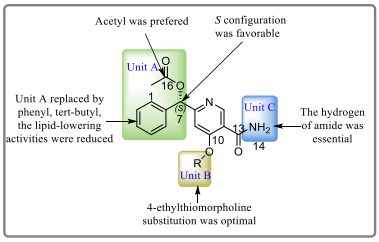


Fig. 4. Structure-activity relationship of penipyridone derivatives.

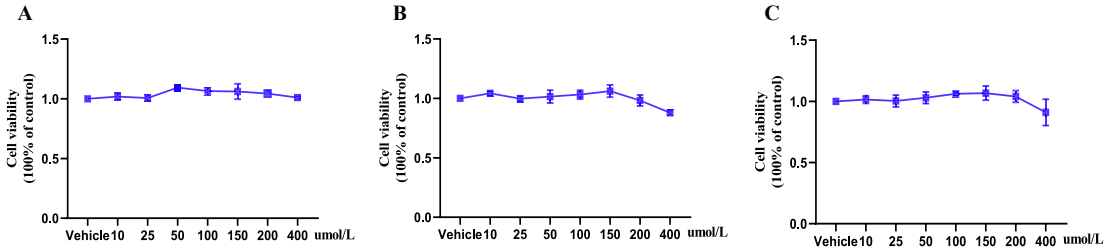


Fig. 5. Effects of compounds **4**, **15k** and **15o** on cytotoxicity of HepG2 cells determined by MTT assays. HepG2 cells were treated with different concentrations compounds for 24 h. (A) Compound **4**; (B) Compound **15k**; (C) Compound **15o**. Data were expressed as the mean ± SD (n = 6).

2.3. Biological evaluation

The cytotoxicity of compounds **4**, **15k**, **15o** on HepG2 cells were detected by MTT assays. The cell viability after treatment with the dose of 400 μM (**4**, **15k**, **15o**) was not significantly different from those in the vehicle group, indicating that compounds **4**, **15k**, **15o** had no significant cytotoxicity up to 400 μM(Fig. 5A-5C).

To determine the lipid-lowering effects of compounds **4**, **15k**, **15o** on the lipid accumulation in insulin resistant cell model, we treated cells with different concentrations of OA for 24 h to induce lipid accumulation. The results showed that the lipid contents of HepG2 cells increased significantly after treatment with 100 μM OA for 24 h (Fig. 6A). Therefore, in the following experiments, we selected 100 μM OA to induce hyperlipidemia model. Then the contents of triglyceride were measured in HepG2 cells that were treated with 100 μM OA for 24 h with or without compounds **4**, **15k**, **15o** at 10 μM. The results suggested that treated with compounds **4**, **15k**, **15o** could significantly reverse the triglyceride accumulation induced by OA in insulin resistant HepG2 cells at 10 μM, while **15o** had the best lipid-lowering effect among them (Fig. 6B). After that, HepG2 cells induced by OA were treated with different doses of compound **15o**, and it was found that **15o** had the best triglyceride-lowering and cholesterol-lowering effect at 20 μM, proving that the activity of **15o** was dose-dependent (Fig. 6C and D). Compared with the vehicle group, the triglyceride inhibition rate of **15o** was about 30.2%, and the total cholesterol inhibition rate was about 14.8% at 20 μM, with the similar efficacy with the positive drug Simvastatin at 10 μM (TG: 29.1%; TC: 12.1%). It should be noted that the inhibitory effect of **15o** on TC at 5 μM (12.8%) was better than that of Simvastatin at 10 μM (12.1%).

In order to observe the accumulation of lipid in HepG2 cells intuitively, we used oil red O staining to monitor the lipid-lowering effects. Compared with the vehicle group, lipid accumulation was significantly increased after OA treatment, while lipid content was significantly decreased after drug treatment with different doses of compound **15o** (Fig. 6E). These results suggested that compound **15o** could significantly reduce intracellular lipid accumulation.

2.4. Molecular docking

The mechanism of lipid metabolism disorder caused by obesity is complicated. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an intracellular glucocorticoid reactivating enzyme, has attracted wide attention. It is a key enzyme to regulate glucocorticoids, which can catalyze the conversion of inactive component cortisone into bioactive glucocorticoids. Compared with subcutaneous adipose tissue, visceral adipose tissue has higher glucocorticoid receptor, and glucocorticoids play an important role in visceral adipose tissue differentiation. A large number of experimental results showed that the activity and expression of 11β-HSD1 was high in patients with hyperlipidemia and dyslipidemia.[20] Veilleux A found that the mRNA level of 11β-HSD1 was different in visceral and peripheral fat of obese patients, and the mRNA content of 11β-HSD1 was positively correlated with visceral fat. The differentiation and maturation of adipocytes were related to the increased 11β-HSD1 mRNA level and oxidoreductase activity.[21] Therefore, 11β-HSD1 can be

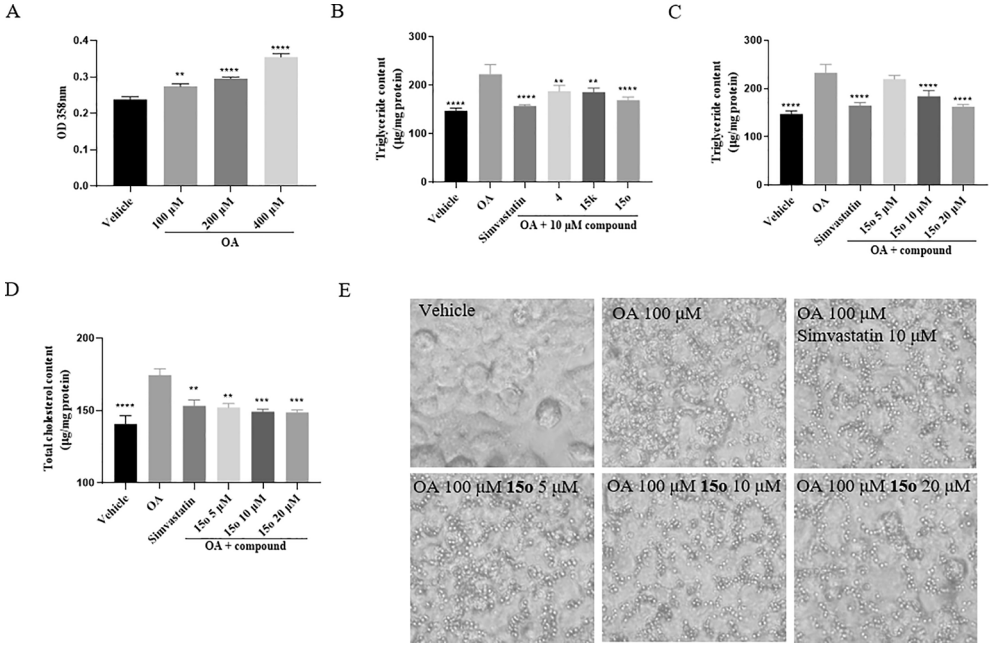


Fig. 6. (A) HepG2 cells treated with different concentrations of OA promoted intracellular lipid accumulation (100, 200 and 400 μM). (B) TG content in HepG2 cells. HepG2 cells were treated with 100 μM OA for 24 h with or without compounds **4**, **15k**, **15o** (10 μM). (C-D) TG and TC content in HepG2 cells. HepG2 cells were treated with 100 μM OA for 24 h with different doses of **15o**. (E) Lipid accumulation in HepG2 cells was observed by Oil Red O staining (40 X). Data were expressed as the mean ± SD (n = 4). Significant difference compared with OA-treated cells, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

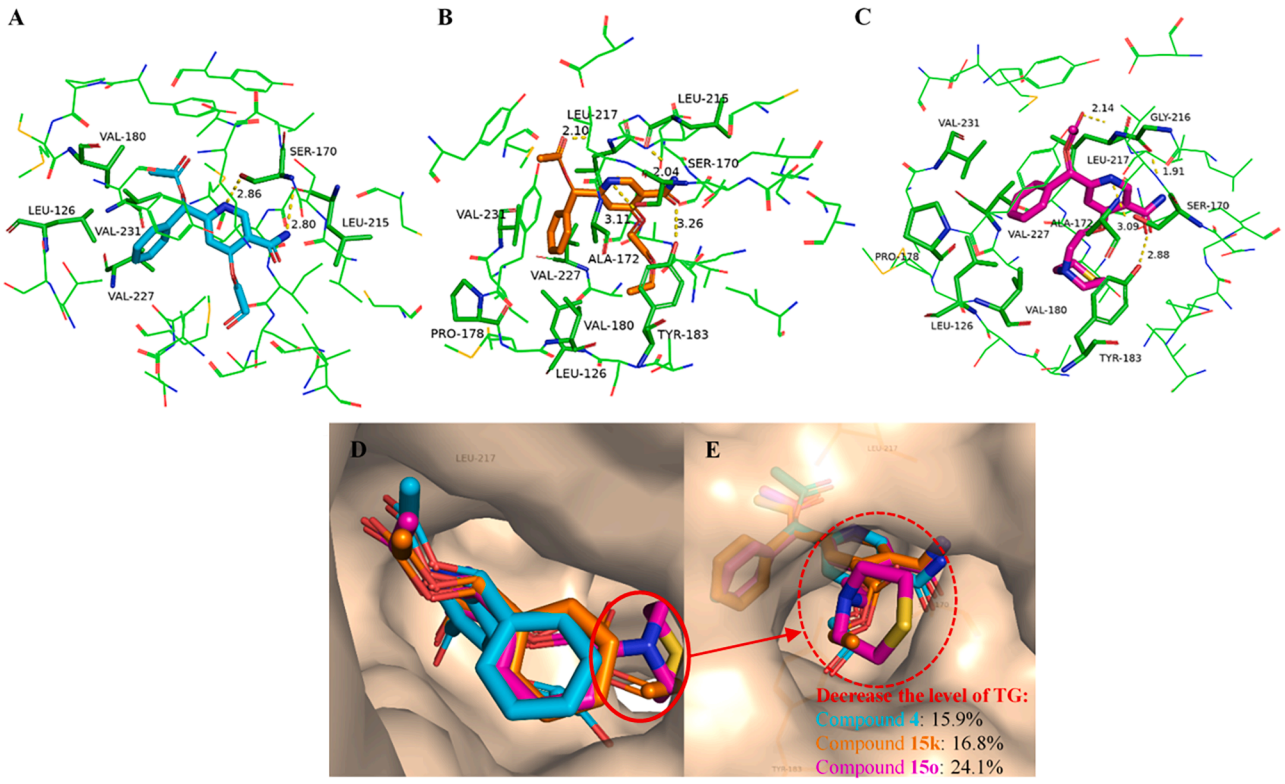


Fig. 7. The predicted models of compounds **4**, **15k**, **15o** with 11β-HSD1(PDB code: 3TFQ). The main protein residues are depicted in green sticks, while the ligands are depicted as: (A) Compound **4** (cyan sticks); (B) Compound **15k** (orange sticks); (C) Compound **15o** (magenta sticks), respectively. (D) and (E) are the representations of compounds **4**, **15k**, **15o** docked into the binding site of 11β-HSD1. All pictures were prepared with PyMOL. For clarity, the $NADP^+$ has been omitted from this representation, and only a few of the surrounding residues are included in the figures.

a novel target for the treatment of central obesity.[22]

Further in, we performed a reverse docking to search the potential lipid-metabolism related target by a designed computational approach in the Supercomputing Center of Pilot National Laboratory for Marine Science and Technology. By the analysis of the results, we found that 11β-hydroxysteroid dehydrogenase type 1 is the prime candidate target.

Now in order to visualize binding modes and to obtain an insight into the relationships between penipyridone derivatives and their potencies, molecular docking study was carried out with the highly potent compounds **4**, **15k**, **15o** within the active site of the crystal structure of 11β-HSD1 complexed with a ligand, 3-[1-(4fluorophenyl)cyclopropyl]-4-(1-methylethyl)-5-[4-(trifluorom-ethoxy)phenyl]-4H-1,2,4 triazole (PDB code: 3TFQ), using Molecular Operating Environment (MOE®) 2016. 08 software. The favored poses from the docking calculations were shown in Fig. 7, along with the residues in the binding pocket that contributed most significantly to the binding energy.

As seen in Fig. 7, the molecular docking results indicated that the phenyl in Unit A was an excellent and privileged moiety for the cavity, which was a hydrophobic cavity formed by Leu126, Val180, Val227, Val231 and their adjacent lipophilic amino acids. In addition, hydrogen of Ser170 formed interaction with the N atom of pyridine ring, which could further stabilize the ligand–protein (Fig. 7A-7C). The carbonyl of carbamoyl (C-13) and acetyl (C-16) formed hydrogen bonds with Tyr183 and Leu217, respectively (Fig. 7B and 7C), which was non-existent in compound **4**. Moreover, both hydrogens of NH_2 in Unit C formed hydrogen bonds with Leu215 (compound **4**, N—H···O 2.86 Å; compound **15k**, N—H···O 2.04 Å) and Gly216 (compound **15o**, N—H···O 1.91 Å), and the lengths of hydrogen bonds in compounds **4** and **15k** were longer than that of **15o**.

The superimposed structures of compounds **4**, **15k** and **15o** docked into the11β-HSD1 binding site indicated that the three compounds were well incorporated in the binding pocket and were able to form similar interactions (Fig. 7D and 7E). However, the binding features R group of compounds **4**, **15k** and **15o** were apparently different. These results suggested that moderate size, flexibility and hydrophilicity (thiomorpholine ring) R substituted groups of Unit B might be suitable to the cavity, which was formed by Asn123, Thr124, Ser125, Tyr183, Tyr221 and other adjacent residues (Fig. 7E).

3. Conclusion

In summary, a series of novel penipyridone derivatives were designed and synthesized. The lipid-lowering activities were evaluated by the adipose accumulation in the insulin resistant HepG2 cell models, and compound **4**, **15k**, **15o** displayed significant lipid-lowering activity without obvious cytotoxicity. In particular, compound **15o** showed the similar effect with Simvastatin on decreasing the TG concentration in HepG2 cells, and the inhibitory effect of **15o** on TC at 5 μM was better than that of Simvastatin at 10 μM. As the same time, molecular docking analysis predicted that compound **15o** showed higher binding affinity with 11β-HSD1 than compounds **4** and **15k**. Taken together, compound **15o** as a potential 11β-HSD1 inhibitor could be considered as a promising lead compound for the treatment of hyperlipidemia.

4. Experimental section

4.1. Chemistry

General information. Commercial reagents and solvents were purchased from Sigma Aldrich, Fluka, and Alfa Aesar used as received, without further purification. The ^1H and ^{13}C NMR spectra were recorded at 500 MHz for ^1H and at 125 MHz for ^{13}C. The chemical shifts (δ) for ^1H and ^{13}C are given in ppm relative to residual signals of the solvents ($CDCl_3$ at 7.26 ppm 1H NMR, 77.16 ppm ^{13}C NMR. DMSO-d_6 at 2.50

ppm ^{1}H NMR, 39.52 ppm ^{13}C NMR). Coupling constants are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. High-resolution mass spectra (HRMS) were obtained from the Waters Q-Tof Ultima Global. Optical rotations are reported as follows: [α] (*c* in g per 100 mL, solvent: $CHCl_3$, MeOH). All the reactions were set up under air and using freshly distilled solvents, without any precautions to exclude moisture, unless otherwise noted open air chemistry on the bench-top. Chromatographic purification of products was accomplished using force-flow chromatography (FC) on silica gel (300–400 mesh). For thin layer chromatography (TLC) analysis throughout this work, Merck pre-coated TLC plates (silica gel 60 GF254, 0.25 mm) were used, using UV light as the visualizing agent and phosphomolybdic acid or basic aqueous potassium permanganate ($KMnO_4$) as stain developing solutions. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator.

Note: NMR signals containing common solvent contaminants were list·H_2O in $CDCl_3$ at 1.56 ppm ^{1}H NMR, and in DMSO-d_6 at 3.33 ppm ^{1}H NMR; Ethyl acetate in $CDCl_3$ at 2.05 (s), 4.12 (q), 1.26 (t) ppm ^{1}H NMR; Dichloromethane in $CDCl_3$ at 5.30 (s) ppm ^{1}H NMR.

4.1.1. General synthetic procedure of compounds **2** and **7b-7d**.

Methyl acetoacetate (17.22 mmol, 1.0 eq.) and N, *N*-dimethylformamide dimethyl acetal (20.10 mmol, 1.2 eq.) were added to a 25 mL dry round bottom flask, then 500 μM DMF was dropped into the flask at room temperature with stirring. After 20 h (monitored by TLC), the mixture was concentrated and purified by column chromatography on silica gel (ethyl acetate/petroleum ether) to obtain compound **5**.

Corresponding acids (2.57 mmol, 1.0 eq.) was added 10 mL anhydrous dichloromethane in a 25 mL dry round bottom flask under nitrogen protection. When the solid was dissolved, oxalyl chloride (328 μL, 3.86 mmol, 1.5 eq.) and 500 μM DMF were dropwise into the stirred solution at 0 °C. Then the reaction was moved to at room temperature for reaction. The reaction progress was monitored by TLC. Upon completion, the reaction was concentrated under reduced pressure to obtain product acyl chlorides **6b** and **6e**.

A solution of LiHMDS (1.0 M in THF, 5.20 mmol, 2.0 eq.) was cooled to −78 °C in 50 mL flask under nitrogen protection, and then freshly THF solutions of prepared chlorides **6b-6e** (2.60 mmol, 1.0 eq.) and **5** (3.12 mmol, 1.2 eq.) were added simultaneously slowly at −78 °C. After stirring for an additional 30 min, the mixture was moved at room temperature. Glacial acetic acid (7.80 mmol, 3.0 eq.) and ammonium acetate (5.20 mmol, 2.0 eq.) were added to the reaction mixture, which was then heated to reflux and stirred for 30 min (monitored by TLC). The mixture was poured into water and extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromate-graphy on silica gel (ethyl acetate/petroleum ether) to obtain compounds **2**, **7b-7d**.

*4.1.1.1. Methyl (S)-6-(acetoxy(phenyl)methyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (**2**).* Compound **2** was prepared from **6e**. Colorless oil, yield: 23%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.13 (s, 1H), 8.89 (s, 1H), 7.42 (d, J = 7.3 Hz, 2H), 7.37–7.29 (m, 3H), 7.06 (s, 1H), 6.78 (s, 1H), 3.97 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.88, 169.80, 167.96, 165.61, 152.11, 138.27, 128.81, 128.64, 127.68, 109.34, 109.32, 77.64, 52.82, 21.31. HRMS: $[M+H]^+$ calcd. For $C_{16}H_{16}NO_5$ *m/z*: 302.1023; found: 302.1020. [α] + 55 (*c* = 0.1 in $CHCl_3$).

*4.1.1.2. Methyl (R)-6-(acetoxy(phenyl)methyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (**7b**).* Compound **7b** was prepared from **6b**. Colorless oil, yield: 30%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.13 (s, 1H), 8.89 (s, 1H), 7.42 (d, J = 7.3 Hz, 2H), 7.37–7.29 (m, 3H), 7.06 (s, 1H), 6.78 (s, 1H), 3.97 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.88, 169.80, 167.96, 165.61, 152.11, 138.27, 128.81, 128.64, 127.68, 109.34, 109.32, 77.64, 52.82, 21.30. HRMS: $[M+H]^+$ calcd. For $C_{16}H_{16}NO_5$ *m/z*: 302.1023; found: 302.1020. [α] + 47 (*c* = 0.1 in $CHCl_3$).

*4.1.1.3. Methyl 4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylate (**7c**).* Compound **7c** was prepared from **6c**. Yellow solid, yield 67%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 11.05 (s, 1H), 8.97 (s, 1H), 8.17 (d, J = 5.2 Hz, 3H), 7.50 (ddd, J = 6.8, 4.6, 1.8 Hz, 5H), 4.03 (s, 3H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 170.50, 168.39, 152.67, 138.86, 130.97, 129.60, 128.10, 109.83, 108.85, 53.14. HRMS: $[M+H]^+$ calcd. For $C_{13}H_{12}NO_3$ *m/z*: 230.0822; found: 230.0820.

*4.1.1.4. Methyl 6-(tert-butyl)-4-oxo-1,4-dihydropyridine-3-carboxylate (**7d**).* Compound **7d** was prepared from **6d**. Yellow solid, yield 33%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.91 (s, 1H), 8.84 (s, 1H), 6.94 (s, 1H), 4.00 (s, 3H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 176.86, 170.60, 168.08, 151.72, 108.63, 108.09, 52.99, 38.49, 29.93. HRMS: $[M+H]^+$ calcd. For $C_{11}H_{16}NO_3$ *m/z*: 210.1125; found: 210.1129.

4.1.2. General synthetic procedure of compounds **3**, (*S*)**9**, (*R*)**10** and **8a–8d**.

To compound **2** and **7b-7d** (0.33 mmol, 1.0 eq.) in a 50 mL dry seal tube, 10 mL saturated ammonia methanol solution was added. The reaction mixture was then heated to 80 °C and stirred for 48 h (monitored by TLC). Upon completion, the reaction mixture was concentrated and purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to obtain compounds **8c**, **8d**, (*S*)**9** and (*R*)**10**.

To a solution of compound (*S*)**9** or (*R*)**10** (0.41 mmol, 1.0 eq.), EDCI (0.62 mmol, 1.5 eq.), DMAP (0.21 mmol, 0.50 eq.) and TEA (0.82 mmol, 2.0 eq.) in 10 mL anhydrous dichloromethane protected with nitrogen and corresponding acid (0.82 mmol, 2.0 eq.) was slowly added under ice bath, and stirred for 1 h (monitored by TLC). 1 N dilute hydrochloric acid solution was added to the reaction mixture to quenching reaction. The solution was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. A crude solid that was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to obtain compounds **3**, **8a** and **8b**.

*4.1.2.1. (S)-(5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate (**3**).* Compound **3** was prepared from (*S*)**9**. Colorless oil, yield 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.43 (s, 1H), 9.40 (s, 1H), 8.37 (s, 1H), 7.41 (ddd, J = 20.4, 16.5, 7.0 Hz, 6H), 6.64 (s, 1H), 6.39 (s, 1H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.74, 169.76, 165.51, 149.58, 142.30, 137.13, 129.28, 129.21, 127.46, 118.58, 117.12, 72.85, 21.16. HRMS: $[M+H]^+$ calcd. For $C_{15}H_{15}N_2O_4$ *m/z*: 287.1026; found: 287.1023. [α] + 18 (*c* = 2 in $CHCl_3$).

*4.1.2.2. (S)-6-(hydroxy(phenyl)methyl)-4-oxo-1,4dihydropyridine-3-carboxamide((S)**9**).* Compound (*S*)**9** was prepared from **2**. White solid, yield 75%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.04 (s, 1H), 8.90 (s, 1H), 7.39–7.31 (m, 4H), 7.29 (d, J = 7.0 Hz, 1H), 6.80 (s, 1H), 5.69 (s, 1H), 3.98 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.64, 167.17, 157.54, 146.32, 142.58, 129.54, 129.02, 127.31, 116.74, 116.56, 72.90, 52.64. HRMS: $[M+H]^+$ calcd. For $C_{14}H_{14}NO_4$ *m/z*: 260.0917; found: 260.0913. [α] −27 (*c* = 0.1 in $CHCl_3$).

*4.1.2.3. (R)-6-(hydroxy(phenyl)methyl)-4-oxo-1,4dihydropyridine-3-carboxamide((R)**10**).* Compound (*R*)**10** was prepared from **7b**. Brown solid, yield 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H), 9.49 (s, 1H), 8.30 (s, 1H), 7.44 (d, J = 7.6 Hz, 2H), 7.38 (dd, J = 14.1, 6.6 Hz, 3H), 7.30 (t, J = 7.2 Hz, 1H), 6.60 (s, 1H), 6.35 (s, 1H), 5.66 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.43, 165.36, 153.37, 141.81, 141.48, 128.43, 127.88, 126.38, 117.79, 115.89, 70.76. HRMS: $[M+H]^+$ calcd. For $C_{13}H_{13}N_2O_3$ *m/z*: 245.0921; found: 245.0924. [α] − 27 (*c* = 0.1 in $CHCl_3$).

*4.1.2.4. (S)-(5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl)(phenyl)methyl 3-methylbutanoate (**8a**).* Compound **8a** was prepared from (*S*)**9** and 3-methylbutanoic acid. Colorless oil, yield 72%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.76 (s, 1H), 10.05 (d, J = 4.8 Hz, 1H), 8.52 (s, 1H), 7.40–7.33 (m, 5H), 6.79 (s, 1H), 6.47 (s, 1H), 5.90 (d, J = 4.9 Hz, 1H), 2.31 (d, J = 7.2 Hz, 2H), 2.15–2.07 (m, 1H), 0.91 (dd, J = 6.6, 3.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.58, 172.29, 167.07, 149.00, 142.69, 135.61, 129.61, 129.26, 127.43, 119.13, 117.91, 72.77, 43.30, 25.87, 22.49, 22.46. HRMS: $[M+H]^+$ calcd. For $C_{18}H_{21}N_2O_4$ *m/z*: 329.1496; found: 329.1491. [α] −43 (c = 0.1 in $CHCl_3$).

*4.1.2.5. (R)-(5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl)(phenyl)methyl acetate (**8b**).* Compound **8b** was prepared from (*S*)**10** and glacial acetic acid. Colorless oil, yield 86%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.86 (s, 1H), 10.03 (d, J = 4.5 Hz, 1H), 8.52 (s, 1H), 7.36 (s, 5H), 6.75 (s, 1H), 6.46 (s, 1H), 5.99 (d, J = 4.5 Hz, 1H), 2.16 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.56, 170.10, 167.15, 149.07, 142.76, 135.52, 129.66, 129.27, 127.50, 118.82, 117.85, 73.06, 21.09. HRMS: $[M+H]^+$ calcd. For $C_{15}H_{15}N_2O_4$ *m/z*: 287.1026; found: 287.1029. [α] −30 (c = 0.1 in $CHCl_3$).

*4.1.2.6. 4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxamide (**8c**).* Compound **8c** was prepared from **7c**. White solid, yield 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.42 (s, 1H), 9.55 (s, 1H), 8.42 (s, 1H), 7.78 (s, 2H), 7.55 (s, 5H), 6.76 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.51, 165.43, 148.23, 142.06, 132.46, 130.59, 129.13, 126.83, 117.76, 116.62. HRMS: $[M+H]^+$ calcd. For $C_{12}H_{11}N_2O_2$ *m/z*: 215.0815; found: 215.0817.

*4.1.2.7. 6-(tert-butyl)-4-oxo-1,4-dihydropyridine-3-carboxamide (**8d**).* Compound **8d** was prepared from **7d**: white solid, 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.73 (s, 1H), 9.52 (s, 1H), 8.27 (s, 1H), 7.39 (s, 1H), 6.26 (s, 1H), 1.26 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.80, 165.36, 158.40, 141.55, 116.80, 114.58, 34.44, 28.32. HRMS: $[M+H]^+$ calcd. For $C_{10}H_{15}N_2O_2$ *m/z*: 195.1128; found: 195.1123.

*4.1.3. Synthetic procedure of compound **1***

To a solution of compound **3** (0.10 mmol, 1.0 eq.), DMAP (0.05 mmol, 0.50 eq.) and TEA (0.20 mmol, 2.0 eq.) in10 mL anhydrous THF protected with nitrogen, isovaleryl chloride (0.30 mmol, 3.0 eq.) was slowly added at room temperature for 6 h (monitored by TLC). The solution was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. A crude solid that was purified by silica gel column chromatography using CH_2Cl_2 and MeOH as the eluent to obtain colorless oil compound **1**.

*4.1.3.1. (S)-(5-((3-methylbutanoyl) carbamoyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate (**1**).* Colorless oil, yield 66%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.09 (s, 1H), 8.48 (s, 1H), 7.45 (d, J = 7.3 Hz, 2H), 7.43–7.34 (m, 3H), 6.63 (s, 1H), 6.48 (s, 1H), 2.60 (dd, J = 6.9, 2.4 Hz, 2H), 2.42 (td, J = 8.2, 3.0 Hz, 1H), 2.18 (d, J = 2.4 Hz, 3H), 0.91 (dd, J = 6.7, 2.5 Hz, 7H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 177.59, 174.04, 169.76, 163.57, 152.31, 145.67, 137.41, 129.19, 129.17, 127.55, 116.99, 116.31, 73.61, 47.04, 24.93, 22.75, 21.18. HRMS: $[M+H]^+$ calcd. For $C_{20}H_{23}N_2O_5$ *m/z*: 371.1601; found: 371.1606. [α] + 40 (c = 0.5 in $CHCl_3$).

*4.1.4. General synthetic procedure of compounds **4**, **11** and **12***

The reaction of **3** and **8a–8d** (0.12 mmol, 1.0 eq.) and K_2CO_3 (0.24 mmol, 2.0 eq.) and bromoethanol (0.24 mmol, 2.0 eq.) in 5 mL anhydrous acetone was heated 60–80 °C and stirred for 12–48 h (monitored by TLC). The reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with brine, dried by Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether) to obtain compounds **4**, **11a-11d, 12a-12c**.

*4.1.4.1. (S)-(5-carbamoyl-4-(2-hydroxyethoxy) pyridin-2-yl) (phenyl) methyl acetate (**4**).* Compound **4** was prepared from **3**. Colorless oil, yield 53%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.72 (s, 1H), 7.60 (s, 1H), 7.46 (d, J = 7.3 Hz, 2H), 7.32 (tt, J = 14.6, 7.2 Hz, 4H), 6.66 (s, 1H), 5.16 (t, J = 5.3 Hz, 1H), 4.28 (t, J = 4.3 Hz, 2H), 3.77 (dd, J = 9.2, 4.9 Hz, 2H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.06, 165.09, 163.67, 163.61, 152.03, 139.01, 128.88, 128.58, 127.75, 118.27, 105.58, 77.63, 71.40, 59.41, 21.33. HRMS: $[M+H]^+$ calcd. For $C_{17}H_{19}N_2O_5$ *m/z*: 331.1288; found: 331.1285. [α] + 43 (c = 1 in $CHCl_3$).

*4.1.4.2. (S)-(5-carbamoyl-4-(2-hydroxyethoxy) pyridin-2-yl) (phenyl) methyl 3-methylbutanoate(**11a**).* Compound **11a** was prepared from **8a**. White solid, yield 51%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.75–7.71 (m, 1H), 7.59 (s, 1H), 7.47 (d, J = 7.4 Hz, 2H), 7.32 (dq, J = 14.4, 7.2 Hz, 4H), 6.70 (s, 1H), 5.09 (t, J = 4.8 Hz, 1H), 4.27–4.20 (m, 2H), 3.79 (d, J = 4.4 Hz, 2H), 2.37 (dd, J = 7.1, 3.3 Hz, 2H), 2.06 (tt, J = 13.5, 6.8 Hz, 1H), 0.91 (d, J = 6.7 Hz, 7H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.35, 164.64, 163.25, 163.19, 151.62, 138.62, 128.42, 128.12, 127.27, 117.84, 105.08, 77.01, 70.92, 59.00, 42.56, 25.27, 22.09. HRMS: $[M+H]^+$ calcd. For $C_{20}H_{25}N_2O_5$ *m/z*: 373.1758; found: 373.1754. [α] −8 (c = 0.1 in $CHCl_3$).

*4.1.4.3. (R)-(5-carbamoyl-4-(2-hydroxyethoxy) pyridin-2-yl) (phenyl) methyl acetate (**11b**).* Compound **11b** was prepared from **8b**. White solid, yield 50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (s, 1H), 7.74–7.71 (m, 1H), 7.59 (s, 1H), 7.46 (d, J = 7.4 Hz, 2H), 7.37–7.28 (m, 4H), 6.67 (s, 1H), 5.09 (t, J = 5.3 Hz, 1H), 4.28 (t, J = 4.6 Hz, 2H), 3.79 (dd, J = 9.3, 5.1 Hz, 2H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6)δ 169.60, 164.66, 163.24, 163.18, 151.62, 138.57, 128.43, 128.13, 127.30, 117.83, 105.13, 77.19, 70.96, 59.02, 20.87. HRMS: $[M+H]^+$ calcd. For $C_{17}H_{19}N_2O_5$ *m/z*: 331.1288; found: 331.1287. [α] −37 (c = 0.1 in $CHCl_3$).

*4.1.4.4. 4-(2-hydroxyethoxy)-6-phenylnicotinamide (**11c**).* Compound **11c** was prepared from **8c**. White solid, yield 16%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.17 (d, J = 6.9 Hz, 2H), 7.76 (s, 1H), 7.67 (d, J = 3.3 Hz, 1H), 7.66 (d, J = 3.2 Hz, 1H), 7.49 (dq, J = 14.0, 6.8 Hz, 3H), 5.14 (t, J = 5.3 Hz, 1H), 4.44–4.36 (m, 2H), 3.83 (dd, J = 9.4, 4.9 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.80, 163.50, 160.09, 152.19, 131.49, 129.72, 128.65, 127.12, 116.85, 104.79, 70.98, 59.11. HRMS: $[M+H]^+$ calcd. For $C_{14}H_{15}N_2O_3$ *m/z*: 259.1077; found: 259.1076.

*4.1.4.5. 6-(tert-butyl)-4-(2-hydroxyethoxy) nicotinamide (**11d**).* Compound **11d** was prepared from **8d**. White solid, yield 55%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (s, 1H), 7.66 (s, 1H), 7.58 (s, 1H), 7.09 (s, 1H), 5.10 (t, J = 5.2 Hz, 1H), 4.29–4.24 (m, 2H), 3.78 (dd, J = 8.8, 4.5 Hz, 2H), 1.31 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.36, 165.01, 162.96, 151.08, 115.80, 103.57, 70.60, 59.09, 37.71, 29.81. HRMS: $[M+H]^+$ calcd. For $C_{12}H_{19}N_2O_3$ *m/z*: 239.1390; found: 239.1397.

*4.1.4.6. (S)-(5-carbamoyl-1-(2-hydroxyethyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl3-methyl Butanoate(**12a**).* Compound **12a** was prepared from **8a**. Colorless oil, yield 31%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.87 (d, J = 4.7 Hz, 1H), 8.46 (s, 1H), 7.42–7.37 (m, 3H), 7.31 (dt, J = 5.9, 3.0 Hz, 2H), 6.93 (s, 1H), 6.78 (s, 1H), 5.93 (d, J = 4.3 Hz, 1H), 4.06–3.98 (m, 2H), 3.92–3.84 (m, 2H), 2.30 (d, J = 7.2 Hz, 2H), 2.10 (dt, J = 13.6, 6.8 Hz, 1H), 0.95–0.91 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.44, 171.98, 167.85, 149.66, 147.92, 135.08, 129.50, 128.97, 128.15, 120.24, 118.44, 71.51, 61.26, 55.77, 43.26, 25.81, 22.49, 22.44. HRMS: $[M+H]^+$ calcd. For $C_{20}H_{25}N_2O_5$ *m/z*: 373.1758; found: 373.1757. [α] + 7 (c = 0.1 in $CHCl_3$).

*4.1.4.7. (R)-(5-carbamoyl-1-(2-hydroxyethyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**12b**).* Compound **12b** was prepared from **8b**. Colorless oil, yield 20%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (d, J = 4.5 Hz, 1H), 8.47 (s, 1H), 7.49 (d, J = 4.5 Hz, 1H), 7.45–7.39 (m, 5H), 6.95 (s, 1H), 6.44 (s, 1H), 5.15 (t, J = 5.1 Hz, 1H), 4.08–4.01 (m, 2H), 3.61 (dt, J = 16.3, 5.5 Hz, 1H), 3.54–3.47 (m, 1H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.35, 169.27, 164.87, 149.55, 147.95, 135.68, 129.00, 128.64, 127.68, 119.18, 118.14, 71.04, 59.95, 54.92, 20.65. HRMS: $[M+H]^+$ calcd. For $C_{17}H_{19}N_2O_5$ *m/z*: 331.1288; found: 331.1286. [α] −68 (c = 0.1 in $CHCl_3$).

*4.1.4.8. 1-(2-hydroxyethyl)-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxamide(**12c**).* Compound **12c** was prepared from **8c**. Colorless oil, yield 33%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (d, J = 4.4 Hz, 1H), 8.57 (s, 1H), 7.54–7.51 (m, 3H), 7.51–7.47 (m, 3H), 6.26 (s, 1H), 4.98 (t, J = 5.2 Hz, 1H), 3.94 (t, J = 5.2 Hz, 2H), 3.40 (dd, J = 10.2, 5.1 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.21, 165.18, 151.83, 146.89, 131.67, 131.50, 129.65, 128.85, 128.71, 128.64, 121.36, 118.12, 59.58, 55.55. HRMS: $[M+H]^+$ calcd. For $C_{14}H_{15}N_2O_3$ *m/z*: 259.1077; found: 259.1078.

*4.1.5. General synthetic procedure of compounds **13** and **14***

The reaction of compound **4** (0.10 mmol, 1.0 eq.) and MeONa (0.15 mmol, 1.5 eq.) in 10 mL MeOH, was stirred reaction at room temperature for 4 h, monitoring by TLC. The reaction mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to obtain compound **13**.

The reaction of compound **4** (0.1 mmol, 1.0 eq.) and palladium carbon (6.60 mg, 20%) in 6 mL MeOH using H_2 gas as the hydrogen source, was put at room temperature and stirred for 2 h (monitored by TLC). The reaction solution was filtered with diatomite and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to obtain compounds **14**.

*4.1.5.1. (S)-6-(hydroxy(phenyl)methyl)-4-(2-hydroxyethoxy) nicotinamide (**13**).* White solid, yield 81%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.69 (s, 1H), 7.69 (s, 1H), 7.58 (s, 1H), 7.40 (t, J = 7.0 Hz, 2H), 7.35 (s, 1H), 7.29 (t, J = 7.5 Hz, 2H), 7.21 (t, J = 7.3 Hz, 1H), 6.19 (d, J = 4.3 Hz, 1H), 5.68 (d, J = 4.1 Hz, 1H), 5.09 (t, J = 5.4 Hz, 1H), 4.29–4.22 (m, 2H), 3.79 (dd, J = 9.6, 4.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.69, 164.83, 163.21, 151.29, 143.84, 128.04, 127.06, 126.67, 116.95, 103.89, 75.46, 70.72, 58.98. HRMS: $[M+H]^+$ calcd. For $C_{15}H_{17}N_2O_4$ *m/z*: 289.1183; found: 289.1186. [α] + 37 (c = 1.1 in $CHCl_3$).

*4.1.5.2. 6-benzyl-4-(2-hydroxyethoxy) nicotinamide (**14**).* White solid, yield 88%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 (s, 1H), 7.69 (s, 1H), 7.56 (s, 1H), 7.32–7.25 (m, 4H), 7.21–7.17 (m, 1H), 7.16 (s, 1H), 5.09 (t, J = 5.4 Hz, 1H), 4.23–4.18 (m, 2H), 4.06 (s, 2H), 3.77 (dd, J = 9.5, 5.1 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.12, 164.91, 162.97, 151.88, 139.38, 128.89, 128.37, 126.21, 116.30, 107.51, 70.72, 59.01, 43.80. HRMS: $[M+H]^+$ calcd. For $C_{15}H_{17}N_2O_3$ *m/z*: 273.1234; found: 273.1236.

*4.1.6. General synthetic procedure of compounds **15** and **16***

The reaction of compound **3** (0.12 mmol, 1.0 eq.) and K_2CO_3 (0.24 mmol, 2.0 eq.) and corresponding halides (0.24 mmol, 2.0 eq.) in 5 mL anhydrous acetone was heated 60–80 °C and stirred for 12–48 h (monitored by TLC). The reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether) to obtain a series of compounds **15** and **16**.

*4.1.6.1. (S)-(5-carbamoyl-4-(2-(dimethylamino) ethoxy) pyridin-2-yl) (phenyl)methyl acetate(**15f**).* Compound **15f** was prepared from compound **3** and 2-chloro-N,*N*-dimethylethan-1-amine. Colorless oil, yield 29%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (s, 1H), 7.63 (s, 1H), 7.48 (s, 1H), 7.30 (t, J = 8.2 Hz, 2H), 7.23–7.09 (m, 4H), 6.51 (d, J = 6.7 Hz, 1H), 4.16 (d, J = 3.1 Hz, 2H), 3.10 (s, 2H), 2.34 (s, 3H), 2.03 (d, J = 13.6 Hz, 8H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.59, 164.69, 163.03, 151.43, 138.59, 128.43, 128.14, 127.32, 118.04, 105.23, 77.17, 66.60, 57.07, 45.18, 20.89. HRMS: $[M+H]^+$ calcd. For $C_{19}H_{24}N_3O_4$ *m/z*: 358.1761; found: 358.1766. [α] + 15 (c = 1.2 in $CHCl_3$).

*4.1.6.2. (S)-(5-carbamoyl-1-(2-(dimethylamino) ethyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16f**).* Compound **16f** was prepared from compound **3** and 2-chloro-N,*N*-dimethylethan-1-amine. Colorless oil, yield 62%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.42 (s, 1H), 7.50 (s, 1H), 7.43 (d, J = 4.9 Hz, 6H), 6.87 (s, 1H), 6.49 (s, 1H), 4.16–4.06 (m, 1H), 3.98 (d, J = 14.2 Hz, 1H), 2.47–2.39 (m, 1H), 2.37–2.29 (m, 1H), 2.19 (s, 3H), 2.10 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.27, 169.44, 164.85, 149.29, 147.84, 135.44, 129.29, 129.08, 127.82, 118.98, 118.10, 71.15, 58.39, 50.56, 45.20, 20.65. HRMS: $[M+H]^+$ calcd. For $C_{19}H_{24}N_3O_4$ *m/z*: 358.1761; found: 358.1768. [α] −26 (c = 0.1 in $CHCl_3$).

*4.1.6.3. (S)-(5-carbamoyl-4-(2-(pyrrolidin-1-yl) ethoxy) pyridin-2-yl) (phenyl)methyl acetate(**15g**).* Compound **15g** was prepared from compound **3** and 1-(2-chloroethyl) pyrrolidine. White solid, yield 22%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.57 (s, 1H), 7.31 (s, 1H), 7.12 (d, J = 7.4 Hz, 2H), 7.04–6.93 (m, 4H), 6.33 (d, J = 7.5 Hz, 1H), 4.00 (s, 2H), 2.16 (d, J = 1.7 Hz, 6H), 1.84 (s, 3H), 1.53 (s, 1H), 1.33 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.60, 164.74, 163.06, 151.44, 138.60, 128.44, 128.14, 127.32, 118.23, 105.38, 77.17, 67.63, 53.48, 53.40, 23.10, 20.90. HRMS: $[M+H]^+$ calcd. For $C_{21}H_{26}N_3O_4$ *m/z*: 384.1918; found: 384.1911. [α] + 10 (c = 1.4 in $CHCl_3$).

*4.1.6.4. (S)-(5-carbamoyl-4-oxo-1-(2-(pyrrolidin-1-yl) ethyl)-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16g**).* Compound **16g** was prepared from compound **3** and 1-(2-chloroethyl) pyrrolidine. Colorless oil, yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (d, J = 3.6 Hz, 1H), 8.44 (s, 1H), 7.49 (d, J = 3.9 Hz, 1H), 7.43 (d, J = 8.2 Hz, 5H), 6.89 (s, 1H), 6.47 (s, 1H), 4.10 (dd, J = 13.9, 7.0 Hz, 1H), 4.05–3.95 (m, 1H), 2.63 (dt, J = 12.7, 6.2 Hz, 1H), 2.54 (d, J = 6.2 Hz, 1H), 2.40 (d, J = 17.0 Hz, 4H), 2.19 (s, 3H), 1.63 (s, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.26, 169.40, 164.80, 149.29, 147.75, 135.51, 129.26, 129.06, 127.76, 119.02, 118.16, 71.15, 54.84, 53.58, 51.59, 23.20, 20.65. HRMS: $[M+H]^+$ calcd. For $C_{21}H_{26}N_3O_4$ *m/z*: 384.1918; found: 384.1914. [α] −46 (c = 0.1 in $CHCl_3$).

*4.1.6.5. (S)-(4-(allyloxy)-5-carbamoylpyridin-2-yl) (phenyl)methyl acetate(**15h**).* Compound **15h** was prepared from compound **3** and 3-bromoprop-1-ene. White solid, yield 33%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.66 (s, 1H), 7.57 (s, 1H), 7.47–7.43 (m, 2H), 7.37–7.32 (m, 2H), 7.32–7.28 (m, 1H), 7.26 (s, 1H), 6.65 (s, 1H), 6.06 (ddt, J = 17.2, 10.6, 5.4 Hz, 1H), 5.45 (dd, J = 17.3, 1.6 Hz, 1H), 5.32 (dd, J = 10.6, 1.4 Hz, 1H), 4.84 (d, J = 5.3 Hz, 2H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.57, 165.03, 162.70, 162.38, 150.74, 138.58, 132.43, 128.44, 128.15, 127.30, 119.09, 118.74, 105.05, 77.22, 69.11, 20.87. HRMS: $[M+H]^+$ calcd. For $C_{18}H_{19}N_2O_4$ *m/z*: 327.1339; found: 327.1331. [α] + 27 (c = 1.0 in $CHCl_3$).

*4.1.6.6. (S)-(1-allyl-5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16h**).* Compound **16h** was prepared from compound **3** and 3-bromoprop-1-ene. Colorless oil, yield 50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 (d, J = 4.4 Hz, 1H), 8.44 (s, 1H), 7.56 (d, J = 4.4 Hz, 1H), 7.48–7.37 (m, 5H), 6.84 (s, 1H), 6.48 (s, 1H), 5.80 (ddd, J = 15.5, 10.4, 5.1 Hz, 1H), 5.22 (d, J = 10.5 Hz, 1H), 5.02 (d, J = 17.2 Hz, 1H),

4.72 (dd, J = 16.8, 4.9 Hz, 1H), 4.64–4.52 (m, 1H), 2.17 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.34, 169.26, 164.66, 149.40, 147.24, 135.51, 132.85, 129.23, 129.07, 127.62, 119.54, 118.90, 118.20, 71.07, 54.62, 20.66. HRMS: $[M+H]^+$ calcd. For $C_{18}H_{19}N_2O_4$ *m/z*: 327.1339; found: 327.1336. [α] + 66 (c = 0.2 in $CHCl_3$).

*4.1.6.7. (S)-(4-(2-(1,3-dioxolan-2-yl) ethoxy)-5carbamoylpyridin-2-yl) (phenyl)methyl acetate(**15i**).* Compound **15i** was prepared from compound **3** and 2-(2-chloroethyl)-1,3-dioxolane. Colorless oil, yield 52%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.95 (s, 1H), 7.41 (d, J = 7.3 Hz, 2H), 7.36–7.27 (m, 3H), 6.97 (s, 1H), 6.82 (s, 1H), 6.06 (s, 1H), 5.04 (t, J = 3.9 Hz, 1H), 4.30 (ddd, J = 9.5, 6.9, 2.9 Hz, 2H), 3.97 (dd, J = 8.7, 5.2 Hz, 2H), 3.87 (dd, J = 8.7, 5.1 Hz, 2H), 2.27 (dd, J = 9.7, 5.2 Hz, 2H), 2.20 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.90, 165.38, 164.21, 163.49, 154.24, 138.35, 128.75, 128.55, 127.44, 115.98, 104.01, 102.44, 77.79, 65.07, 64.29, 32.57, 21.31. HRMS: $[M+H]^+$ calcd. For $C_{20}H_{23}N_2O_6$ *m/z*: 387.1551; found: 387.1553. [α] + 3 (c = 0.1 in $CHCl_3$).

*4.1.6.8. (S)-(1-(2-(1,3-dioxolan-2-yl) ethyl)-5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16i**).* Compound **16i** was prepared from compound **3** and 2-(2-chloroethyl)-1,3-dioxolane. Colorless oil, yield 28%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (d, J = 4.2 Hz, 1H), 8.45 (s, 1H), 7.43–7.39 (m, 3H), 7.34 (dd, J = 6.5, 3.1 Hz, 3H), 6.82 (s, 1H), 6.81 (s, 1H), 5.98 (d, J = 4.2 Hz, 1H), 4.90 (t, J = 3.5 Hz, 1H), 4.04–3.93 (m, 4H), 3.93–3.82 (m, 4H), 2.17 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.44, 169.68, 166.15, 149.36, 147.21, 134.84, 130.04, 129.50, 128.40, 119.92, 119.18, 101.16, 71.41, 65.33, 65.28, 48.58, 33.85, 21.07. HRMS: $[M+H]^+$ calcd. For $C_{20}H_{23}N_2O_6$ *m/z*: 387.1551; found: 387.1555. [α] + 35 (c = 0.1 in $CHCl_3$).

*4.1.6.9. (S)-(4-((3,5-bis(trifluoromethyl)benzyl) oxy)-5-carbamoylpyridin-2-yl) (phenyl)methyl acetate(**15j**).* Compound **15j** was prepared from compound **3** and 1-(bromomethyl)-3,5-bis(trifluoromethyl)benzene. Yellow solid, yield 42%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.27 (s, 2H), 8.11 (s, 1H), 7.72 (d, J = 5.9 Hz, 1H), 7.72–7.67 (m, 1H), 7.41 (d, J = 7.1 Hz, 2H), 7.35–7.27 (m, 4H), 6.66 (s, 1H), 5.57 (s, 2H), 2.16 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.48, 165.20, 162.74, 161.95, 150.43, 139.51, 138.51, 131.70, 131.48, 130.54, 130.28, 128.64, 128.46, 128.44, 128.39, 128.15, 127.18, 120.02, 104.90, 77.23, 68.21, 20.77. HRMS: $[M+H]^+$ calcd. For $C_{24}H_{19}F_6N_2O_4$ *m/z*: 513.1244; found: 513.1242. [α] + 17 (c = 0.8 in $CHCl_3$).

*4.1.6.10. (S)-(1-(3,5-bis(trifluoromethyl)benzyl)-5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16j**).* Compound **16j** was prepared from compound **3** and 1-(bromomethyl)-3,5-bis(trifluoromethyl)benzene. Colorless oil, yield 15%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (d, J = 4.5 Hz, 1H), 8.59 (s, 1H), 7.98 (s, 1H), 7.63–7.57 (m, 3H), 7.30 (ddd, J = 13.1, 6.1, 3.0 Hz, 6H), 6.83 (s, 1H), 6.63 (s, 1H), 5.65 (d, J = 17.2 Hz, 1H), 5.54 (d, J = 17.2 Hz, 1H), 2.03 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.44, 169.20, 164.43, 149.75, 147.81, 139.49, 134.76, 131.69, 131.49, 130.53, 130.26, 129.06, 128.75, 128.64, 127.80, 127.12, 119.46, 119.35, 71.29, 54.38, 20.33. HRMS: $[M+H]^+$ calcd. For $C_{24}H_{19}F_6N_2O_4$ *m/z*: 513.1244; found: 513.1240. [α] + 22 (c = 0.1 in $CHCl_3$).

*4.1.6.11. Ethyl (S)-2-((2-(acetoxy(phenyl)methyl)-5-carbamoylpyridin-4-yl) oxy) acetate (**15k**).* Compound **15k** was prepared from compound **3** and ethyl 2-bromoacetate. Colorless oil, yield 48%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (s, 1H), 7.78 (d, J = 14.6 Hz, 2H), 7.45 (d, J = 7.2 Hz, 2H), 7.32 (dt, J = 23.7, 7.2 Hz, 3H), 7.25 (s, 1H), 6.65 (s, 1H), 5.11 (s, 2H), 4.23–4.18 (m, 2H), 2.17 (s, 3H), 1.22 (t, J = 7.1 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.86, 167.09, 164.83, 164.58, 162.23, 154.62, 138.13, 128.84, 128.68, 127.43, 116.27, 104.38, 77.65, 65.46, 62.46, 21.32, 14.23. HRMS: $[M+H]^+$ calcd. For $C_{19}H_{21}N_2O_6$ *m/z*: 373.1394; found: 373.1398. [α] −12 (c = 0.1 in $CHCl_3$).

*4.1.6.12. Ethyl (S)-2-(2-(acetoxy(phenyl)methyl)-5-carbamoyl-4-oxopyridin-1(4H)-yl) acetate (**16k**).* Compound **16k** was prepared from compound **3** and ethyl 2-bromoacetate. Colorless oil, yield 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (d, J = 4.5 Hz, 1H), 8.51 (s, 1H), 7.55 (d, J = 4.5 Hz, 1H), 7.45–7.37 (m, 3H), 7.37–7.33 (m, 2H), 6.91 (s, 1H), 6.49 (s, 1H), 5.00 (q, J = 18.1 Hz, 2H), 3.94 (qd, J = 7.0, 1.3 Hz, 2H), 2.14 (s, 3H), 1.11 (t, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.58 (s), 168.96 (s), 167.28 (s), 164.57 (s), 149.07 (s), 148.66 (s), 134.97 (s), 128.94 (d, J = 17.0 Hz), 127.19 (s), 120.27 (s), 118.53 (s), 71.54 (s), 61.50 (s), 53.27 (s), 20.47 (s), 13.81 (s). HRMS: $[M+H]^+$ calcd. For $C_{19}H_{21}N_2O_6$ *m/z*: 373.1394; found: 373.1390. [α] + 17 (c = 0.1 in $CHCl_3$).

*4.1.6.13. (S)-(5-carbamoyl-4-(2-methoxyethoxy) pyridin-2-yl) (phenyl) methyl acetate(**15l**).* Compound **15l** was prepared from compound **3** and 1-bromo-2-methoxyethane. White solid, yield 46%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.68 (s, 1H), 7.70 (s, 1H), 7.52 (s, 1H), 7.46 (d, J = 7.4 Hz, 2H), 7.32 (dq, J = 14.5, 7.2 Hz, 4H), 6.67 (s, 1H), 4.38 (dd, J = 5.1, 3.3 Hz, 2H), 3.78–3.70 (m, 2H), 3.31 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.59, 164.72, 163.07, 162.95, 151.27, 138.56, 128.44, 128.14, 127.32, 118.13, 105.02, 77.20, 69.75, 68.05, 58.21, 20.88. HRMS: $[M+H]^+$ calcd. For $C_{18}H_{21}N_2O_5$ *m/z*: 345.1445; found: 345.1441. [α] + 31 (c = 0.2 in $CHCl_3$).

*4.1.6.14. (S)-(5-carbamoyl-1-(2-methoxyethyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16l**).* Compound **16l** was prepared from compound **3** and 1-bromo-2-methoxyethane. Colorless oil, yield 19%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 (d, J = 4.4 Hz, 1H), 8.45 (s, 1H), 7.52 (d, J = 4.4 Hz, 1H), 7.43 (dt, J = 11.6, 6.9 Hz, 5H), 6.94 (s, 1H), 6.44 (s, 1H), 4.22 (ddd, J = 14.3, 7.1, 3.4 Hz, 1H), 4.17–4.09 (m, 1H), 3.54 (ddd, J = 10.5, 7.2, 3.1 Hz, 1H), 3.48–3.38 (m, 1H), 3.20 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.32, 169.35, 164.76, 149.62, 147.85, 135.60, 129.22, 129.04, 127.68, 119.19, 118.34, 71.03, 70.52, 58.40, 52.37, 21.05. HRMS: $[M+H]^+$ calcd. For $C_{18}H_{21}N_2O_5$ *m/z*: 345.1445; found: 345.1448. [α] + 104 (c = 0.2 in $CHCl_3$).

*4.1.6.15. (S)-(4-(benzyloxy)-5-carbamoylpyridin-2-yl)(phenyl)methyl acetate(**15m**).* Compound **15m** was prepared from compound **3** and (bromomethyl)benzene. White solid, yield 56%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (s, 1H), 7.65 (s, 1H), 7.59 (s, 1H), 7.51 (d, J = 7.1 Hz, 2H), 7.44–7.38 (m, 4H), 7.38–7.27 (m, 5H), 6.63 (s, 1H), 5.39 (s, 2H), 2.15 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.50, 165.01, 162.74, 162.44, 150.70, 138.49, 135.70, 128.58, 128.42, 128.22, 128.13, 127.87, 127.27, 119.22, 105.18, 77.22, 70.02, 20.84. HRMS: $[M+H]^+$ calcd. For $C_{22}H_{21}N_2O_4$ *m/z*: 377.1496; found: 377.1493. [α] + 31 (c = 0.5 in $CHCl_3$).

*4.1.6.16. (S)-(1-benzyl-5-carbamoyl-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate(**16m**).* Compound **16m** was prepared from compound **3** and (bromomethyl)benzene. Colorless oil, yield 53%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (d, J = 4.5 Hz, 1H), 8.53 (s, 1H), 7.58 (d, J = 4.5 Hz, 1H), 7.39 (ddd, J = 6.2, 4.6, 2.0 Hz, 3H), 7.36 (d, J = 7.6 Hz, 2H), 7.32 (dd, J = 5.5, 2.2 Hz, 3H), 7.03 (d, J = 7.3 Hz, 2H), 6.85 (s, 1H), 6.46 (s, 1H), 5.40–5.26 (m, 2H), 1.94 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.34, 169.14, 164.59, 149.68, 147.74, 136.02, 135.49, 129.10, 128.99, 128.93, 127.94, 127.31, 126.35, 120.27, 118.95, 71.11, 55.40, 20.24. HRMS: $[M+H]^+$ calcd. For $C_{22}H_{21}N_2O_4$ *m/z*: 377.1496; found: 377.1491. [α] + 43 (c = 0.1 in $CHCl_3$).

*4.1.6.17. (S)-(4-(2-bromoethoxy)-5-carbamoylpyridin-2-yl) (phenyl)methyl acetate(**15n**).* Compound **15n** was prepared from compound **3** and 1,2-dibromoethane. White solid, yield 55%. ^{1}H NMR (500 MHz, DMSO-d_6) δ

8.71 (s, 1H), 7.80 (s, 1H), 7.51–7.44 (m, 3H), 7.35 (t, J = 7.3 Hz, 2H), 7.32–7.26 (m, 2H), 6.67 (s, 1H), 4.62–4.58 (m, 2H), 3.96–3.91 (m, 2H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.60, 164.45, 163.22, 162.28, 151.53, 138.49, 128.43, 128.16, 127.35, 117.87, 104.90, 77.15, 68.67, 30.90, 20.90. HRMS: $[M+H]^+$ calcd. For $C_{17}H_{18}BrN_2O_4$ *m/z*: 393.0444; found: 393.0448. [α] + 17 (c = 1.0 in $CHCl_3$).

*4.1.6.18. (S)-(5-carbamoyl-4-(2-thiomorpholinoethoxy) pyridin-2-yl) (phenyl)methyl acetate(**15o**).* The reaction of compound **15n** (0.12 mmol, 1.0 eq.) and potassium carbonate (0.24 mmol, 2.0 eq.) and thiomorpholine (0.36 mmol, 3.0 eq.) in 5 mL anhydrous acetone was heated 60 °C to reflux and stirred for 12 h (monitored by TLC). The reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether) to obtain compound **15o**, colorless oil, yield 67%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.02 (s, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.31 (ddd, J = 10.9, 9.7, 5.7 Hz, 3H), 6.95 (s, 1H), 6.82 (s, 1H), 6.08 (s, 1H), 4.24 (dd, J = 9.4, 4.9 Hz, 2H), 2.83 (t, J = 5.4 Hz, 2H), 2.81–2.75 (m, 4H), 2.67–2.60 (m, 4H), 2.21 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.94, 165.45, 164.27, 163.49, 154.21, 138.26, 128.80, 128.61, 127.44, 116.34, 104.81, 77.76, 65.22, 57.09, 55.01, 28.04, 21.34. HRMS: $[M+H]^+$ calcd. For $C_{21}H_{26}N_3O_4S$ *m/z*: 416.1639; found: 416.1637. [α] −2 (c = 0.2 in $CHCl_3$).

*4.1.6.19. (S)-(5-carbamoyl-4-(2-morpholinoethoxy) pyridin-2-yl) (phenyl) methyl acetate(**15p**).* The synthetic method was similar to that of compound **15o**, and starting from morpholine. Colorless oil, yield 95%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.11 (s, 1H), 7.41 (d, J = 7.2 Hz, 2H), 7.36–7.26 (m, 3H), 6.96 (s, 1H), 6.82 (s, 1H), 6.19 (s, 1H), 4.32–4.20 (m, 2H), 3.70–3.62 (m, 4H), 2.81 (t, J = 5.4 Hz, 2H), 2.51 (s, 4H), 2.20 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.91, 165.50, 164.23, 163.50, 154.14, 138.25, 128.77, 128.58, 127.41, 116.39, 104.81, 77.73, 66.91, 65.05, 56.73, 53.42, 21.31. HRMS: $[M+H]^+$ calcd. For $C_{21}H_{26}N_3O_5$ *m/z*: 400.1867; found: 400.1869. [α] −21 (c = 0.1 in $CHCl_3$).

*4.1.6.20. (S)-(5-carbamoyl-4-oxo-1-(2-(piperidin-1-yl) ethyl)-1,4-dihydrop yridin-2-yl) (phenyl)methyl acetate(**16q**).* Compound **16q** was prepared from 1-(2-chloroethyl) piperidine. Colorless oil, yield 73%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (d, J = 4.7 Hz, 1H), 8.41 (s, 1H), 7.46–7.42 (m, 6H), 6.87 (s, 1H), 6.48 (s, 1H), 2.35–2.30 (m, 3H), 2.26 (d, J = 5.1 Hz, 2H), 2.18 (s, 3H), 1.98 (s, 2H), 1.91 (s, 8H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.37, 169.45, 164.91, 149.24, 148.54, 135.49, 129.31, 129.06, 127.91, 118.64, 117.57, 71.22, 59.75, 57.33, 53.87, 25.41, 21.05, 20.65. HRMS: $[M+H]^+$ calcd. For $C_{22}H_{28}N_3O_4$ *m/z*: 398.2074; found: 398.2077. [α] + 55 (c = 0.3 in $CHCl_3$).

*4.1.6.21. (S)-(5-carbamoyl-1-(4-methoxybenzyl)-4-oxo-1,4-dihydropyridin-2-yl)(phenyl)methyl acetate(**16r**).* Compound **16r** was prepared from 1-(bromomethyl)-4-methoxybenzene. Colorless oil, yield 84%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.36 (d, J = 4.6 Hz, 1H), 8.49 (s, 1H), 7.57 (d, J = 4.6 Hz, 1H), 7.45–7.39 (m, 3H), 7.38–7.32 (m, 2H), 7.01 (d, J = 8.7 Hz, 2H), 6.94–6.90 (m, 2H), 6.89 (s, 1H), 6.47 (s, 1H), 5.22 (q, J = 15.9 Hz, 2H), 3.73 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 176.32, 169.22, 164.63, 159.05, 149.67, 147.32, 135.54, 129.14, 129.03, 128.27, 127.55, 127.44, 119.95, 118.90, 114.37, 71.12, 55.17, 54.91, 20.40. HRMS: $[M+H]^+$ calcd. For $C_{23}H_{23}N_2O_5$ *m/z*: 407.1601; found: 407.1609. [α] + 48 (c = 0.3 in $CHCl_3$)

*4.1.7. Synthetic of compounds **17**–**19***

To compound **2** (0.33 mmol, 1.0 eq.) in a 25 mL dry seal tube, 10 mL of 2 M dimethylamine methanol solution was added. The reaction mixture was then heated to 80 °C and stirred for 48 h (monitored by TLC). Upon completion, the reaction was concentrated under reduced pressure and purified by column chromatography on silica gel (CH_2Cl_2/MeOH) to obtain compound **17**.

To a solution of compound **17** (0.10 mmol, 1.0 eq.), DMAP (0.05 mmol, 0.5 eq.) and TEA (0.20 mmol, 2.0 eq.) in 5 mL anhydrous dichloromethane protected with nitrogen, and corresponding acid (0.20 mmol, 2.0 eq.) was slowly added under ice bath and stirred for 1 h (monitored by TLC). 1 N dilute hydrochloric acid solution was added to the reaction mixture to quenching reaction. The solution was then extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was washed with brine, dried (Na_2SO_4), and concentrated. A crude solid that was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to obtain compound **18**.

The reaction of compound **18** (0.15 mmol, 1.0 eq.) and K_2CO_3 (0.30 mmol, 2.0 eq.) and corresponding halides (0.30 mmol, 2.0 eq.) in 5 mL anhydrous acetone was heated 60–80 °C and stirred for 12 h (monitored by TLC). The reaction mixture was extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with brine, dried by Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether) to obtain compound **19**.

*4.1.7.1. (S)-6-(hydroxy(phenyl)methyl)-N,N-dimethyl-4-oxo-1,4-dihydropyridine-3-carboxamide (**17**).* Yellow solid, yield 37%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 7.61 (s, 1H), 7.44 (d, J = 7.4 Hz, 2H), 7.35 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 6.9 Hz, 1H), 6.48 (s, 1H), 6.16 (s, 1H), 5.58 (s, 1H), 2.84 (d, J = 48.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.8, 167.2, 153.3, 142.6, 137.4, 128.8, 128.3, 126.9, 114.7, 71.5, 38.0, 34.8. HRMS: $[M+H]^+$ calcd. For $C_{15}H_{17}N_2O_3$ *m/z*: 273.1234; found: 273.1237. [α] −5 (c = 0.2 in $CHCl_3$).

*4.1.7.2. (S)-(5-(dimethylcarbamoyl)-4-oxo-1,4-dihydropyridin-2-yl) (phenyl)methyl acetate (**18**).* Colorless oil, yield 65%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.40–7.36 (m, 2H), 7.35–7.27 (m, 3H), 6.67 (s, 2H), 3.05 (s, 6H), 2.14 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.1, 178.2, 149.4, 139.6, 139.5, 138.3, 137.7, 50.8, 50.6, 50.4, 31.1, 11.6. HRMS: $[M+H]^+$ calcd. For $C_{17}H_{19}N_2O_4$ *m/z*: 315.1339; found: 315.1333. [α] –22 (c = 0.1 in $CHCl_3$).

*4.1.7.3. (S)-(5-(dimethylcarbamoyl)-4-(2-hydroxyethoxy)pyridin-2-yl) (phenyl)methyl acetate(**19**).* Colorless oil, yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.47 (d, J = 7.3 Hz, 2H), 7.38–7.29 (m, 3H), 7.28 (s, 1H), 6.68 (s, 1H), 4.92 (t, J = 5.2 Hz, 1H), 4.20 (t, J = 4.8 Hz, 2H), 3.72(dd, J = 9.7, 5.0 Hz, 2H), 2.96 (s, 3H), 2.78 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.61, 165.52, 161.42, 161.24, 148.17, 138.81, 128.44, 128.09, 127.27, 121.68, 104.63, 77.28, 70.25, 59.22, 37.60, 34.24, 20.91. HRMS: $[M+H]^+$ calcd. For $C_{19}H_{23}N_2O_5$ *m/z*: 359.1601; found: 359.1607. [α] + 10 (c = 0.4 in $CHCl_3$).

4.2. Biological assays

4.2.1. HepG2 cell culture and treatments

The HepG2 cells were obtained from ATCC and grown in Dulbecco Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and antibiotics (100 μg/L penicillin, 100 μg/L streptomycin) and maintained at 37 °C in a humidified atmosphere of 95% air − 5% carbon dioxide. Culture medium was changed every 2 days, and the number of viable cells was determined using the MTT method and approximately 2.5 × 10^4 cells were plated in each well. Control cells received only with the solvent (DMSO) and the OA cells treated only with 100 μM oleic acid (Sigma, USA) (OA-treated cells). In parallel, the experiment cells were treated with or without different target compounds (10 μM) dissolved in DMSO and oleic acid.[23,24] Simvastatin was used as a positive control drug (10 μM). A single experiment was repeated six times to calculate the standard deviation.

4.2.2. Oil red O staining

For quantification, The HepG2 cells were fixed with 10% neutral formalin for 1 h at room temperature, washed with phosphate-buffered saline and then stained for 1 h with 0.5% oil red O in 60% isopropanol. After washing with distilled water, the stained cells were observed under a microscope (*N*-MSI-vectra).[24] The lipid droplets in cells were stained with oil red O for 30 min and then washed with 70% ethanol for quantification by measuring its absorbance at 358 nm.

4.2.3. Cell viability assay

The cytotoxic effect was reported as percentage of viable cells after exposure to myclobutanil with respect to vehicle-treated cells.[25] HepG2 cells (human hepatoma cell line), (seeded at a density of 1.2×10^4 cells per well, 100 µL per well) were grown in 96-well plates for 24 h and then treated with serial dilutions of compounds **4**, **15k**, **15o** and DMSO as a control, in DMEM supplemented with 10% FBS. A stock solution of each complex was prepared in DMSO and filtered with Minisart filters (0.22 µm) and the final concentration of the compounds were 10 µM. After incubation at 37 °C for 48 h, 5 mg/mL of MTT in PBS was added into each well and incubated at 37 °C for further 4 h. Successively, a solubilizing solution (10% SDS, 0.01 N HCl) was added to dissolve the formazan salt and lyse the cells and incubated 5% CO_2, 95% air at 37 °C. Finally, absorbance was read at the wavelength of 490 nm using an ELISA microplate reader. Values obtained from the wells treated with only DMSO were set as 100% of viable cells.[26]

4.2.4. Triglycerides and total cholesterol contents assays

HepG2 cells were inoculated in 12-well plates at the density of 2×10^5 cells/well at 37 °C. After 24 h, the cells were treated with 100 µmol/L oleic acid (OA) and different doses of **15o** for 24 h. After rinsing twice with PBS, the cells were treated with lysis buffer (Beyotime Biotechnology, China) for 20 min on ice to lyse the cells. Triglycerides (TG) and total cholesterol (TC) contents in cell lysates were detected by assay kits, E1013 and E1015 (Applygen Technologies Inc., Beijing, China).[27]

4.3. Molecular docking

Molecular docking was performed using MOE employing AMBER12: EHT forcefield.[28], 11β-hydroxysteroid dehydrogenase type 1 enzyme was screened as the potential target by the computational reverse docking method. The crystal structure of 11β-HSD1 (PDB code:3TFQ) was obtained from the Protein Data Bank (http://www.rcsb.org) for the docking studies. Compounds were minimized. The binding sites were identified through site-finder tool of MOE and mutagenesis data validation. Both the ligand and the protein were protonated at physiological pH prior to docking. Prior to docking the probable binding site of the target protein were automatically searched using the 'binding site finding' module in MOE. Then induced fit docking approach was applied for consideration of the flexibility of the side chains of the residues at the predicted binding sites. The produced conformation of with the best score was selected for the analysis. The docking pose for each ligand was analyzed by using PyMOL.

4.4. Statistical analysis

All data were presented as mean ± SD. Differences between variants were analyzed by a Student's *t*-test (Graph Pad Prism 5) for unpaired data. Data of cell experiments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's (SPSS 19.0). Values of $p < 0.05$ were considered statistically significant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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Appendix A. Supplementary material

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