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PII: S0223-5234(19)31128-6

DOI: https://doi.org/10.1016/j.ejmech.2019.111976

Reference: EJMECH 111976

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 9 September 2019

Revised Date: 6 November 2019

Accepted Date: 16 December 2019

Please cite this article as: J. Wang, X. Lv, J. Xu, X. Liu, T. Du, G. Sun, J. Chen, X. Shen, J. Wang, L. Hu, Design, synthesis and biological evaluation of vincamine derivatives as potential pancreatic β-cells protective agents for the treatment of type 2 diabetes mellitus, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2019.111976.

Graphical Abstract

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Design, synthesis and biological evaluation of vincamine derivatives as potential pancreatic β-cells protective agents for the treatment of type 2 diabetes mellitus

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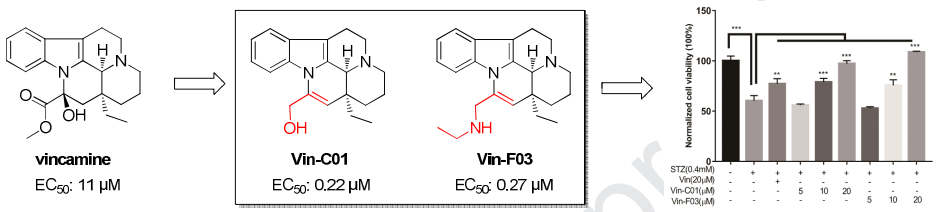

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European Journal of Medicinal Chemistry

Journal homepage: www.elsevier.com

Design, synthesis and biological evaluation of vincamine derivatives as potential pancreatic β-cells protective agents for the treatment of type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Type 2 diabetes mellitus
Pancreatic β-cells protective agents
Vincamine derivatives
Structural modifications
Structure-activity relationships

ABSTRACT

A series of vincamine derivatives were designed, synthesized and evaluated as pancreatic β-cells protective agents for type 2 diabetes mellitus. Most of the compounds displayed potent pancreatic β-cells protective activities and five derivatives were found to exhibit 20~50-fold higher activities than vincamine. Especially for compounds **Vin-C01** and **Vin-F03**, exhibited a remarkable EC_{50} value of 0.22 μM and 0.27 μM, respectively. Their pancreatic β-cells protective activities increased approximately 2 times than vincamine. In cell viability assay, compounds **Vin-C01** and **Vin-F03** could effectively promote β-cell survival and protect β-cells from STZ-induced apoptosis. Further cellular mechanism of action studies demonstrated that their potent β-cells protective activities were achieved by regulating IRS2/PI3K/Akt signaling pathway. The present study evidently showed that compounds **Vin-C01** and **Vin-F03** were two more potent pancreatic β-cells protective agents compared to vincamine and might serve as promising lead candidates for the treatment of type 2 diabetes mellitus.

1. Introduction

Diabetes is one of the largest global health emergencies affecting over 300 million people worldwide [1]. More than 90% of the cases are diagnosed as type 2 diabetes mellitus (T2DM), which causes significant morbidity and mortality. Although many antihyperglycemic agents have been approved for the treatment of T2DM, such as biguanides, sulfonylureas, thiazolidinediones, meglitinides, α-glucosidase inhibitors and insulin, they usually have limitations and undesirable side effects [2]. Thus, T2DM remains a significant unmet medical need.

T2DM is mainly characterized by insulin resistance and dysfunction of pancreatic β-cells [3]. Normally, β-cells can increase insulin release to maintain normal glucose tolerance, once β-cells fail to compensate for the decreased insulin sensitivity, T2DM may occur [4]. Thus, β-cells dysfunction plays a crucial role in the progression of T2DM. Pancreatic β-cells dysfunction includes decreased β-cells mass and impaired β-cells function [5]. The mass of β-cells is mainly regulated by apoptosis, size modification, replication and neogenesis. Among which β-cell apoptosis determines the onset and rate of T2DM progression [6]. A series of genetic alterations including G protein-coupled receptors, Akt, IRS2, PTEN, Fas/FasL, NF-κb, Bcl2 family and caspase family have been proved to be associated with β-cells apoptosis [7,8]. Therefore, the successful protection of pancreatic β-cells from apoptosis may potentially prevent T2DM [9].

Clinically, several anti-diabetic drugs have been used for improving β-cells dysfunction, such as sulfonylurea derivatives (SUs), DPP4 inhibitors and incretin hormone GLP-1 analogs [10-13]. However, none of them can successfully control long-term microvascular and macrovascular complications, many patients still remain unable to safely achieve and maintain tight glycemic control [14]. Natural products are important sources of lead compounds in drug development, the discovery of efficient β-

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cells protectors from natural resources attracts much attention. Recently, many medicinal herbs and their extracts have been applied in the treatment of diabetes and lots of active compounds have been determined, such as curcumin and berberine [15-19].

In our previous work [20], the preliminary high throughput screening (HTS) leaded to the discovery of a small molecule compound vincamine [21] which exhibited good protective activity against STZ-induced apoptosis in pancreatic β-cells. In vivo pharmacodynamic evaluation indicated that vincamine showed significant effects on lowering the level of fasting blood glucose, improving oral glucose tolerance, and decreasing the level of HbA1c and LDL in serum, restoring the histology structure of damaged islet of pancreas and increasing positive regions of insulin on the models of STZ/HFD-induced type 2 diabetic mice and *db/db* type 2 diabetic mice. Based on its in vitro and vitro efficacy, safety and simple structures, vincamine could be identified as the lead structure for further structure-activity relationship (SAR) research and the discovery of more potent β-cells protective agents for the treatment of type 2 diabetes mellitus.

2. Results and discussion

2.1. Design of novel pancreatic β-cells protective agents

Vincamine was obtained through high throughput screening and the structure-activity relationship was not clear. In this paper, we focus on the structural modifications of vincamine to find more potent pancreatic β-cells protective agents. Based on the comprehensive understanding of vincamine chemistry, we conducted systematic structural modifications at its C-14 position, designing and synthesizing 51 derivatives of various types (Fig. 1).

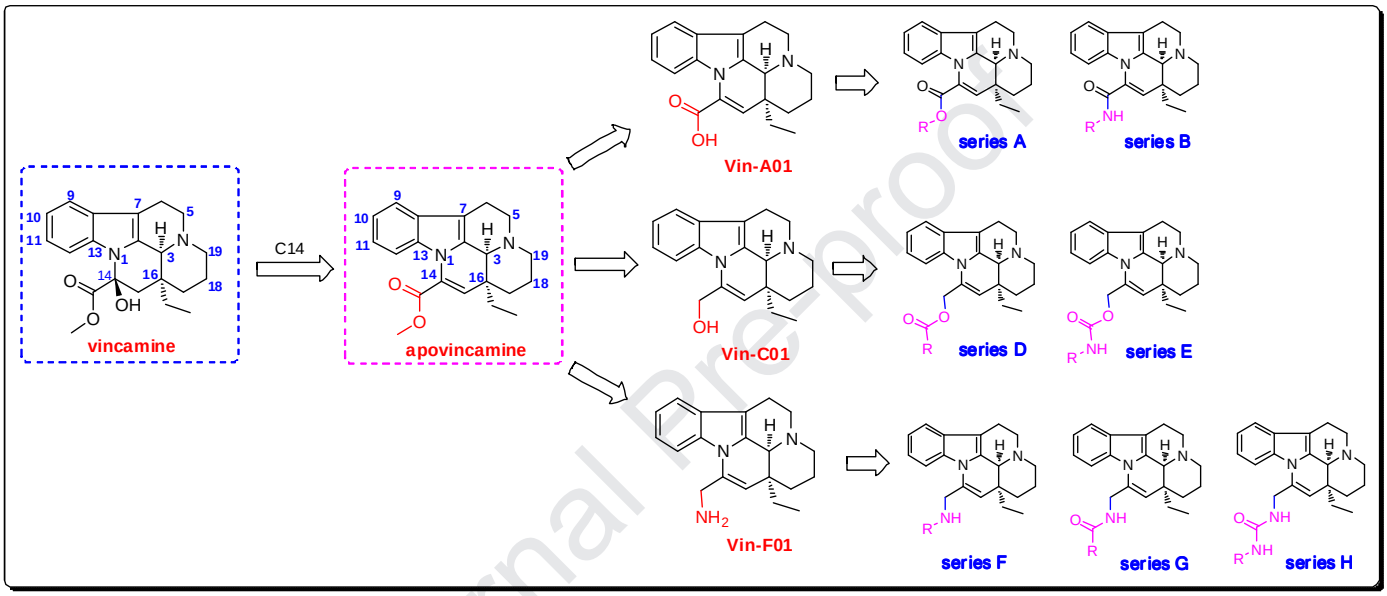


Fig. 1. Design of novel pancreatic β-cells protective agents

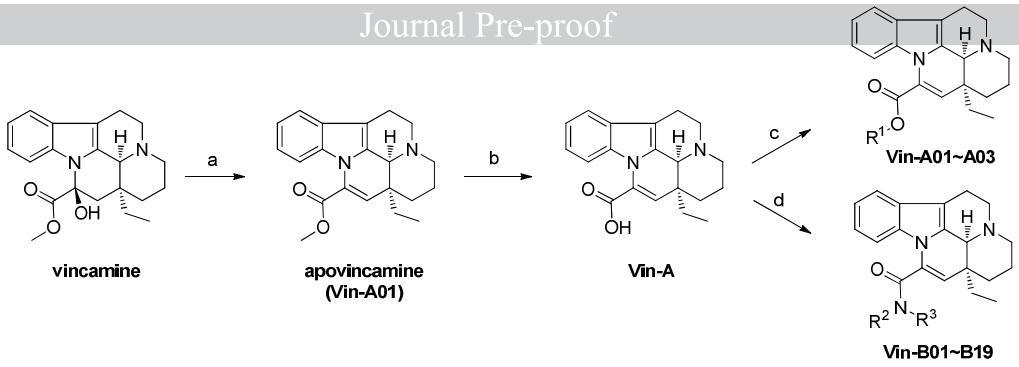
2.2. Chemistry

The synthetic routes for the 51 compounds were depicted in Scheme 1-4. Firstly, dehydration reaction of commercially available vincamine in the presence of *para*-toluenesulfonic acid (TsOH) gave apovincamine, which was further hydrolyzed to give the corresponding acid **Vin-A**. Then, esterification of **Vin-A** with different alcohols in the presence of concentrated sulfuric acid (con.H_2SO_4) afforded the target compounds **Vin-A01~A03** in good yields. While **Vin-A** was activated with *para*-toluensulfonyl chloride (TsCl) and subsequently coupled with different amines in the presence of K_2CO_3 to afford the target compounds **Vin-B01~B19** in good yields (Scheme 1).

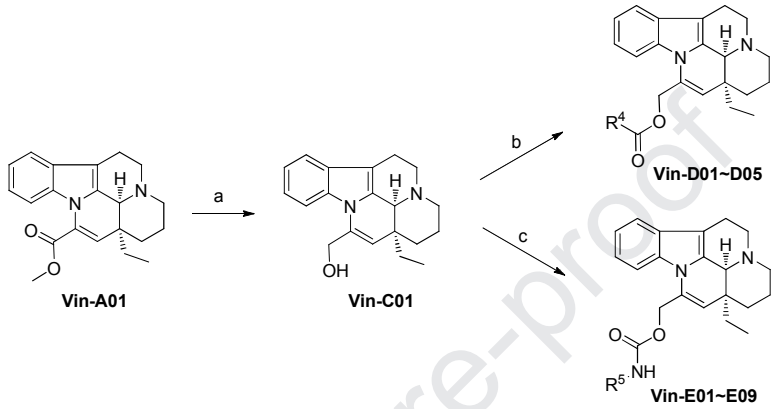
Compounds **Vin-C01**, **Vin-D01~D05** and **Vin-E01~E09** were prepared according to Scheme 2. Treatment of vincamine with lithium aluminum hydride ($LiAlH_4$) produced the corresponding product **Vin-C01**, which was reacted with different acyl chlorides to give the corresponding products **Vin-D01~D05**.

Activation of **Vin-C01** with 4-nitrophenyl carbonate and subsequently coupled with different amines gave compounds **Vin-E01~E09** in moderate yields. As depicted in Scheme 3, reaction of **Vin-C01** with phthalimide in the presence of diisopropyl azodiformate (DIAD) and triphenylphosphine (PPh_3) afforded **Vin-F01**, which was reacted with different acyl chlorides to give the corresponding products **Vin-G01~G05**. Compounds **Vin-F02~F05** were obtained by tosylation of **Vin-F01** and subsequently nucleophilic attacked by different amines. Reaction of **Vin-F01** with 4-nitrophenyl carbamate and then substituted with different amines to give **Vin-H01~H04**.

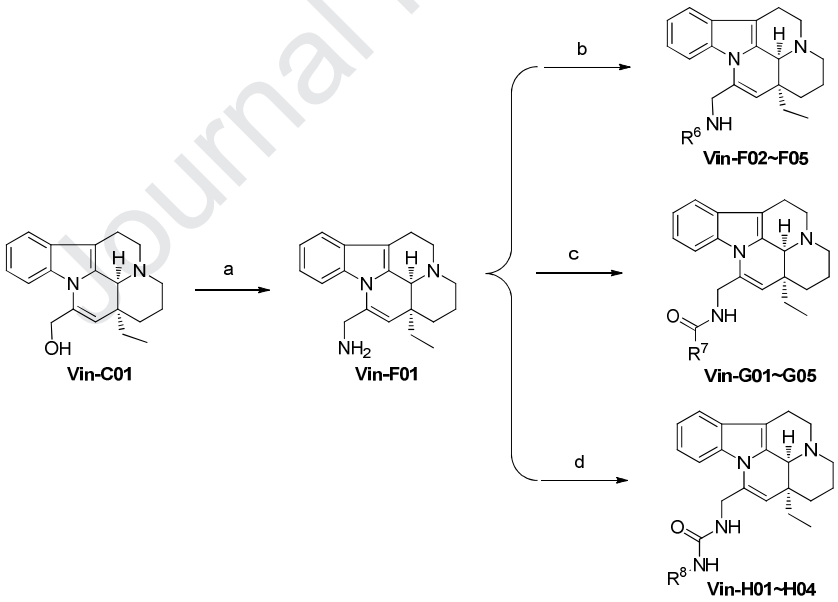
All synthetic compounds were purified by chromatographic techniques (see the experiment section for details) and characterized by their spectroscopic data (^{1}H-NMR, ^{13}C-NMR and MS).



Scheme 1. Reagents and conditions: (a) TsOH, toluene, reflux, 2 h, 92%; (b) NaOH, EtOH, reflux, 2 h; HOAc, pH 3~4, 96%; (c) R^1OH, con.H_2SO_4, reflux, 8 h, 74-81%; (d) TsCl, K_2CO_3, DCM, r.t., 4 h; R^2R^3NH, Et_3N, r.t., 3 h, 72-92%.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, -50 °C, 0.5 h, 96%; (b) R^4COCl, Et_3N, DCM, r.t., 2 h, 75-97%; (c) 4-nitrophenyl carbamate, Py, DCM, r.t., 3 h; R^5NH_2, DCM, r.t., 1 h; 43-65%.



Scheme 3. Reagents and conditions: (a) phthalimide, PPh_3, DIAD, THF, reflux, 1.5 h; NH_2-$NH_2 \cdot H_2O$, N_2, 80 °C, 1h, 76%; (b) TsCl, K_2CO_3, DCM, r.t., 5 h; R^6NH_2, Et_3N, r.t., 8 h, 45-62%; (c) R^7COCl, DIPEA, DCM, r.t., 0.5 h, 80-90%; (d) 4-nitrophenyl carbamate, Py, DCM, r.t., 0.5 h; R^8NH_2, DCM, r.t., 1 h; 75-87%.

2.3. Inhibition of apoptosis in INS-1 cells induced by STZ

The pancreatic β-cell protective activity of the 51 compounds against STZ-induced apoptosis was evaluated in INS-832/13 cells and vincamine was used as the positive control. The pancreatic β-cells protection rate of vincamine at 20 μM was 63%. We chose the compounds which showed stronger protective activity than vincamine at 20 μM to determine the EC_{50} value. The relative protective activity and the EC_{50} values were summarized in Tables 1-3. On the basis of these results, following SAR has been derived to analyze the influence of the modifications on C14 position.

Initial modification started from the elimination of the hydroxyl groups on C14 position of vincamine giving apovincamine (**Vin-A01**). In comparison to vincamine, **Vin-A01** showed much higher potency of inhibiting apoptosis in INS-1

cells with EC_{50} of 5.03 μM. When changing the R^1 substituent, the ester compounds **Vin-A02** and **Vin-A03** with aliphatic groups showed similar potency to **Vin-A01** with EC_{50} of 5.44 μM and 4.55 μM, respectively. The amide compounds with small aliphatic groups displayed higher potency (EC_{50} < 1 μM) than vincamine, especially for compound **Vin-B03**, it displayed a significant EC_{50} value of 0.20 μM. While compounds **Vin-B05**, **Vin-B06**, **Vin-B07** and **Vin-B08** which had long or large aliphatic groups showed similar potency to vincamine. The introduction of hydroxyl at the end of the aliphatic group did not improve activity, for example, the potency of **Vin-B09** and **Vin-B10** both decreased compared to their parent compound **Vin-B04** and **Vin-B05**. The activities of aromatic and di-substituted amide compounds also decreased a lot, which indicates that this position is not suitable for modification of large groups.

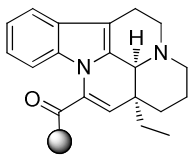
Vin-C01, which was the reduction product of **Vin-A01**, showed potent inhibitory activity of apoptosis with EC_{50} value of 0.22 μM. Thus, a series of esters and amino esters derivatives of **Vin-C01** were designed, synthesized and evaluated. As shown in Table 2, the potency of the aliphatic ester compounds (**Vin-D01~D04**) improved a lot compared to vincamine, especially for compound **Vin-D01**, it displayed potent activity with a significant EC_{50} value of 0.36 μM.

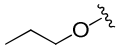
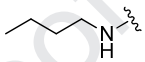
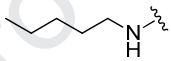
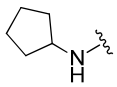
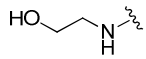
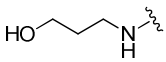
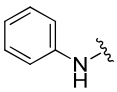
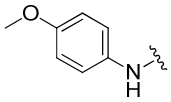
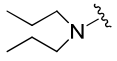
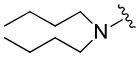
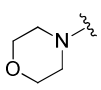
Small aliphatic amino esters derivatives **Vin-E02~04** showed increased activity compared to vincamine and it was also observed that the activity decreased with the increase of the substituent chain length. The results of **Vin-D05** and **Vin-E09** showed that the aromatic ring is not conducive to maintain the activity, which is consistent with the SAR of series B derivatives.

Further, the hydroxyl group of **Vin-C01** is converted to the amine group and a series of amine, amide and urea derivatives were synthesized to investigate the effect on the activity. As shown in Table 3, amine derivatives **Vin-F02** and **Vin-F03** which had methyl or ethyl group showed stronger activity than its parent compound **Vin-F01**, propyl and benzyl substituted compounds and all the urea derivatives lost the pancreatic β-cell protective activity. While, most of the amide derivatives showed improved activity compared to vincamine, except for aromatic substituted product **Vin-G05**. The results of modifications on C14 position indicated that structural modifications can be tolerable in this region and appropriate modification is benefit to improve the activity.

In general, most of the derivatives showed improved potency, however, the EC_{50} value was not completely corresponding to the strength of protective activity. The good news is that in the modification of C14 position, several compounds with high potency were obtained, especially for compound **Vin-C01** (0.22 μM) and **Vin-F03** (0.27 μM), their pancreatic β-cell protective activity increased 2 times approximately than vincamine. Overall consideration of the EC_{50} value and the relative protective activity, compounds **Vin-C01** and **Vin-F03** were selected for further investigation.

Table 1
The structures and the effects of compounds **Vin-A01~A03** and **Vin-B01~B19** on STZ-induced cell death in INS-832/13 cells.



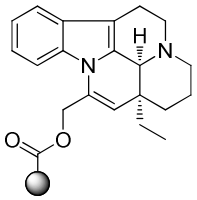
Compd	OR^1/NR^2R^3	Protective activity[a]	EC_{50}(μM)[b]
Vincamine			11.0 ± 1.21
Vin-A01		+	5.03 ± 0.37
Vin-A02		+	5.44 ± 0.03
Vin-A03		+	4.55 ± 0.45
Vin-B01		+	10.23 ± 0.85
Vin-B02		+	0.66 ± 0.04
Vin-B03		+	0.20 ± 0.02
Vin-B04		+	0.37 ± 0.03
Vin-B05		+	9.40 ± 1.08
Vin-B06		−	ND
Vin-B07		+	10.76 ± 0.62
Vin-B08		+	12.78 ± 0.47
Vin-B09		+	0.96 ± 0.02
Vin-B10		+	10.18 ± 0.35
Vin-B11		−	ND
Vin-B12		−	ND
Vin-B13		−	ND
Vin-B14		+	11.50 ± 1.05
Vin-B15		+	7.10 ± 0.33
Vin-B16		−	ND
Vin-B17		+	13.17 ± 0.92
Vin-B18		+	4.89 ± 0.28
Vin-B19		+	10.9 ± 0.76

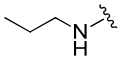
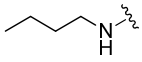
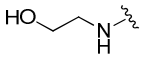
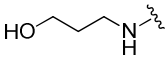
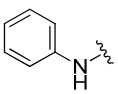
[a] The pancreatic β-cells protection rate of vincamine at 20 μM was 63%, "+" means the protective activity at 20 μM was stronger than vincamine, "−" means the protective activity 20 μM was weaker than vincamine.

[b] EC_{50} was measured by the MTT assay with seven concentrations (0.1, 1, 5, 10, 20, 40, 50 μM). Data were shown as means ± S.E.M. with three independent experimental replicates.

Table 2

The structures and the effects of compounds **Vin-D01~D05** and **Vin-E01~E09** on STZ-induced cell death in INS-832/13 cells.



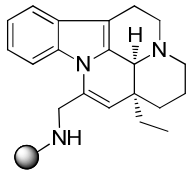
Compd	R^4/NHR^5	Protective activity[a]	EC_{50} (μM)[b]
Vin-D01		+	0.36 ± 0.02
Vin-D02		+	6.84 ± 0.13
Vin-D03		+	3.77 ± 0.56
Vin-D04		+	1.74 ± 0.03
Vin-D05		−	ND
Vin-E01		+	8.75 ± 0.67
Vin-E02		+	1.65 ± 0.11
Vin-E03		+	1.69 ± 0.14
Vin-E04		+	5.85 ± 0.47
Vin-E05		−	ND
Vin-E06		+	5.32 ± 0.38
Vin-E07		+	9.05 ± 0.57
Vin-E08		+	10.76 ± 0.35
Vin-E09		−	ND

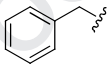
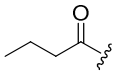
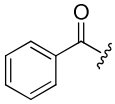
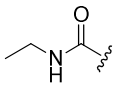
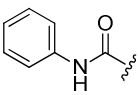
[a] The pancreatic β-cells protection rate of vincamine at 20 μM was 63%, "+" means the protective activity at 20 μM was stronger than vincamine, "−" means the protective activity at 20 μM was weaker than vincamine.

[b] EC_{50} was measured by the MTT assay with seven concentrations (0.1, 1, 5, 10, 20, 40, 50 μM). Data were shown as means ± S.E.M. with three independent experimental replicates.

Table 3

The structures and the effects of compounds **Vin-F01~F05**, **Vin-G01~G05** and **Vin-H01~H04** on STZ-induced cell death in INS-832/13 cells



Compd	R^6	Protective activity[a]	EC_{50} (μM)[b]
Vin-F01	H	+	7.62 ± 0.23
Vin-F02		+	1.60 ± 0.07
Vin-F03		+[c]	0.27 ± 0.05
Vin-F04		−	ND
Vin-F05		−	ND
Vin-G01		+	3.02 ± 0.11
Vin-G02		+	8.70 ± 0.13
Vin-G03		+	7.20 ± 0.23
Vin-G04		+	4.40 ± 0.18
Vin-G05		−	ND
Vin-H01		−	ND
Vin-H02		−	ND
Vin-H03		−	ND
Vin-H04		−	ND

[a] The pancreatic β-cells protection rate of vincamine at 20 μM was 63%, "+" means the protective activity at 20 μM was stronger than vincamine, "−" means the protective activity at 20 μM was weaker than vincamine.

[b] EC_{50} was measured by the MTT assay with seven concentrations (0.1, 1, 5, 10, 20, 40, 50 μM). Data were shown as means ± S.E.M. with three independent experimental replicates.

[c] The protective activity was 2 times more than vincamine.

2.4. Cell viability assay

To investigate the potential protection of **Vin-C01** and **Vin-F03** against β-cells damage, MTT assay was carried out in INS-832/13 cells with or without STZ (0.4 mM) stimulus and vincamine was used as the positive control [22]. As depicted in Fig. 2A and 2B, **Vin-C01** and **Vin-F03** showed no effect on INS-832/13 cells viability, whereas they could reversed STZ-induced decrease in cell viability. **Vin-C01** and **Vin-F03** achieved the same effect at 10 μM as the effect of vincamine at 20 μM in reversing STZ-induced decrease in cell viability. The results demonstrated that **Vin-C01** and **Vin-F03** had the capability in enhancing INS-832/13 cells viability against STZ treatment, which was much stronger than vincamine.

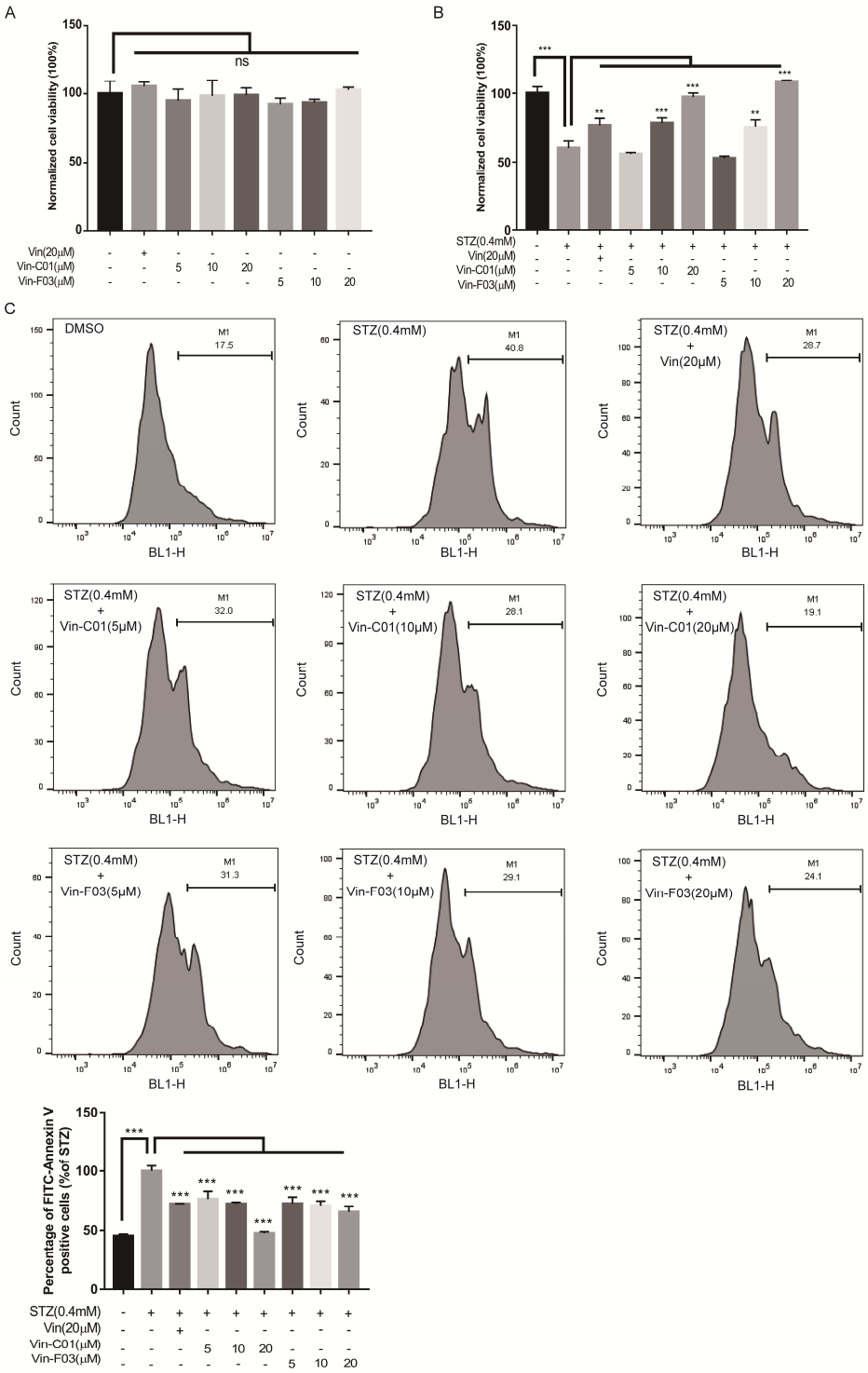


Fig. 2. Vin-C01 and **Vin-F03** promote β-cell survival. (A) INS-832/13 cells were incubated with **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) for 24 h, and then MTT assay was applied. (B) INS-832/13 cells were incubated with **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) with STZ (0.4 mM) for 24 h, and then MTT assay was applied. (C) INS-832/13 cells treated as B were stained with Annexin V-FITC, followed by the determination of flow cytometry. (M1: FITC-Annxin V positive cells.). Data were shown as means ± S.E.M. with three independent experimental replicates. Significant differences between groups are represented as $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, and $p > 0.05$ stood for no significance (ns). (**Vin** is short for vincamine)

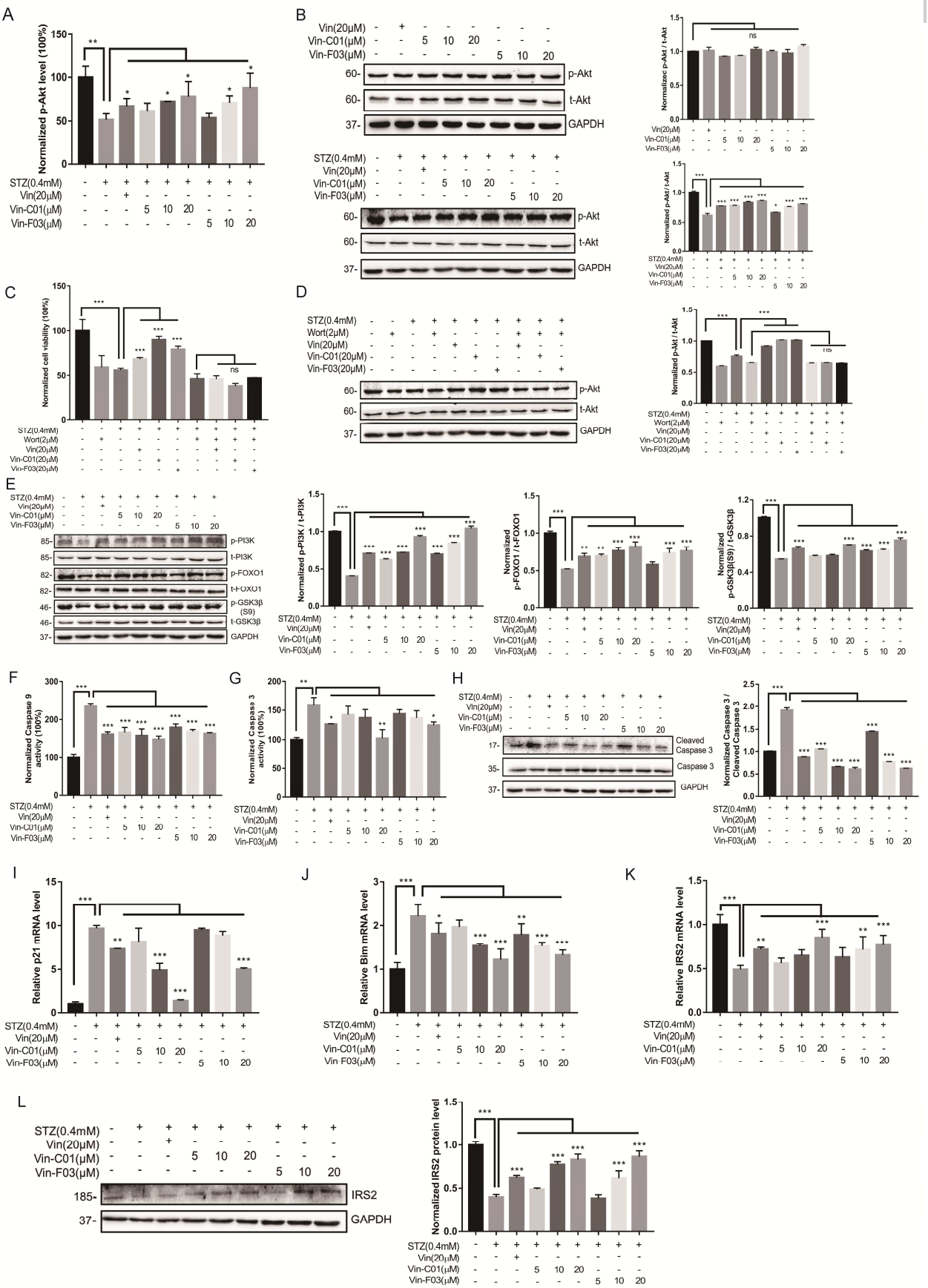


Fig. 3. Vin-C01 and **Vin-F03** protect β-cells through IRS2/PI3K/Akt signaling pathway. (A) After incubation with STZ (0.4 mM) and **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) in INS-832/13 cells for 24 h, the level of p-Akt was determined by AlphaLISA assay. (B) INS-832/13 cells with or without STZ treatment were incubated with **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) for 24 h, and then p-Akt was detected by Western blot. (C) **Vin**, **Vin-C01** and **Vin-F03**-induced β-cell survival was measured in the presence of wortmannin (2 μM). (D) The level of p-Akt was detected by Western blot in INS-832/13 cells incubated with STZ (0.4 mM) and **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) for 24 h in the presence of wortmannin (2 μM). (F, G and H) INS-832/13

cells were incubated with STZ (0.4 mM) and **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) for 6 h, and then the activity of Caspase 9 (F) and Caspase 3 (G) were examined using corresponding assay kits and the expression of cleaved Caspase 3 (H) was detected by Western blot. (E, I, J, K and L) INS-832/13 cells were treated with STZ (0.4 mM) and **Vin** (20 μM), **Vin-C01** (5, 10, 20 μM) and **Vin-F03** (5, 10, 20 μM) for 24 h. IRS2/PI3K/Akt signaling pathway-related proteins (E, L) were then assessed by Western blot, and the mRNA expressions of p21 (I), Bim (J) and IRS2 (K) were detected by quantitative real-time PCR (qRT-PCR). Data were shown as means ± S.E.M. with three independent experimental replicates. Significant differences between groups are represented as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, and $p > 0.05$ stands for no significance (ns).

2.5. Apoptosis assay

Next, we performed flow cytometry (FCM) analysis of Annexin V-FITC/PI double staining to further investigate the protective effect of **Vin-C01** and **Vin-F03** on INS-832/13 cells. As shown in Fig. 2C, these two compounds efficiently decreased the mass of apoptotic cells induced by STZ (0.4 mM). **Vin-C01** and **Vin-F03** achieved the same effect at 10 μM as that of vincamine at 20 μM, which indicated that they had better effect than vincamine in protecting INS-832/13 cells against STZ-induced apoptosis.

2.6. Cellular mechanism of action studies.

*2.6.1 **Vin-C01** and **Vin-F03** reversed the STZ-induced reduction of p-Akt*

Given that Akt plays an important role in the signaling transductions of β-cells growth and apoptosis [23], we next investigated the potential regulation of **Vin-C01** and **Vin-F03** against Akt signaling. Firstly, AlphaLISA-based assay was performed to investigate the potency of the two compounds in stimulating p-Akt. As indicated in Fig. 3A, both compounds reversed STZ-induced reduction of p-Akt. Next, we performed western blot assay to further verify the AlphaLISA results. As shown in Fig. 3B, **Vin-C01** and **Vin-F03** exhibited no effect on p-Akt, while, they could recover STZ-induced reduction in p-Akt. These results demonstrated that **Vin-C01** and **Vin-F03** possessed the capability of reversing STZ-induced reduction of p-Akt in β-cells and their effects were much better than vincamine.

*2.6.2 **Vin-C01** and **Vin-F03** promoted β-cells survival by regulating PI3K/Akt signaling*

Considering that Akt is often regulated by PI3K in cell survival and growth, we further investigated whether these two compounds mediated β-cells survival and p-Akt restoration were of PI3K dependence. As shown in Fig. 3C, MTT assay result revealed that PI3K inhibitor wortmannin could impeded the activity of **Vin-C01** and **Vin-F03** in reversing STZ-induced cell apoptosis. In addition, the western blot result (Fig. 3D) demonstrated that these two compounds-mediated p-Akt restoration can be completely reversed by wortmannin. It was obvious that **Vin-C01** and **Vin-F03** efficiently recovered STZ-induced reduction of p-PI3K and their effects were much better than vincamine (Fig. 3E).

Next, we investigated the regulation of **Vin-C01** and **Vin-F03** against the downstream effectors of PI3K/Akt signaling, including FOXO1, GSK3β, Caspase 9/3, Bim and p21 [24]. The results showed that **Vin-C01** and **Vin-F03** could reverse STZ-induced reduction of phosphorylated FOXO1 (Ser256)/GSK3β (Ser9) (Fig. 3E) and deplete STZ-induced increases in enzyme activity of Caspase 9/3 (Fig. 3F and 3G) and the protein level of cleaved Caspase 3 (Fig. 3H). Moreover, they could also reduce the elevation of Bim and p21 mRNA stimulated by STZ (Fig. 3I and 3J).

Taken together, all results demonstrated that **Vin-C01** and **Vin-F03** could protect β-cells against STZ-induced apoptosis by regulating PI3K/Akt signaling and their protective activities were much stronger than vincamine.

*2.6.3 **Vin-C01** and **Vin-F03** regulated insulin receptor substrate 2 (IRS2)*

Insulin receptor substrate 2 (IRS2) is an interface between activated tyrosine kinase receptors and the downstream signaling molecules including PI3K [25]. Thus, we performed relevant assays to investigate whether IRS2 was the upstream protein of PI3K/Akt signaling which responded to the regulation of **Vin-C01** and **Vin-F03**. The results indicated that both **Vin-C01** and **Vin-F03** reversed STZ-induced IRS2 reduction in either mRNA or protein level in INS-832/13 cells (Fig. 3K and 3L). Thus, we concluded that IRS2 was in the upstream of PI3K/Akt signaling responding to vincamine, **Vin-C01** and **Vin-F03** regulation. In addition, **Vin-C01** and **Vin-F03** exhibited much better effect than vincamine in reversing STZ-induced reduction of IRS2.

3. Conclusions

In conclusion, to develop effective drugs for the treatment of type 2 diabetes mellitus, 51 vincamine derivatives were designed, synthesized and evaluated. Among all the tested compounds, **Vin-C01** and **Vin-F03** showed most potent pancreatic β-cells protective effect with the EC_{50} values of 0.22 μM and 0.27 μM, respectively. Further cell viability assay demonstrated that **Vin-C01** and **Vin-F03** effectively enhanced INS-832/13 cells viability and protected INS-832/13 cells against STZ-induced apoptosis, and their effect were much stronger than vincamine. In addition, we found that **Vin-C01** and **Vin-F03** could protect INS-832/13 cells function by regulating IRS2/PI3K/Akt signaling pathway through the cellular mechanism of action studies. Taking together, these results suggest that **Vin-C01** and **Vin-F03** could be considered as two potential pancreatic β-cells protective agents for the development of type 2 diabetes drugs.

3. Experimental section

3.1. Materials and physical measurements

All cell culture reagents were purchased from Gibco (Invitrogen, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), STZ, wortmannin, SB216763 and chloroquine were purchased from Sigma-Aldrich company (USA). All chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (China), Alfa Aesar, Adamas-beta, J&K and TCI. All solvents were used without further purifications. Reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated silica gel GF254 (Yantai, China) plates and the spots were detected under UV light (254 nm). Column chromatography was performed on silica gel (200-300 mesh) from Yantai (China). Melting point were recorded on a WRS-1B melting point apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured in DMSO-d_6 or $CDCl_3$ solutions using an AVANCE III 400 spectrometer. Chemical shifts are reported in δ scale (ppm) relative to internal TMS, J values are given in Hertz, and spin multiplicities are expressed as s (singlet), d (doublet), t (triplet), or m (multiplet). Low and high-resolution mass spectra were obtained in the ESI mode.

3.2. Chemistry

*3.2.1. General procedure for the synthesis of **Vin-A01***

To a stirred solution of vincamine (1.77 g, 5 mmol) in toluene (20 mL) was added TsOH (1.72 g, 10 mmol) at room temperature. The mixture was heated to reflux for 2 h, and TLC indicated that the reaction was completed. After cooling to room temperature, the reaction mixture was evaporated to dryness under vacuum and diluted with H_2O (20 mL). When the solution was basified to pH 8 by 5% NaOH solution, a large amount of white solid dissolve out. The precipitate was filtered and washed with with H_2O (15 mL) and EtOH (3 mL), then dried under vacuum to give **Vin-A01** as a white solid (Yield 92%). m.p.: 163-165 °C. HPLC: 98.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.2 Hz, 1H), 7.23(d, *J* = 7.2 Hz, 1H), 7.19-7.11 (m, 2H), 6.15 (s, 1H), 4.14 (s, 1H), 3.95 (s, 3H), 3.38-3.33 (m, 1H), 3.29-3.21 (m, 1H), 3.07-2.99 (m, 1H), 2.63-2.58 (m, 2H), 2.53-2.48 (m, 1H), 1.97-1.85 (m, 2H), 1.78-1.69 (m, 1H), 1.53-1.50 (m, 1H), 1.43-1.38 (m, 1H), 1.02 (t, *J* = 7.2 Hz, 3H), 0.98-0.97 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.8, 134.1, 130.7, 129.0, 128.2, 128.1, 121.9, 120.2, 118.2, 112.4, 108.6, 55.7, 52.5, 51.4, 44.9, 37.7, 28.5, 27.2, 20.2, 16.3, 8.7; ESI-MS (*m/z*): 337 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}N_2O_2$: 337.1916, Found: 337.1912.

*3.2.2. General procedure for the synthesis of **Vin-A***

To a stirred solution of **Vin-A01** (1.68 g, 5 mmol) in EtOH (10 mL) was added NaOH (2.04 g, 6 mmol) at room temperature. The mixture was heated to reflux for 2 h, and TLC indicated that the reaction was completed. After cooling to room temperature, the reaction mixture was evaporated to dryness under vacuum and diluted with H_2O (6 mL). When the solution was acidified to pH 3~4 by acetic acid, a large amount of off-white solid dissolve out. The precipitate was filtered and washed with with H_2O (15 mL), then dried under vacuum to give **Vin-A** as a light yellow solid (Yield 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, *J* = 7.2 Hz, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.09-7.02 (m, 2H), 6.01 (s, 1H), 4.19 (s, 1H), 3.26-3.21 (m, 2H), 2.97-2.89 (m, 1H), 2.62-2.60 (m, 2H), 2.53-2.50 (m, 2H), 1.85-1.79 (m, 2H), 1.60-1.46 (m, 2H), 1.37-1.33 (m, 1H), 0.95-0.91 (t, *J* = 7.2 Hz, 3H), 0.85-0.78 (m, 1H); ESI-MS (*m/z*): 323 $[M+H]^+$.

*3.2.3. General procedure for the synthesis of **Vin-A02~A03***

To a stirred solution of vincamine (354 mg, 1 mmol) in anhydrous R^1OH (6 mL) was added con. H_2SO_4 (1 mL) at room temperature. The mixture was heated to reflux for 8 h, and TLC indicated that the reaction was completed. After cooling to room temperature, the reaction mixture was evaporated to dryness under vacuum and diluted with H_2O (6 mL). When the solution was basified to pH 8 by 5% NaOH solution, a large amount of white solid dissolve out. The precipitate was filtered and washed with with H_2O (10 mL) and EtOH (1 mL), then dried under vacuum to give the target compound **Vin-A02~A03**.

*3.2.4. (4^1S,13aS)-propyl 13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (**Vin-A02**)*

The product was a light yellow solid (Yield 81%). m.p.: 189-191 °C. HPLC: 97.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.24 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.17-7.10 (m, 2H), 6.11 (s, 1H), 4.36-4.28 (m, 2H), 4.15 (s, 1H), 3.37-3.32 (m, 1H), 3.28-3.21 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.61 (m, 2H), 2.53-2.48 (m, 1H), 1.96-1.86 (m, 2H), 1.83-1.74 (m, 2H), 1.73-1.66 (m, 1H), 1.53-1.49 (m, 1H), 1.42-1.38 (m, 1H), 1.07-0.95 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.4, 134.0, 130.8, 128.9, 128.3, 127.7, 121.7, 120.1, 118.1, 112.5, 108.5, 67.2, 55.5, 51.3, 44.8, 37.6, 28.6, 27.2, 21.9, 20.2, 16.2, 10.4, 8.7; ESI-MS (*m/z*): 365 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_2$: 365.2229, Found: 365.2226.

*3.2.5. (4^1S,13aS)-isopropyl 13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxylate (**Vin-A03**)*

The product was a light yellow solid (Yield 74%). m.p.: 173-176 °C. HPLC: 97.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.2 Hz, 1H), 7.27 (d, *J* = 7.2 Hz, 1H), 7.17-7.10 (m, 2H), 6.08 (s, 1H), 5.34-5.25 (m, 1H), 4.14 (s, 1H), 3.37- 3.32 (m, 1H), 3.28-3.21 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.61 (m, 2H), 2.53-2.47 (m, 1H), 1.98-1.85 (m, 2H), 1.74-1.68 (m, 1H), 1.53-1.49 (m, 1H), 1.39 (d, *J* = 6 Hz, 3H), 1.37 (d, *J* = 6 Hz, 3H), 1.31-1.26 (m, 1H), 1.01 (t, *J* = 7.2 Hz, 3H), 1.04-0.97 (m, 4H); ESI-MS (*m/z*): 365 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_2$: 365.2229, Found: 365.2226.

*3.2.6. General procedure for the synthesis of **Vin-B01~B19***

To a stirred solution of **Vin-A** (322 mg, 1 mmol) in DCM (6 mL) was added TsCl (380 mg, 2 mmol) at room temperature. The mixture was stirred at room temperature for 4 h. After completion, Et_3N (303 mg, 3 mmol) and amines R^2R^3NH (2 mmol) was added into the reaction mixture. After stirring at room temperature for another 3 h, the mixture was diluted with DCM (40 mL) and washed with H_2O (30 mL), saturated Na_2CO_3 solution (30 mL) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude products. The appropriate compounds (**Vin-B01~B19**) were obtained following purification by silica gel chromatography.

*3.2.7. (4^1S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo-[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B01**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light red solid (Yield 83%). m.p.: 197-199 °C. HPLC: 97.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.6 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.18-7.10 (m, 2H), 6.38 (s, 1H), 6.02 (s, 1H), 5.82 (s, 1H), 4.15 (s, 1H), 3.37-3.32 (m, 1H), 3.28-3.20 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.60 (m, 2H), 2.52-2.47 (m, 1H), 1.96-1.83 (m, 2H), 1.76-1.65 (m, 1H), 1.51-1.48 (m, 1H), 1.41-1.37 (m, 1H), 1.04-0.96 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.4, 133.3, 131.0, 130.9, 128.5, 121.2, 120.2, 119.6, 117.9, 112.2, 107.4, 55.3, 50.8, 44.5, 36.5, 29.1, 26.8, 19.9, 15.8, 8.4; ESI-MS (*m/z*): 322 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{24}N_3O$: 322.1919, Found: 322.1915.

*3.2.8. (4^1S,13aS)-13a-ethyl-N-methyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B02**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 82%). m.p.: 186-188 °C. HPLC: 95.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.6 Hz, 1H), 7.26-7.11 (m, 3H), 6.04-6.03 (m, 1H), 5.68 (s, 1H), 4.15 (s, 1H), 3.38-3.33 (m, 1H), 3.28-3.20 (m, 1H), 3.03-3.01 (m, 3H), 2.63-2.61 (m, 2H), 2.52-2.48 (m, 1H), 2.05 (s, 1H), 1.95-1.81 (m, 2H), 1.73-1.67 (m, 1H), 1.51-1.48 (m, 1H), 1.41-1.37 (m, 1H), 1.01-0.96 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.8, 133.8, 131.7, 131.2, 129.3, 122.3, 122.0, 120.5, 118.6, 111.9, 109.0, 56.0, 51.7, 45.1, 37.4, 29.4, 27.5, 26.7, 20.5, 16.5, 8.8; ESI-MS (*m/z*): 336 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{26}N_3O$: 336.2076, Found: 336.2074.

*3.2.9. (4^1S,13aS)-N,13a-diethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B03**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 92%). m.p.: 210-212 °C. HPLC: 96.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.26 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.17-7.10 (m, 2H), 5.95 (s, 1H), 5.68 (s, 1H), 4.15 (s, 1H), 3.50-3.48 (m, 2H), 3.38-3.33 (m, 1H), 3.28-3.20 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.61 (m, 2H), 2.52-2.47 (m, 1H), 1.95-1.82 (m, 2H), 1.74-1.70 (m, 1H), 1.52-1.48 (m, 1H), 1.42-1.37 (m, 1H), 1.28-1.26 (t, *J* = 7.2 Hz, 3H), 1.04-0.96 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 133.7, 131.9, 131.3, 129.3, 122.1, 121.9, 120.4, 118.5, 112.0, 108.9, 56.0, 51.6, 45.1, 37.4, 35.0, 29.4, 27.5, 20.5, 16.5, 14.8, 8.8; ESI-MS (*m/z*): 350 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}N_3O$: 350.2232, Found: 350.2227.

*3.2.10. (4^{1}S,13aS)-13a-ethyl-N-propyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B04**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 89%). m.p.: 266-268 °C. HPLC: 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.2 Hz, 1H), 7.27-7.25 (m, 1H), 7.17-7.10 (m, 2H), 5.99-5.96 (t, *J* = 5.6 Hz, 1H), 5.67 (s, 1H), 4.16 (s, 1H), 3.50-3.33 (m, 3H), 3.28-3.21 (m, 1H), 3.07-2.98 (m, 1H), 2.64-2.61 (m, 2H), 2.53-2.47 (m, 1H), 1.94-1.82 (m, 2H), 1.78-1.73 (m, 1H), 1.68-1.62 (m, 2H), 1.52-1.49 (m, 1H), 1.42-1.37 (m, 1H), 1.05-0.97 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1, 133.7, 131.9, 131.1, 129.2, 122.1, 121.7, 120.4, 118.5, 112.0, 108.9, 56.0, 51.6, 45.1, 41.8, 37.4, 29.4, 27.5, 22.8, 20.5, 16.4, 11.6, 8.8; ESI-MS (*m/z*): 364 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O$: 364.2389, Found: 364.2384.

*3.2.11. (4^{1}S,13aS)-N-butyl-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B05**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 86%). m.p.: 259-261 °C. HPLC: 93.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.22 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.13-7.06 (m, 2H), 6.31-6.24 (m, 1H), 5.60 (s, 1H), 4.11 (s, 1H), 3.43-3.28 (m, 3H), 3.23-3.15 (m, 1H), 3.04-2.95 (m, 1H), 2.60-2.56 (m, 2H), 2.48-2.43 (m, 1H), 1.93-1.75 (m, 2H), 1.72-1.61 (m, 1H), 1.58-1.51 (m, 2H), 1.45-1.42 (m, 1H), 1.38-1.30 (m, 3H), 1.09-0.91 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1, 133.6, 131.8, 131.0, 129.1, 121.9, 121.2, 120.2, 118.3, 111.9, 108.6, 55.8, 51.5, 45.0, 39.7, 37.1, 31.4, 29.4, 27.3, 20.4, 20.1, 16.3, 13.7, 8.7; ESI-MS (*m/z*): 378 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O$: 378.2545, Found: 378.2542.

*3.2.12. (4^{1}S,13aS)-13a-ethyl-N-pentyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B06**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 88%). m.p.: 227-230 °C. HPLC: 90.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 6.4, 2.0 Hz, 1H), 7.25 (dd, *J* = 6.4, 2.0 Hz, 1H), 7.17-7.10 (m, 2H), 5.96 (m, 1H), 5.67 (s, 1H), 4.15 (s, 1H), 3.54-3.40 (m, 2H), 3.38-3.33 (m, 1H), 3.28-3.20 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.61 (m, 2H), 2.52-2.47 (m, 1H), 1.97-1.83 (m, 2H), 1.82-1.80 (m, 1H), 1.77-1.67 (m, 1H), 1.65-1.58 (m, 2H), 1.52-1.48 (m, 1H), 1.37-1.34 (m, 4H), 1.05-0.98 (m, 4H), 0.92 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 392 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{34}N_3O$: 392.2702, Found: 392.2698.

*3.2.13. (4^{1}S,13aS)-13a-ethyl-N-isopropyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]-naphthyridine-12-carboxamide (**Vin-B07**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 85%). m.p.: 220-222 °C. HPLC: 96.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.28 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.17-7.10 (m, 2H), 5.66 (s, 1H), 4.36-4.28 (m, 1H), 4.15 (s, 1H), 3.37-3.33 (m, 1H), 3.27-3.20 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.61 (m, 2H), 2.52-2.47 (m, 1H), 1.97-1.80 (m, 2H), 1.76-1.65 (m, 1H), 1.52-1.48 (m, 1H), 1.41-1.36 (m, 1H), 1.27 (d, *J* = 6.4 Hz, 3H), 1.25 (d, *J* = 6.4 Hz, 3H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.96-1.03 (m, 4H); ESI-MS (*m/z*): 364 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O$: 364.2389, Found: 364.2386.

*3.2.14. (4^{1}S,13aS)-N-cyclopentyl-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]-naphthyridine-12-carboxamide (**Vin-B08**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 81%). m.p.: 152-154 °C. HPLC: 96.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.27 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.17-7.10 (m, 2H), 5.87-5.85 (m, 1H), 5.68 (s, 1H), 4.49-4.40 (m, 1H), 4.15 (s, 1H), 3.38-3.33 (m, 1H), 3.28-3.20 (m, 1H), 3.07-2.98 (m, 1H), 2.63-2.60 (m, 2H), 2.52-2.47 (m, 1H), 2.15-2.09 (m, 2H), 1.94-1.80 (m, 3H), 1.72-1.65 (m, 5H), 1.52-1.46 (m, 2H), 1.44-1.37 (m, 1H), 1.02-0.96 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 133.6, 131.8, 131.4, 129.2, 122.0, 121.9, 120.4, 118.4, 112.1, 108.9, 56.0, 51.7, 51.6, 45.1, 37.4, 33.2, 32.8, 29.3, 27.5, 23.9, 23.8, 20.5, 16.5, 8.8; ESI-MS (*m/z*): 390 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{32}N_3O$: 390.2545, Found: 390.2541.

*3.2.15. (4^{1}S,13aS)-13a-ethyl-N-(2-hydroxyethyl)-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]-naphthyridine-12-carboxamide (**Vin-B09**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 79%). m.p.: 191-193 °C. HPLC: 97.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 6.4 Hz, 1H), 7.24 (d, *J* = 6.4 Hz, 1H), 7.16-7.08 (m, 2H), 6.54-6.52 (m, 1H), 5.69 (s, 1H), 4.11 (s, 1H), 3.77 (t, *J* = 5.6 Hz, 2H), 3.67- 3.59 (m, 1H), 3.56-3.49 (m, 1H), 3.43 (s, 1H), 3.33- 3.28 (m, 1H), 3.20-3.13 (m, 1H), 3.04-2.95 (m, 1H), 2.61-2.57 (m, 2H), 2.50-2.44 (m, 1H), 1.94-1.78 (m, 2H), 1.74-1.62 (m, 1H), 1.51-1.43 (m, 1H), 1.42-1.34 (m, 1H), 1.02-0.94 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.0, 133.7, 131.6, 130.9, 129.2, 122.2 (2C), 120.5, 118.5, 111.9, 108.9, 61.7, 55.9, 51.5, 45.0, 42.6, 37.4, 29.2, 27.4, 20.4, 16.4, 8.8; ESI-MS (*m/z*): 366 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}N_3O_2$: 366.2182, Found: 366.2177.

*3.2.16. (4^{1}S,13aS)-13a-ethyl-N-(3-hydroxypropyl)-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B10**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 75%). m.p.: 203-205 °C. HPLC: 95.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 6.4 Hz, 1H), 7.24 (d, *J* = 6.4 Hz, 1H), 7.17-7.10 (m, 2H), 6.52-6.49 (m, 1H), 5.72 (s, 1H), 4.13 (s, 1H), 3.73 (t, *J* = 5.6 Hz, 2H), 3.69-3.54 (m, 2H), 3.36-3.31 (m, 1H), 3.25-3.18 (m, 1H), 3.06-2.97 (m, 1H), 2.62-2.56 (m, 2H), 2.53-2.46 (m, 1H), 1.96-1.85 (m, 2H), 1.83-1.78 (m, 2H), 1.75-1.64 (m, 1H), 1.53-1.44 (m, 1H), 1.42-1.34 (m, 1H), 1.03-0.95 (m, 4H); ESI-MS (*m/z*): 380 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O_2$: 380.2338, Found: 380.2336.

*3.2.17. (4^{1}S,13aS)-13a-ethyl-N-phenyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B11**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 72%). m.p.: 176-178 °C. HPLC: 97.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.49-7.46 (m, 1H), 7.30 (d,

J = 8.0 Hz, 1H), 7.28-7.27 (m, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 7.13-7.08 (m, 2H), 5.81 (s, 1H), 4.14 (s, 1H), 3.36-3.33 (m, 1H), 3.25-3.17 (m, 1H), 3.07-3.00 (m, 1H), 2.65-2.55 (m, 2H), 2.51-2.46 (m, 1H), 1.95-1.78 (m, 2H), 1.74-1.64 (m, 1H), 1.51-1.47 (m, 1H), 1.38-1.34 (m, 1H), 1.03-0.93 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.0, 137.3, 133.6, 131.8, 131.1, 129.2, 129.1 (2C), 124.9, 122.9, 122.3, 120.5, 120.3 (2C), 118.5, 111.8, 109.0, 55.8, 51.5, 45.0, 37.5, 29.2, 27.4, 20.4, 16.4, 8.7; ESI-MS (*m/z*): 398 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{28}N_3O$: 398.2232, Found: 398.22328.

*3.2.18. (4^1S,13aS)-13a-ethyl-N-(4-methoxyphenyl)-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]-naphthyridine-12-carboxamide (**Vin-B12**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 81%). m.p.: 193-195 °C. HPLC: 93.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.48 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.48 (d, *J* = 8.8. Hz, 2H), 7.27 (dd, *J* = 7.2, 2.0 Hz, 1H), 7.13-7.08 (m, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 5.76 (s, 1H), 4.11 (s, 1H), 3.75 (s, 3H), 3.35-3.30 (m, 1H), 3.24-3.16 (m, 1H), 3.05-2.98 (m, 1H), 2.60-2.55 (m, 2H), 2.49-2.45 (m, 1H), 1.92-1.77 (m, 2H), 1.72-1.62 (m, 1H), 1.47-1.44 (m, 1H), 1.35-1.32 (m, 1H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.92-0.82 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.9, 156.7, 133.6, 131.8, 131.1, 130.4, 129.2, 122.5, 122.2, 121.9 (2C), 120.4, 118.4, 114.2 (2C), 111.9, 108.9, 55.8, 55.5, 51.5, 45.0, 37.4, 29.2, 27.3, 20.4, 16.4, 8.7; ESI-MS (*m/z*): 428 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{30}N_3O_2$: 428.2338, Found: 428.2333.

*3.2.19. (4^1S,13aS)-13a-ethyl-N,N-dimethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5] naphthyridine-12-carboxamide (**Vin-B13**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give an off-white solid (Yield 85%). m.p.: 268-27 °C. HPLC: 94.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 7.2 Hz, 1H), 7.15-7.08 (m, 3H), 5.29 (s, 1H), 4.22 (s, 1H), 3.39-3.34 (m, 1H), 3.30-3.23 (m, 1H), 3.16 (s, 3H), 3.07-2.98 (m, 2H), 2.73-2.67 (m, 3H), 2.53-2.48 (m, 1H), 1.98-1.90 (m, 2H), 1.85-1.68 (m, 2H), 1.57-1.53 (m, 1H), 1.46-1.41 (m, 1H), 1.22-1.14 (m, 1H), 0.99 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 133.4 (2C), 130.2, 128.9, 122.5, 120.4, 118.5 (2C), 110.1, 108.6, 55.9, 51.9, 45.3, 37.3, 35.1, 29.8, 27.5 (2C), 20.6, 16.5, 8.9; ESI-MS (*m/z*): 350 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}N_3O$: 350.2232, Found: 350.2227.

*3.2.20. (4^1S,13aS)-N,N,13a-triethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridine-12-carboxamide (**Vin-B14**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 90%). m.p.: 226-228 °C. HPLC: 98.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.2 Hz, 1H), 7.18-7.28 (m, 3H), 5.24 (s, 1H), 4.80 (s, 1H), 3.78-3.81 (m, 1H), 3.66-3.67 (m, 2H), 3.33-3.36 (m, 1H), 3.07-3.20 (m, 3H), 2.12-2.31 (m, 4H), 1.68-1.77 (m, 1H), 1.09-1.39 (m, 11H), 0.84-0.91 (m, 1H); ESI-MS (*m/z*): 378 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O$: 378.2545, Found: 378.2542.

*3.2.21. (4^1S,13aS)-13a-ethyl-N,N-dipropyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5] naphthyridine-12-carboxamide (**Vin-B15**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 86%). m.p.: 302-304 °C. HPLC: 96.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.0 Hz, 1H), 7.08-7.28 (m, 3H), 5.19 (s, 1H), 4.23 (s, 1H), 3.54-3.61 (m, 1H), 3.27-3.40 (m, 3H), 3.00-3.09 (m, 2H), 2.50-2.68 (m, 3H), 1.93-2.02 (m, 1H), 1.77-1.83 (m, 4H), 1.44-1.55 (m, 4H), 1.18-1.24 (m, 1H), 0.90-1.02 (t, *J* = 7.6 Hz, 6H), 0.75-0.90 (m, 4H); ESI-MS (*m/z*): 406 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{36}N_3O$: 406.2858, Found: 406.2854.

*3.2.22. (4^1S,13aS)-N,N-dibutyl-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5] naphthyridine-12-carboxamide (**Vin-B16**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 85%). m.p.: 252-254 °C. HPLC: 93.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.0 Hz, 1H), 7.10-7.27 (m, 3H), 5.10-5.20 (m, 1H), 4.19-4.25 (m, 1H), 3.52-3.61 (m, 2H), 3.26-3.39 (m, 3H), 2.94-3.08 (m, 4H), 2.67-2.74 (m, 2H), 2.50-2.54 (m, 1H), 1.93-1.97 (m, 1H), 1.62-1.87 (m, 5H), 1.42-1.51 (m, 4H), 0.90-1.08 (m, 9H), 0.75-0.89 (m, 2H); ESI-MS (*m/z*): 434 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{40}N_3O$: 434.3171, Found: 434.3167.

*3.2.23. ((4^1S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)(pyrrolidin-1-yl)methanone (**Vin-B17**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a white solid (Yield 81%). m.p.: 185-187 °C. HPLC: 95.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.44 (m, 1H), 7.12-7.09 (m, 3H), 5.32 (s, 1H), 4.20 (s, 1H), 3.75-3.62 (m, 2H), 3.38-3.30 (m, 2H), 3.29-3.22 (m, 1H), 3.07-2.98 (m, 2H), 2.71-2.67 (m, 1H), 2.65-2.64 (m, 1H), 2.53-2.47 (m, 1H), 2.00-1.89 (m, 2H), 1.86-1.77 (m, 4H), 1.74-1.66 (m, 1H), 1.56-1.53 (m, 1H), 1.44-1.39 (m, 1H), 1.19-1.11 (m, 1H), 0.99 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.0, 133.4, 131.2, 130.4, 128.8, 122.4, 120.3, 118.5, 117.6, 110.1, 108.4, 55.9, 51.8, 48.0, 46.0, 45.2, 37.2, 29.7, 27.5, 25.9, 24.4, 20.6, 16.4, 8.8; ESI-MS (*m/z*): 376 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{30}N_3O$: 376.2389, Found: 376.2385.

*3.2.24. ((4^1S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)(piperidin-1-yl)methanone (**Vin-B18**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 85%). m.p.: 246-247 °C. HPLC: 99.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 6.4 Hz, 1H), 7.25-7.10 (m, 3H), 5.30 (s, 1H), 5.09 (s, 1H), 4.30-4.22 (m, 1H), 3.91-3.63 (m, 3H), 3.41-3.34 (m, 1H), 3.31-3.25 (m, 1H), 3.09-3.01 (m, 1H), 2.80-2.68 (m, 2H), 2.56-2.50 (m, 1H), 2.02-1.94 (m, 2H), 1.83-1.44 (m, 9H), 1.25-1.19 (m, 1H), 1.01 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 390 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{32}N_3O$: 390.2545, Found: 390.2542.

*3.2.25. ((4^1S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)(morpholino)methanone (**Vin-B19**)*

The crude product was purified by flash chromatography (DCM/MeOH = 50/1) to give a light yellow solid (Yield 78%). m.p.: 296-297 °C. HPLC: 99.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 6.4 Hz, 1H), 7.19-7.10 (m, 3H), 5.37 (s, 1H), 5.10 (s, 1H), 4.28-4.16 (m, 1H), 3.86-3.68 (m, 7H), 3.39-3.34 (m, 1H), 3.29-3.23 (m, 1H), 3.07-2.98 (m, 1H), 2.66-2.65 (m, 2H), 2.53-2.48 (m, 1H), 1.98-1.89 (m, 1H), 1.85-1.69 (m, 2H), 1.57-1.53 (m, 1H), 1.45-1.42 (m, 1H), 1.21-1.13 (m, 1H), 0.99 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 392 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{30}N_3O_2$: 392.2338, Found: 392.2334.

*3.2.26. General procedure for the synthesis of **Vin-C01***

To a stirred solution of **Vin-A01** (1.68 g, 5 mmol) in anhydrous THF (20 mL) was added $LiAlH_4$ (475 mg, 12.5 mmol) at -50°C. The mixture was stirred at -50°C for 0.5 h, and TLC

indicated that the reaction was completed. A small amount of MeOH was added to quench the reaction. The reaction mixture was evaporated to dryness under vacuum and diluted with H_2O (20 mL), and then extracted with DCM (15 mL × 3). The combined organic phase was washed with H_2O (30 mL) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain **Vin-C01** as a light yellow solid (Yield 96%). m.p.: 203-204 °C. HPLC: 99.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.21-7.17 (m, 1H), 7.14-7.10 (m, 1H), 5.09 (s, 1H), 4.81 (d, *J* = 13.2 Hz, 1H), 4.59 (d, *J* = 13.2 Hz, 1H), 4.12 (s, 1H), 3.32-3.27 (m, 1H), 3.21-3.13 (m, 1H), 3.04-2.95 (m, 1H), 2.73-2.67 (m, 1H), 2.63-2.61 (m, 1H), 2.48-2.44 (m, 1H), 1.90-1.73 (m, 2H), 1.71-1.62 (m, 1H), 1.46-1.34 (m, 2H), 1.14-1.07 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.6, 133.8, 131.1, 129.0, 122.3, 119.9, 118.3, 116.6, 112.5, 108.0, 62.5, 56.5, 51.7, 45.1, 36.6, 29.7, 27.4, 20.5, 16.4, 8.8; ESI-MS (*m/z*): 309 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{25}N_2O$: 309.1967, Found: 309.1960.

3.2.27. General procedure for the synthesis of ***Vin-D01~D05***

To a stirred solution of **Vin-C01** (308 mg, 1 mmol) and Et_3N (202 mg, 2 mmol) in DCM (10 mL) was added acyl chloride R_4COCl (1.5 mmol) at room temperature. The mixture was stirred at room temperature for 2 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL), and then washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain compounds **Vin-D01~D04**. While compound **Vin-D05** was obtained following purification by silica gel chromatography.

*3.2.28. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl acetate (**Vin-D01**)*

The product was a brown oil (Yield 97%). HPLC: 96.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.0 Hz, 1H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.12-7.21 (m, 2H), 5.37 (d, *J* = 13.2 Hz, 1H), 5.21 (s, 1H), 4.96 (d, *J* = 13.2 Hz, 1H), 4.23 (s, 1H), 3.35-3.40 (m, 1H), 3.23-3.31 (m, 1H), 3.01-3.10 (m, 1H), 2.67-2.77 (m, 2H), 2.50-2.55 (m, 1H), 2.11 (s, 3H), 1.93-2.00 (m, 1H), 1.70-1.81 (m, 2H), 1.42-1.50 (m, 2H), 1.11-1.19 (m, 1H), 1.01-1.04 (t, *J* = 7.6, 3H); ESI-MS (*m/z*): 351 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{27}N_2O_2$: 351.2073, Found: 351.2070.

*3.2.29. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl propionate (**Vin-D02**)*

The product was a tawny oil (Yield 95%). HPLC: 94.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.20-7.11 (m, 2H), 5.34 (d, *J* = 13.2 Hz, 1H), 5.18 (s, 1H), 4.94 (d, *J* = 13.2 Hz, 1H), 4.44 (s, 1H), 3.44-3.41 (m, 1H), 3.40-3.35 (m, 1H), 3.07-2.98 (m, 1H), 2.95-2.92 (m, 1H), 2.82-2.75 (m, 1H), 2.71-2.66 (m, 1H), 2.36-2.31 (m, 2H), 2.03-1.94 (m, 1H), 1.88-1.83 (m, 1H), 1.82-1.73 (m, 1H), 1.46-1.42 (m, 1H), 1.28-1.25 (m, 1H), 1.13 (t, *J* = 7.2 Hz, 3H), 1.05-1.03 (m, 1H), 0.99 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 365 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_2$: 365.2229, Found: 365.2224.

*3.2.30. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl butyrate (**Vin-D03**)*

The product was a yellow oil (Yield 96%). HPLC: 97.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 7.18-7.10 (m, 2H), 5.35 (d, *J* = 14.4 Hz, 1H), 5.18 (s, 1H), 4.94 (d, *J* = 14.4 Hz, 1H), 4.26 (s, 1H), 3.39-3.34 (m, 1H), 3.31-3.24 (m, 1H), 3.07-2.98 (m, 1H), 2.73-2.71 (m, 2H), 2.57-2.52 (m, 1H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.00-1.91 (m, 1H), 1.80-1.71 (m, 2H), 1.68-1.61 (m, 2H), 1.48-1.40 (m, 2H), 1.17-1.10 (m, 1H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 379 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{31}N_2O_2$: 379.2386, Found: 379.2378.

*3.2.31. ((41S,13aS)-13a-ethyl-2,3,41,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl isobutyrate (**Vin-D04**)*

The product was a yellow oil (Yield 93%). HPLC: 96.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.19-7.08 (m, 2H), 5.34 (d, *J* = 12.8 Hz, 1H), 5.17 (s, 1H), 4.92 (d, *J* = 12.8 Hz, 1H), 4.31 (s, 1H), 3.42-3.35 (m, 1H), 3.35-3.27 (m, 1H), 3.08-2.97 (m, 1H), 2.81-2.70 (m, 2H), 2.63-2.50 (m, 2H), 2.02-1.91 (m, 1H), 1.83-1.70 (m, 2H), 1.50-1.40 (m, 2H), 1.17 (t, *J* = 7.2 Hz, 3H), 1.13 (t, *J* = 7.2 Hz, 3H), 0.99 (t, *J* = 7.2 Hz, 3H), 0.97-0.92 (m, 1H); ESI-MS (*m/z*): 365 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{31}N_2O_2$: 379.2386, Found: 379.2378.

*3.2.32. ((41S,13aS)-13a-ethyl-2,3,41,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl benzoate (**Vin-D05**)*

The crude product was purified by flash chromatography (PE/acetion = 5/1) to give a light yellow solid (Yield 75%). m.p.: 83-85 °C. HPLC: 97.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.58 (d, *J* = 8 Hz, 1H), 7.53-7.49 (m, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.20-7.11 (m, 2H), 5.55 (d, *J* = 13.2 Hz, 1H), 5.30 (s, 1H), 5.27 (d, *J* = 13.2 Hz, 1H), 4.35 (s, 1H), 3.46-3.41 (m, 1H), 3.38-3.30 (m, 1H), 3.11-3.02 (m, 1H), 2.81-2.80 (m, 1H), 2.78-2.75 (m, 1H), 2.63-2.57 (m, 1H), 2.07-1.98 (m, 1H), 1.86-1.75 (m, 2H), 1.52-1.44 (m, 2H), 1.23-1.15 (m, 1H), 1.03 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 413 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{29}N_2O_2$: 413.2229, Found: 413.2225.

3.2.33. General procedure for the synthesis of ***Vin-E01~E09***

To a stirred solution of **Vin-C01** (308 mg, 1 mmol) and 4-nitrophenyl carbonochloridate (503 mg, 2.5 mmol) in DCM (10 mL) was added pyridine (237 mg, 3 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL), and then washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum. The intermediate was dissolved in DCM (10 mL) and then amine R^5NH2 (3 mmol) was added. The mixture was stirred at room temperature for 1 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL), and then washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude products. The appropriate compounds (**Vin-E01~Vin-E09)** were obtained following purification by silica gel chromatography.

*3.2.34. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl carbamate (**Vin-E01**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 47%). m.p.: 128-130 °C. HPLC: 97.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.18-7.14 (m, 1H), 7.11-7.07 (m, 1H), 5.46 (d, *J* = 12.8 Hz, 1H), 5.12 (s, 1H), 4.70

(d, *J* = 12.8 Hz, 1H), 3.96 (s, 1H), 2.74-2.69 (m, 1H), 2.62-2.48 (m, 2H), 2.43-2.35 (m, 2H), 1.89-1.66 (m, 2H), 1.62-1.52 (m, 2H), 1.40-1.29 (m, 2H), 1.06-0.96 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.0, 133.8, 131.0, 130.5, 129.0, 122.3, 119.9, 119.8, 118.3, 111.9, 108.3, 64.5, 55.3, 50.4, 44.8, 36.8, 29.5, 27.1, 20.4, 15.7, 8.8; ESI-MS (*m/z*): 352 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{26}N_3O_2$: 352.2025, Found: 352.2021.

*3.2.35. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl methylcarbamate (**Vin-E02**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 51%). m.p.: 169-171 °C. HPLC: 92.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.32 (m, 3H), 7.15-7.12 (m, 1H), 7.10-7.05 (m, 1H), 5.47 (d, *J* = 12.8 Hz, 1H), 5.07 (s, 1H), 4.67 (d, *J* = 12.8 Hz, 1H), 3.88 (s, 1H), 2.76 (d, *J* = 4.4 Hz, 3H), 2.60-2.55 (m, 1H), 2.52-2.46 (m, 2H), 2.41-2.38 (m, 1H), 2.26-2.18 (m, 1H), 1.82-1.60 (m, 2H), 1.56-1.49 (m, 1H), 1.44-1.26 (m, 3H), 1.03-0.99 (m, 1H), 0.95 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.9, 133.7, 131.2, 130.4, 128.9, 122.2, 119.7, 119.4, 118.1, 112.0, 108.1, 64.2, 55.1, 50.1, 44.9, 36.7, 29.5, 27.3, 27.0, 20.4, 15.6, 8.7; ESI-MS (*m/z*): 366 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}N_3O_2$: 366.2182, Found: 366.2177.

*3.2.36. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl ethylcarbamate (**Vin-E03**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 64%). m.p.: 218-220 °C. HPLC: 98.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.46 (m, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 7.16-7.12 (m, 1H), 7.09-7.06 (m, 1H), 5.51 (d, *J* = 12.8 Hz, 1H), 5.07 (s, 1H), 4.61 (d, *J* = 12.8 Hz, 1H), 3.89 (s, 1H), 3.26-3.19 (m, 2H), 2.53-2.42 (m, 3H), 2.36-2.34 (m, 1H), 2.19-2.11 (m, 1H), 1.82-1.64 (m, 2H), 1.56-1.47 (m, 1H), 1.37-1.27 (m, 3H), 1.13 (t, *J* = 7.2 Hz, 3H), 1.03-0.95 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.2, 133.7, 131.2, 130.5, 129.0, 122.1, 119.7, 119.4, 118.2, 112.1, 108.2, 64.3, 55.0, 50.1, 44.8, 36.8, 35.7, 29.5, 27.1, 20.4, 15.5, 15.1, 8.7; ESI-MS (*m/z*): 380 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O_2$: 380.2338, Found: 380.2335.

*3.2.37. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl propylcarbamate (**Vin-E04**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 62%). m.p.: 192-195 °C. HPLC: 97.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.32 (m, 3H), 7.15-7.11 (m, 1H), 7.09-7.05 (m, 1H), 5.50 (d, *J* = 12.8 Hz, 1H), 5.08 (s, 1H), 4.61 (d, *J* = 12.8 Hz, 1H), 3.89 (s, 1H), 3.19-3.09 (m, 2H), 2.56-2.45 (m, 3H), 2.36-2.34 (m, 1H), 2.21-2.13 (m, 1H), 1.84-1.63 (m, 2H), 1.56-1.43 (m, 3H), 1.37-1.28 (m, 3H), 1.03-0.92 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 133.7, 131.2, 130.6, 129.0, 122.1, 119.7, 119.4, 118.2, 112.1, 108.2, 64.3, 55.1, 50.1, 44.9, 42.8, 36.8, 29.5, 27.1, 23.3, 20.5, 15.6, 11.4, 8.7; ESI-MS (*m/z*): 394 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O_2$: 394.2495, Found: 394.2491.

*3.2.38. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl butylcarbamate (**Vin-E05**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 62%). m.p.: 166-167 °C. HPLC: 99.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.6, 1.2Hz, 2H), 7.17-7.06 (m, 2H), 6.17-6.11 (m, 1H), 5.42 (d, *J* = 12.8 Hz, 1H), 5.12 (s, 1H), 4.75 (d, *J* = 12.8 Hz, 1H), 4.02 (s, 1H), 3.23-3.11 (m, 3H), 2.93-2.85 (m, 1H), 2.78-2.54 (m, 3H), 2.52-2.45 (m, 1H), 1.89-1.81 (m, 2H), 1.75-1.67 (m, 2H), 1.65-1.57 (m, 1H), 1.51-1.41 (m, 2H), 1.39-1.31 (m, 2H), 1.10-1.00 (m, 1H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 408 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{25}H_{34}N_3O_2$: 408.2651, Found: 408.2647.

*3.2.39. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl (2-hydroxyethyl)carbamate (**Vin-E06**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 65%). m.p.: 107-109 °C. HPLC: 97.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.0 Hz, 2H), 7.18-7.06 (m, 3H), 5.47 (d, *J* = 12.8 Hz, 1H), 5.11 (s, 1H), 4.72 (d, *J* = 12.8 Hz, 1H), 3.93 (s, 1H), 3.72-3.66 (m, 2H), 3.37-3.27 (m, 2H), 2.78-2.70 (m, 1H), 2.67-2.55 (m, 1H), 2.54-2.48 (m, 1H), 2.47-2.35 (m, 3H), 1.86-1.77 (m, 1H), 1.66-1.51 (m, 2H), 1.42-1.29 (m, 2H), 1.06-0.99 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 396 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O_3$: 396.2287, Found: 396.2285.

*3.2.40. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl (3-hydroxypropyl)carbamate (**Vin-E07**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 43%). m.p.: 98-100 °C. HPLC: 95.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.32 (m, 2H), 7.26-7.20 (m, 1H), 7.18-7.12 (m, 1H), 7.11-7.05 (m, 1H), 5.50 (d, *J* = 12.8 Hz, 1H), 5.11 (s, 1H), 4.67 (d, *J* = 12.8 Hz, 1H), 3.92 (s, 1H), 3.67 (d, *J* = 6.0 Hz, 2H), 3.39-3.25 (m, 2H), 2.69-2.48 (m, 4H), 2.44-2.38 (m, 1H), 2.34-2.26 (m, 1H), 1.84-1.75 (m, 1H), 1.73-1.48 (m, 5H), 1.42-1.30 (m, 2H), 1.06-1.00 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 410 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O_3$: 410.2444, Found: 410.2441.

*3.2.41. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl isopropylcarbamate (**Vin-E08**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 55%). m.p.: 225-227 °C. HPLC: 96.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.26-7.06 (m, 2H), 6.34 (s, 1H), 5.45 (d, *J* = 12.8 Hz, 1H), 5.10 (s, 1H), 4.68 (d, *J* = 12.8 Hz, 1H), 3.99 (s, 1H), 3.88-3.81 (m, 1H), 2.80-2.77 (m, 1H), 2.69-2.62 (m, 1H), 2.58-2.51 (m, 2H), 2.49-2.44 (m, 1H), 1.89-1.80 (m, 1H), 1.75-1.66 (m, 2H), 1.61-1.52 (m, 1H), 1.40-1.32 (m, 2H), 1.28-1.24 (m, 1H), 1.14 (d, *J* = 6.4 Hz, 6H), 1.07-1.03 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H), 0.87-0.82 (m, 1H); ESI-MS (*m/z*): 394 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O_2$: 394.2495, Found: 394.2491.

*3.2.42. ((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl phenylcarbamate (**Vin-E09**)*

The crude product was purified by flash chromatography (PE/EtOAc = 5/1) to give a light yellow solid (Yield 60%). m.p.: 196-199 °C. HPLC: 98.9%. ^{1}H NMR (400 MHz, DMSO) δ 9.80 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.30-7.23 (m, 2H), 7.13-7.08 (m, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 7.02-6.96 (m, 1H), 5.48 (d, *J* = 12.8 Hz, 1H), 5.41 (s, 1H), 4.98 (d, *J* = 12.8 Hz, 1H), 4.05 (s, 1H), 3.25-3.18 (m, 1H), 3.17-3.08 (m, 1H), 2.97-2.87 (m, 1H), 2.59-2.53 (m, 2H), 2.45-2.38 (m, 1H), 1.86-1.70 (m, 2H), 1.66-1.51 (m, 1H), 1.47-1.41 (m, 1H), 1.39-1.32 (m, 1H), 1.03-0.92 (m, 4H); ESI-MS (*m/z*): 428 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{30}N_3O_2$: 428.2338, Found: 428.2334.

*3.2.43. General procedure for the synthesis of **Vin-F01***

To a stirred solution of **Vin-C01** (308 mg, 1 mmol), phthalimide (22 mg, 1.5 mmol) and PPh_3 (394 mg, 1.5 mmol) in anhydrous THF (4 mL) was added DIAD (303 mg, 1.5 mmol) after stirring at room temperature for 15 min. The mixture was heated to reflux for 1.5 h, and TLC indicated that the reaction was completed. Hydrazine hydrate (2 mL) was added and the reaction mixture was heated to reflux for another 1 h under N_2 atmosphere. After completion, the reaction mixture was evaporated to dryness under vacuum and then diluted with DCM (50 mL). The organic layer was washed with H_2O (30 mL × 2), and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude product which was further purified by silica gel chromatography (DCM/MeOH = 10/1) to give compound **Vin-F01** as a red brown oil (Yield 45%). HPLC: 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.25-7.13 (m, 2H), 5.07 (s, 1H), 4.50 (s, 1H), 4.18 (d, *J* = 14.8 Hz, 1H), 3.86 (d, *J* = 14.8 Hz, 1H), 3.59-3.54 (m, 1H), 3.50-3.43 (m, 1H), 3.10-3.01 (m, 2H), 2.89-2.77 (m, 2H), 2.02-1.96 (m, 1H), 1.95-1.83 (m, 2H), 1.54-1.50 (m, 1H), 1.32-1.30 (m, 1H), 1.21-1.13 (m, 1H), 1.02 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 308 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{26}N_3$: 308.2127, Found: 308.2123.

3.2.44. General procedure for the synthesis of ***Vin-F02~F05***

To a stirred solution of **Vin-C01** (308 mg, 1 mmol) and TsCl (953 mg, 5 mmol) in DCM (6 mL) was added amine R^6NH_2 (3mmol) and Et_3N (303 mg, 3 mmol) at room temperature. The mixture was stirred at room temperature for 8 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL) and the organic layer was washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude product which was further purified by silica gel chromatography to give compounds **Vin-F02~Vin-F05**.

*3.2.45. 1-((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)-N-methylmethanamine (**Vin-F02**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a colorless oil (Yield 45%). HPLC: 96.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.19-7.09 (m, 2H), 5.00 (s, 1H), 4.16 (s, 1H), 4.14 (d, *J* = 14.4 Hz, 1H), 3.68 (d, *J* = 14.4 Hz, 1H), 3.38-3.33 (m, 1H), 3.29-3.21 (m, 1H), 3.08-2.99 (m, 1H), 2.74-2.63 (m, 2H), 2.53-2.48 (m, 4H), 2.48 (s, 3H), 1.97-1.90 (m, 1H), 1.77-1.67 (m, 2H), 1.46-1.43 (m, 1H), 1.31-1.25 (m, 1H), 1.17-1.08 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 322 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{28}N_3$: 322.2283, Found: 322.2280.

*3.2.46. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)ethanamine (**Vin-F03**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light-yellow oil (Yield 53%). HPLC: 98.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.19-7.09 (m, 2H), 5.00 (s, 1H), 4.16 (s, 1H), 4.14 (d, *J* = 14.4 Hz, 1H), 3.68 (d, *J* = 14.4 Hz, 1H), 3.38-3.33 (m, 1H), 3.29-3.21 (m, 1H), 3.08-2.99 (m, 1H), 2.74-2.63 (m, 2H), 2.53-2.49 (m, 4H), 2.48 (s, 3H), 1.97-1.90 (m, 1H), 1.77-1.67 (m, 2H), 1.46-1.43 (m, 1H), 1.31-1.25 (m, 1H), 1.17-1.08 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 336 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{30}N_3$: 336.2440, Found: 336.2436.

*3.2.47. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)propan-1-amine (**Vin-F04**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light-yellow oil (Yield 48%). HPLC: 96.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.19-7.09 (m, 2H), 5.00 (s, 1H), 4.16 (s, 1H), 4.14 (d, *J* = 14.4 Hz, 1H), 3.67 (d, *J* = 14.4 Hz, 1H), 3.38-3.33 (m, 1H), 3.28-3.21 (m, 1H), 3.08-2.99 (m, 1H), 2.79-2.70 (m, 2H), 2.69-2.63 (m, 2H), 2.53-2.47 (m, 1H), 1.97-1.88 (m, 1H), 1.78-1.69 (m, 2H), 1.46-1.37 (m, 2H), 1.16-1.09 (m, 4H), 1.12 (t, *J* = 7.2 Hz, 3H), 0.99 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 350 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{32}N_3$: 350.2596, Found: 350.2591.

*3.2.48. N-benzyl-1-((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methanamine (**Vin-F05**)*

The crude product was purified by flash chromatography (PE/acetone = 10/1) to give a colorless oil (Yield 80%). HPLC: 94.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 7.6,1.6 Hz, 1H), 7.46 (dd, *J* = 7.6,1.6 Hz, 1H), 7.43-7.40 (m, 1H), 7.27-7.24 (m, 4H), 7.18-7.10 (m, 2H), 5.19 (s, 1H), 4.18 (d, *J* = 13.2 Hz, 1H), 3.78 (d, *J* = 13.2 Hz, 1H), 3.72 (s, 1H), 3.69 (d, *J* = 13.2 Hz, 1H), 3.48 (d, *J* = 13.2 Hz, 1H), 3.33-3.28 (m, 1H), 3.20-3.13 (m, 1H), 3.07-2.98 (m, 1H), 2.71-2.58 (m, 2H), 2.52-2.47 (m, 1H), 1.91-1.72 (m, 2H), 1.71-1.64 (m, 1H), 1.44-1.31 (m, 2H), 1.11-1.04 (m, 1H), 1.00 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 398 $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{32}N_3$: 398.2596, found: 398.2594.

3.2.49. General procedure for the synthesis of ***Vin-G01~G05***

To a stirred solution of **Vin-F01** (307 mg, 1 mmol) and DIPEA (193 mg, 1.5 mmol) in DCM (10 mL) was added acyl chloride R^7COCl (1.2 mmol) at 0^{o}C. The mixture was stirred at room temperature for 0.5 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL) and the organic layer was washed with H_2O (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude product which was further purified by silica gel chromatography to give compounds **Vin-G01~Vin-G05**.

*3.2.50. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)propionamide (**Vin-G01**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 88%). m.p.: 86-88 ^{o}C. HPLC: 90.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.18-7.14 (m, 1H), 7.13-7.09 (m, 1H), 5.68-5.66 (m, 1H), 5.06 (s, 1H), 4.75 (dd, *J* = 15.26, 4.4 Hz, 1H), 4.35 (dd, *J* = 15.26, 4.4 Hz, 1H), 4.17 (s, 1H), 3.38-3.33 (m, 1H), 3.27-3.19 (m, 1H), 3.07-2.98 (m, 1H), 2.73-2.67 (m, 2H), 2.53-2.48 (m, 1H), 2.20-2.14 (m, 2H), 1.95-1.74 (m, 2H), 1.73-1.66 (m, 1H), 1.47-1.38 (m, 2H), 1.16-1.07 (m, 4H), 0.98 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 133.7, 132.0, 131.3, 129.0, 122.6, 120.1, 118.5, 117.6, 111.7, 108.3, 56.3, 51.5, 45.1, 42.1, 36.8, 29.6, 29.5, 27.3, 20.4, 16.3, 9.9, 8.7; ESI-MS (*m/z*): 364, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{30}N_3O$: 364.2389, found: 364.2483.

*3.2.51. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)butyramide (**Vin-G02**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 86%). m.p.: 92-94 ^{o}C. HPLC: 96.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.45 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.16-7.08 (m, 2H), 5.84-5.82 (m, 1H), 5.05 (s, 1H), 4.73 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.33 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.12 (s, 1H), 3.33-3.29 (m, 1H), 3.20-3.12 (m, 1H), 3.05-2.96 (m, 1H), 2.71-2.60 (m, 2H), 2.48-2.43 (m, 1H), 2.17-2.05 (m, 2H), 1.92-1.73 (m, 2H), 1.71-1.66 (m, 1H), 1.65-1.57 (m, 2H), 1.45-1.37 (m, 2H), 1.13-1.04 (m, 1H), 0.97 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 133.8, 132.0, 131.6, 129.1, 122.6, 120.2, 118.6, 117.9, 111.7, 108.4, 56.4, 51.7, 45.2, 42.1, 38.6, 36.8, 29.7, 27.5, 20.6, 19.2, 16.4, 13.8, 8.8; ESI-MS (*m/z*): 378, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O$: 378.2545, found: 378.2538.

*3.2.52. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)isobutyramide (**Vin-G03**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 80%). m.p.: 194-197 °C. HPLC: 91.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.16-7.07 (m, 2H), 5.75-5.71 (m, 1H), 5.05 (s, 1H), 4.75 (dd, *J* = 15.6, 4.4 Hz, 1H), 4.30 (dd, *J* = 15.6, 4.4 Hz, 1H), 4.14 (s, 1H), 3.36-3.31 (m, 1H), 3.24-3.16 (m, 1H), 3.06-2.97 (m, 1H), 2.72-2.61 (m, 2H), 2.51-2.45 (m, 1H), 2.34-2.24 (m, 1H), 1.93-1.74 (m, 2H), 1.73-1.64 (m, 1H), 1.46-1.37 (m, 2H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.09-1.06 (m, 4H), 0.97 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 133.8, 132.0, 131.7, 129.1, 122.6, 120.2, 118.6, 118.0, 111.8, 108.5, 56.5, 51.8, 45.3, 42.2, 36.9, 35.7, 29.8, 27.5, 20.7, 19.7, 19.6,16.5, 8.8; ESI-MS (*m/z*): 378, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{32}N_3O$: 378.2545, found: 378.2539.

*3.2.53. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)cyclopropanecarboxamide (**Vin-G04**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1)) to give a light yellow solid (Yield 85%). m.p.: 112-114 °C. HPLC: 96.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 7.20-7.10 (m, 2H), 5.84-5.80 (m, 1H), 5.08 (s, 1H), 4.76 (dd, *J* = 17.2, 2.0 Hz, 1H), 4.39 (dd, *J* = 17.2, 2.0 Hz, 1H), 4.16 (s, 1H), 3.39-3.34 (m, 1H), 3.28-3.20 (m, 1H), 3.08-2.99 (m, 1H), 2.73-2.63 (m, 2H), 2.54-2.48 (m, 1H), 1.83-1.74 (m, 2H), 1.73-1.66 (m, 1H), 1.47-1.39 (m, 2H), 1.31-1.24 (m, 1H), 1.14-1.06 (m, 1H), 1.02-0.95 (m, 5H), 0.76-0.66 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 133.8, 132.1, 131.7, 129.1, 122.7, 120.2, 118.6, 118.0, 111.8, 108.4, 56.5, 51.7, 45.3, 42.4, 36.9, 29.8, 27.5, 20.6, 16.5, 14.9, 8.8, 7.5, 7.4; ESI-MS (*m/z*): 376, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{30}N_3O$: 376.2389, found: 376.2383.

*3.2.54. N-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)benzamide (**Vin-G05**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 86%). m.p.: 126-129 °C. HPLC: 93.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.18-7.08 (m, 2H), 6.43-6.40 (m, 1H), 5.13 (s, 1H), 4.89 (dd, *J* = 15.2, 5.2 Hz, 1H), 4.60 (dd, *J* = 15.2, 5.2 Hz, 1H), 4.17 (s, 1H), 3.37-3.33 (m, 1H), 3.26-3.18 (m, 1H), 3.07-3.00 (m, 1H), 2.73-2.67 (m, 2H), 2.48-2.45 (m, 1H), 2.06-1.86 (m, 2H), 1.81-1.73 (m, 1H), 1.48-1.39 (m, 2H), 1.19-1.11 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 412, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{30}N_3O$: 412.2389, found: 412.2384.

*3.2.55. General procedure for the synthesis of **Vin-H01~H04***

To a stirred solution of **Vin-F01** (307 mg, 1 mmol) and 4-nitrophenyl carbonochloridate (302 mg, 1.5 mmol) in DCM (10 mL) was added pyridine (158 mg, 2 mmol) at room temperature. The mixture was stirred at room temperature for 0.5 h, and TLC indicated that the reaction was completed. The reaction mixture was diluted with DCM (40 mL) and the organic layer was washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered, evaporated to dryness and then dissolved in DCM (10 mL). To the solution was added amine R^8NH_2 and the mixture was stirred at room temperature for 1 h. After completion, the reaction mixture was diluted with DCM (40 mL) and the organic layer was washed with H_2O (30 mL × 2), saturated Na_2CO_3 solution (30 mL × 2) and brine (30 mL), then dried over anhydrous Na_2SO_4. The organic layer was filtered and evaporated to dryness under vacuum to obtain the desired crude product which was further purified by silica gel chromatography to give compounds **Vin-H01~H04**.

*3.2.56. 1-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)urea (**Vin-H01**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 76%). m.p.: 208-210 °C. HPLC: 97.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.4 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.12-7.08 (m, 1H), 7.05-7.02 (m, 1H), 5.72-5.70 (m, 1H), 4.99 (s, 1H), 4.95 (s, 2H), 4.46 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.18 (dd, *J* = 15.2, 4.8 Hz, 1H), 3.94 (s, 1H), 3.12-3.07 (m, 1H), 2.90-2.77 (m, 2H), 2.61-2.49 (m, 1H), 2.25-2.22 (m, 2H), 1.80-1.65 (m, 2H), 1.63-1.53 (m, 1H), 1.39-1.30 (m, 2H), 1.03-0.96 (m, 1H), 0.93 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 351, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{27}N_4O$: 351.2185, found: 351.2180.

*3.2.57. 1-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)-3-methylurea (**Vin-H02**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 75%). m.p.: 196-198 °C. HPLC: 98.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.18-7.14 (m, 1H), 7.12-7.08 (m, 1H), 5.05 (s, 1H), 4.95-4.90 (m, 2H), 4.57 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.30 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.03 (s, 1H), 3.26-3.19 (m, 1H), 3.03-2.91 (m, 2H), 2.72 (d, *J* = 4.8 Hz, 3H), 2.69-2.58 (m, 2H), 2.34-2.32 (m, 1H), 1.88-1.73 (m, 2H), 1.71-1.62 (m, 1H), 1.45-1.37 (m, 2H), 1.10-1.03 (m, 1H), 0.97 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 133.8, 133.1, 131.4, 129.0, 122.6, 120.1, 118.5, 117.0, 112.0, 108.2, 56.6, 51.5, 45.2, 42.9, 36.8, 29.6, 27.5, 27.2, 20.5, 16.4, 8.8; ESI-MS (*m/z*): 365, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{29}N_4O$: 365.2341, found: 365.2339.

*3.2.58. 1-ethyl-3-(((4^{1}S,13aS)-13a-ethyl-2,3,4^1,5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)urea (**Vin-H03**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 85%). m.p.: 198-200 °C. HPLC: 95.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 7.10-7.01 (m, 2H), 5.50-5.47 (m, 1H), 5.36-5.34 (m, 1H), 4.97 (s, 1H), 4.46 (dd, *J* = 15.2, 5.2 Hz, 1H), 4.20 (dd, *J* = 15.2, 5.2 Hz, 1H), 3.88 (s, 1H), 3.16-3.04 (m, 3H), 2.88-2.81 (m, 1H), 2.77-2.70 (m, 1H), 2.59-2.49 (m, 2H), 2.23-2.17 (m, 1H), 1.80-1.65 (m, 2H), 1.63-1.53 (m, 1H), 1.37-1.25 (m, 2H), 1.00 (t, *J* = 7.2 Hz, 3H), 0.98-0.93 (m, 1H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.0, 133.9, 133.1, 131.3, 129.0, 122.8, 120.1, 118.4, 116.8, 112.1, 108.2, 56.7, 51.4, 45.2, 42.8, 36.7, 35.3, 29.5, 27.5,

27.2, 20.5, 15.6, 8.8; ESI-MS (*m/z*): 379, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{31}N_4O$: 379.2498, found: 379.2493.

*3.2.59. 1-(((4[1]S,13aS)-13a-ethyl-2,3,4[1],5,6,13a-hexahydro-1H-indolo[3,2,1-de]pyrido[3,2,1-ij][1,5]naphthyridin-12-yl)methyl)-3-phenylurea (**Vin-H04**)*

The crude product was purified by flash chromatography (DCM/MeOH = 10/1) to give a light yellow solid (Yield 87%). m.p.: 314-316 °C. HPLC: 98.8%. ^{1}H NMR (400 MHz, DMSO) δ 8.52 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.22 (t, *J* = 7.6 Hz, 2H), 7.10 (t, *J* = 8.4 Hz, 1H), 7.04 (t, *J* = 8.4 Hz, 1H), 6.90 (t, *J* = 7.6 Hz, 1H), 6.43-6.40 (m, 1H), 5.15 (s, 1H), 4.57 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.38 (dd, *J* = 15.2, 4.8 Hz, 1H), 4.04 (s, 1H), 3.24-3.20 (m, 1H), 3.17-3.08 (m, 1H), 2.98-2.90 (m, 1H), 2.59-2.52 (m, 2H), 2.44-2.39 (m, 1H), 1.84-1.68 (m, 2H), 1.64-1.51 (m, 1H), 1.43-1.40 (m, 1H), 1.35-1.32 (m, 1H), 1.00-0.96 (m, 1H), 0.93 (t, *J* = 7.2 Hz, 3H); ESI-MS (*m/z*): 427, $[M+H]^+$. HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{27}H_{31}N_4O$: 427.2498, found: 427.2496.

3.3. Biological activity assay

3.3.1. Materials

Wortmannin was purchased from Selleck. Antibody against IRS2 was purchased from Santa Cruz Biotechnology and the rest of the antibodies were purchased from Cell Signaling Technology (Boston, USA). Streptozotocin was purchased from Solarbio (Beijing, China). Cell culture medium and supplements were purchased from Hyclone (Logan, Utah, USA).

3.3.2. Cell culture

INS-832/13 cells were cultured in RPMI-1640 medium supplemented with 10% FBS (Gibco, USA), 100 U/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, 1 mM Sodium-pyruvate, 0.05 mM β-mercaptoethanol and 10 mM HEPES. All cells were cultured in a humidified incubator with 5% CO_2 at 37°C.

3.3.3. MTT assay

INS-832/13 cells were plated into 96-well plates at a density of 4×10^4 cells/well. After overnight culture, cells were treated with different concentrations of corresponding compounds and STZ (0.4 mM) for 24 h and then incubated with 0.5 mg/mL MTT for 4 h to form formazan crystals. Finally, these crystals were dissolved in DMSO and the absorbance were determined at 570 nm.

3.3.4. Flow cytometry analysis

INS-832/13 cells were plated into 12-well plates at a density of 1×10^6 cells/well and allowed to grow overnight, then cells were treated with different concentrations of corresponding compounds. The collection and treatment of cells were carried out using FITC Annexin V Apoptosis Detection Kit I (KeyGEN Biotech, China) according to the manufacture's protocol. The number of apoptotic cells was quantified by IntelliCyt iQue Screener PLUS (IntelliCyt, USA).

3.3.5. p-Akt (Ser 473) AlphaLISA assay

INS-832/13 cells were grown in 96-well plates at a density of 5×10^4 cells/well and allowed to attach overnight. Then, cells were incubated with corresponding compounds for 24 h. Cells lysates were collected to examine p-Akt (Ser473) content by using AlphaLISA SureFire Ultra p-Akt (473) Assay Kits (PerkinElmer, USA).

3.3.6. RNA isolation and quantitative real-time PCR

INS-832/13 cells were plated into 96-well plates at a density of 9×10^5 cells/well with overnight culture. The cells were incubated with different concentrations of corresponding compounds for 24 h and washed in PBS, then total mRNA in cells were gathered using TRIzol Reagent (Invitrogen, USA). Synthesis of first-strand cDNA was performed using PrimeScript RT Master Mix (TaKaRa, Japan). The quantity of mRNA was detected by quantitative real-time PCR (qRT-PCR) using the SYBY Premix Ex Taq (Vazyme, USA). PCR primer sequences are listed in Table 4.

Table 4. List of rat primer sequences used in RT-PCR assay

Gene	Forward primer (5'-3')
IRS2	AGCTGGTGGTAGTCATACCC
p21	CAAAGTATGCCGTCGTCTGT
Bim	CCAGGCCTTCAACCATTATCTC
β-actin	GGAGATTACTGCCCTGGCTCCTA

Gene	Reverse primer (5'-3')
IRS2	CAGGTTCATATAGTCAGA
p21	GTCTCAGTGGCGAAGTCAAA
Bim	GCGCAGATCTTCAGGTTCCT
β-actin	GACTCATCGTACTCCTGCTTGCTG

3.3.7. Western blot assay

Total proteins of cells were separated by SDS-PAGE and transferred from gel to nitrocellulose membrane (GE Healthcare). Finally, after incubation with corresponding antibodies overnight, the signals of detected proteins were measured by Tanon-5200Multi with the West-Dura detection system (Tanon, China) (GAPDH antibody dilution, 1:5000; other antibodies dilution, 1:1000).

3.3.8. Caspase 3 and Caspase 9 activity assay

INS-832/13 cells were cultured in white opaque 96-well plates at a density of 4×10^4 cells/well and grown overnight. The activity of Caspase 3 or Caspase 9 in cells treated with corresponding compounds was detected by applying Apo-ONE Homogeneous Caspase-3/7 Assay and Caspase-Glo 9 Assay kit (Promega) according to the manufacture's protocol.

3.3.9. Statistical analysis

Data are reported as the mean ± S.E.M. The significant difference between multiple treatments and the control was analyzed using a one-way ANOVA with Dunnett's post-test. *p* values less than 0.05 were considered statistically significant.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (grant: 81773596), the Natural Science Foundation of Jiangsu Province (grant: BK20180826), Natural science foundation of Jiangsu Higher Education Institutions (grants: 17KJA360004, 18KJB350008), Program for Outstanding Scientific and Technological Innovation Team of Jiangsu Higher Education Institutions, and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

Appendix A. Supplementary data

Supplementary data related to this article can be found at…..

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Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: