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Design, synthesis and structure-activity relationship optimization of phenanthridine derivatives as new Wnt/β-catenin signalling pathway agonists

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ABSTRACT

Phenanthridine derivativeHLY78 has previously been identified as the first Wnt/β-catenin signalling pathway agonist that targets the DAX domain of axin. However, due to the relatively weak activation on the Wnt/β-catenin signalling pathway, HLY78 is insufficient for further pharmacological study. Herein, the structural optimization of HLY78 and analyses of the structure-activity relationships (SARs) of HLY78-derived phenanthridine derivatives as agonists of the Wnt/β-catenin signalling pathway are presented. In this work, 36 derivatives were designed and synthesized with some derivatives exhibiting stronger Wnt activity than the activity of HLY78. In particular, one of them, 8-((1,3-dimethy-pyrazol-5-yl)methoxy)-5-ethyl-4-methyl-5,6-dihydro-phenanthridin-9-ol, exhibited strong Wnt active activity and is 10 times more potent than HLY78. The following SAR analysis suggests that a pyrazole group, especially at the C-8 position, is important for Wnt activation; a methyl group at the C-4position seems to be more beneficial for Wnt activation than ethyl; and oxidation of the C-6 position reduces the Wnt activation.

1. Introduction

Wnt/β-catenin is a highly conserved signalling pathway [1,2] that is important for development. Aberrant Wnt signalling is involved in many diseases, such as cancer (in which the pathway is inappropriately activated), Alzheimer's disease and osteoporosis (in which the pathway is attenuated) [3–5]. In recent years, Wnt antagonists have attracted attention due to the potential applications in the clinical treatment of cancer [6–9]. Appropriate activation of Wnt signalling pathways could also be useful in other clinical applications, such as haematopoietic stem cell (HSC) expansion, the treatment of osteoporosis and vitiligo [10].

Lycorine is a naturally occurring multifunctional benzylphenethylamine alkaloid (Fig. 1a) of considerable interest [11–14]. Previous studies have identified that lycorine derivatives are novel HCV (hepatitis C virus) inhibitors that act via novel mechanisms [15,16]. A new small-molecule activator of the Wnt/β-catenin signalling pathway, 4-ethyl-5-methyl-5,6-dihydro-[1,3] dioxolo [4,5-j] phenanthridine (HLY78), which acts in a Wnt ligand-dependent manner, has also been identified. Mechanistic studies have indicated that HLY78 targets the DAX domain of axin and potentiates axin/LRP6 association, which subsequently promotes LRP6 phosphorylation and Wnt signal transduction (Fig. 1) [18]. These findings not only provide new insights into the regulation of the Wnt/β-catenin signalling pathway via a Wnt-specific small molecule but also may facilitate therapeutic applications, such as HSC expansion and the treatment of osteoporosis and vitiligo.

However, the activity of HLY78 is insufficient for further pharmacological study. To identify a Wnt agonist, the structure of HLY78 must be further optimized using rational drug design approaches. Here, the structural optimization of phenanthridine analogues based on X-ray

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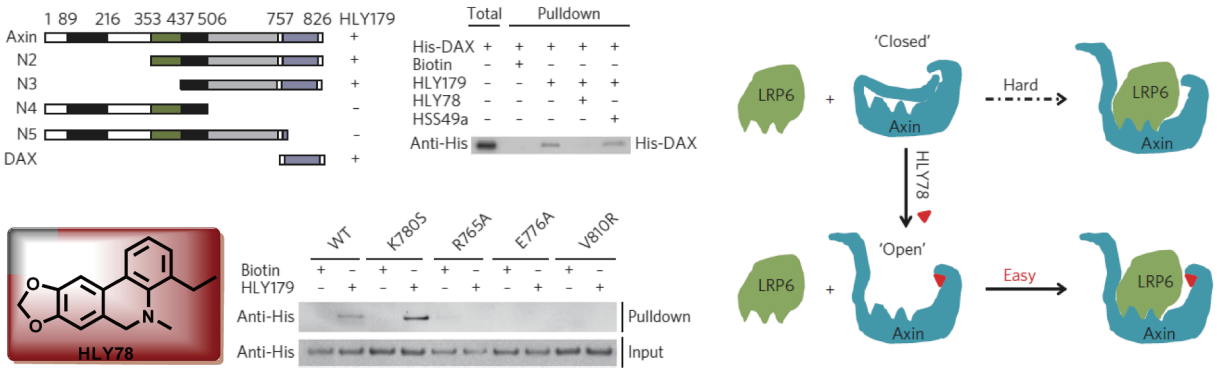


Fig. 1. HLY78 and the mode of action on active the Wnt/**β**-catenin signalling pathway. HLY78 could affect the stability of the LRP6-Axin transcription complex to regulate the activation of Wnt by targeting axin [18].

diffraction and molecular docking techniques is described, and an evaluation of derivatives that exhibit Wnt-activating effects in vitro are presented. The structure-activity relationships (SARs) of these derivatives are also analysed.

2. Results and discussion

2.1. Design and synthesis of phenanthridine derivatives

As shown in Fig. 2, our initial attempts to modify the structure of HLY78 focused on manipulating the C-4substituents (highlighted in red) and C-6substituents (highlighted in pink). In addition, we investigated the effects of modifying the properties of the nitrogen atom at the 5-position of the HLY78 skeleton (highlighted in blue) and the benzodioxole group (highlighted in green). Nine phenanthridine derivatives were ultimately synthesized, bearing various combinations of these modifications, but unfortunately, such modifications did not lead to active compounds [17].

The aforementioned failed attempts caused an in-depth structural analysis of HLY78. Predictionsof the binding modes between HLY78 and the target protein axin were initially conducted. The X-ray crystal structure of axin (PDB 1WSP) [19] and HLY78 were examined to predict the binding modes; molecular docking of HLY78 into the axin binding domain, DAX, was analysed using AutoDock [20]. All the conformations of HLY78 docking to the DAX domain were analysed, and six of the conformations were localized to the same cavity formed by the juxtaposition of residues from two neighbouring protomers. The modelled complex structure of axin-HLY78 revealed that a group of residues in the cavity interact with HLY78 via electrostatic or hydrophobic interactions. The docking results indicated that the binding of the phenanthridine compound to axin likely occurs via the substituents at C-8 and C-9, and due to the steric hindrance in the active cavity of axin, an ethyl group at the C-4 position seems to adversely affect the entrance of compounds into the narrow cavity. In addition, amidation at C-6 also affects the binding of the compounds to the protein (Fig. 2). Consequently, it is necessary to appropriately modify the phenanthridine skeleton of HLY78, and eight phenanthridineskeletons (**5–12**) that focused on the optimization of the C-4, N-5 and C-6 positions were designed and synthesized by referencing the results of the docking analysis (Fig. 3). Another23phenanthridine derivatives bearing different substituents at C-8 or C-9were further designed and synthesized (Scheme1).

2.2. The Wnt activity of phenanthridine derivatives

The effects of all 36 derivatives on the reporter gene of the Wnt/βcatenin signalling pathway were evaluated in HEK293T cells using HLY78 as a positive control. As shown in Table 1, compounds **3f** and **3g** exhibit much stronger Wnt activity than the activity of HLY78, and **3f** in particular could double the activation of the Wnt signalling pathway at the low concentration of 0.5 μM, which is 10 times more potent than HLY78. Furthermore, the Wnt activation effects of compounds **1d**, **2d**, **2f**, **4** and **6f**are equal to HLY78. In addition, nine of these compounds (**1**, **1e**, **2**, **2d**, **3**, **3d**, **3e**, **4e** and **5b**) show weaker Wnt activity than that of HLY78 (Fig. 4). The other remaining phenanthridine derivatives did not activate the Wnt/**β**-catenin pathway, even at high concentrations. In addition, a dose response analysis of compounds 3f and 3g were conducted and the EC_{50} value of 3f and 3g is 0.27 ± 0.11 μM and 0.79 ± 0.21 μM, respectively. To further confirm the activating function, the effects of compounds **3f** and **3g** on the expression of the endogenous Wnt target genes (*Axin2* and *NKD1*) were tested. These compounds could clearly upregulate the expression of endogenous Wnt target genes (Fig. 5).

2.3. SAR analysis of the Wnt activation effects of phenanthridine derivatives

The SAR study initially focused on investigating the importance of

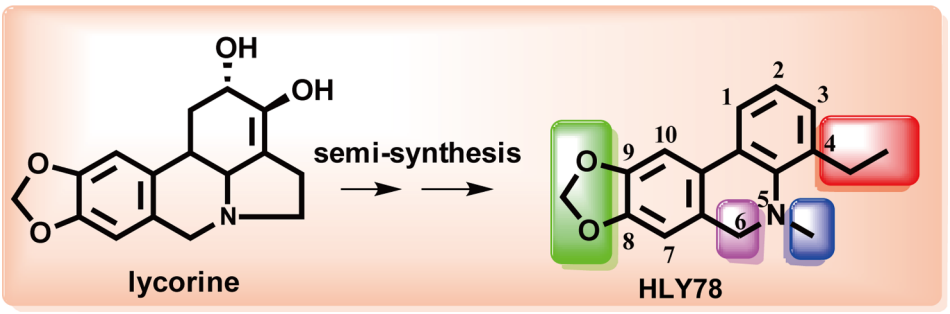


Fig. 2. Regions of the phenanthridine skeleton that were targeted for modification. The C-4substituent (highlighted in red); the N-5substituent (highlighted in blue); the C-6substituent (highlighted in pink); and the benzodioxole group between C-8 and C-9 (highlighted in green).

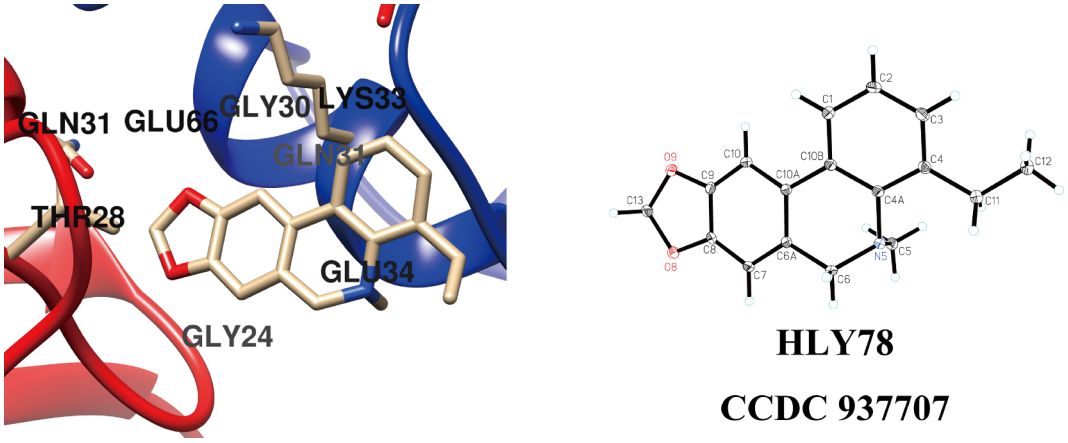


Fig. 3. Molecular docking of HLY78 into the axin binding domain, DAX, using AutoDock.

the substituents at the C-4 and N-5 positions. When comparing the previously reportedC-4 ethyl-substituted compounds, the newly synthesized derivatives bearing a C-4 methyl substituent, such as **3e**, **4e**, and **2g**, exhibit stronger Wnt activation than that of the equivalent compounds that bear C-4 ethyl substituents. From these results, it can be concluded that relative to an ethyl group, a methyl substituent at the C-4 position would improve the Wnt activation of phenanthridine derivatives. On the other hand, substituents at the N-5 position seem to have limited effects on the Wnt activities of the compounds.

The nitrogen atoms of alkaloids are known to have profound effects on their biological activities. The amidated derivatives **5a-d**, **6a-h** and **8a-b**were therefore prepared to determine how the conversion of the parent compound's basic nitrogen into an amide would affect its biological activity. Nearly all of these derivatives had substantially lower activity thanthat of the equivalent non-amidated compounds, regardless of the other substituents. The basic nitrogen at the N-5 position thus seems be very important to Wnt activity.

The SAR analysis of the phenanthridine derivatives was continued by investigating the effects of varying the substituents on the C-8 or C-9 oxygen atoms of the phenanthridine skeleton. Nitrogen heterocyclic, aromatic, and linear alkyl derivatives were therefore prepared and investigated. Among these compounds, the ones bearing smaller nitrogen heterocyclic substituents at C-8 or C-9 (**2f** and **2g**) had a stronger effect on Wnt than bigger heterocycles (**2h**), aromatics (**5c**) and linear alkyl (**1e**, **2e** and **3e**) derivatives. These results indicated that a smaller nitrogen heterocyclic group at the C-8 or C-9 position was good for Wnt activation, and linear alkyl groups are likewise helpful to Wnt activation, except that the improvements are limited. However, the bigger nitrogen heterocycles and aromatic groups would diminish the activity of a compound.

There is a phenomenon that was quite interesting through comparison of compounds **3f** and **3g**. Compound **3f**bears a nitrogen heterocyclic substituent at the C-8 position, and displays better performance than **3g**, which bears the same substituent but at the C-9 position. Subsequent docking analysis of **3f** and **3g**indicated that**3f**can associate with the protein residues in the axin cavity in which HLY78 docks tighter than **3g** does (Fig. 6). These result simply that substituents at the C-8 position of phenanthridine have a greater influence on Wnt activation.

3. Conclusions

HLY78 could become a more efficient Wnt/*β*-catenin signalling pathway agonist through appropriate modifications. According to the process of the structure–activity relationship optimization of phenanthridine derivatives, it could be concluded that the C-4methyl substituent and the basic nitrogen at the N-5 position are crucial to Wnt activation activity. The nitrogen heterocyclic substitution, especially smaller nitrogen heterocycles such as pyrazole or pyridineat the C-8 or C-9 position should improve the Wnt activity of phenanthridine derivatives. These findings provide constructive guidance for further structural modifications of lycorine.

4. Experimental

4.1. Chemistry

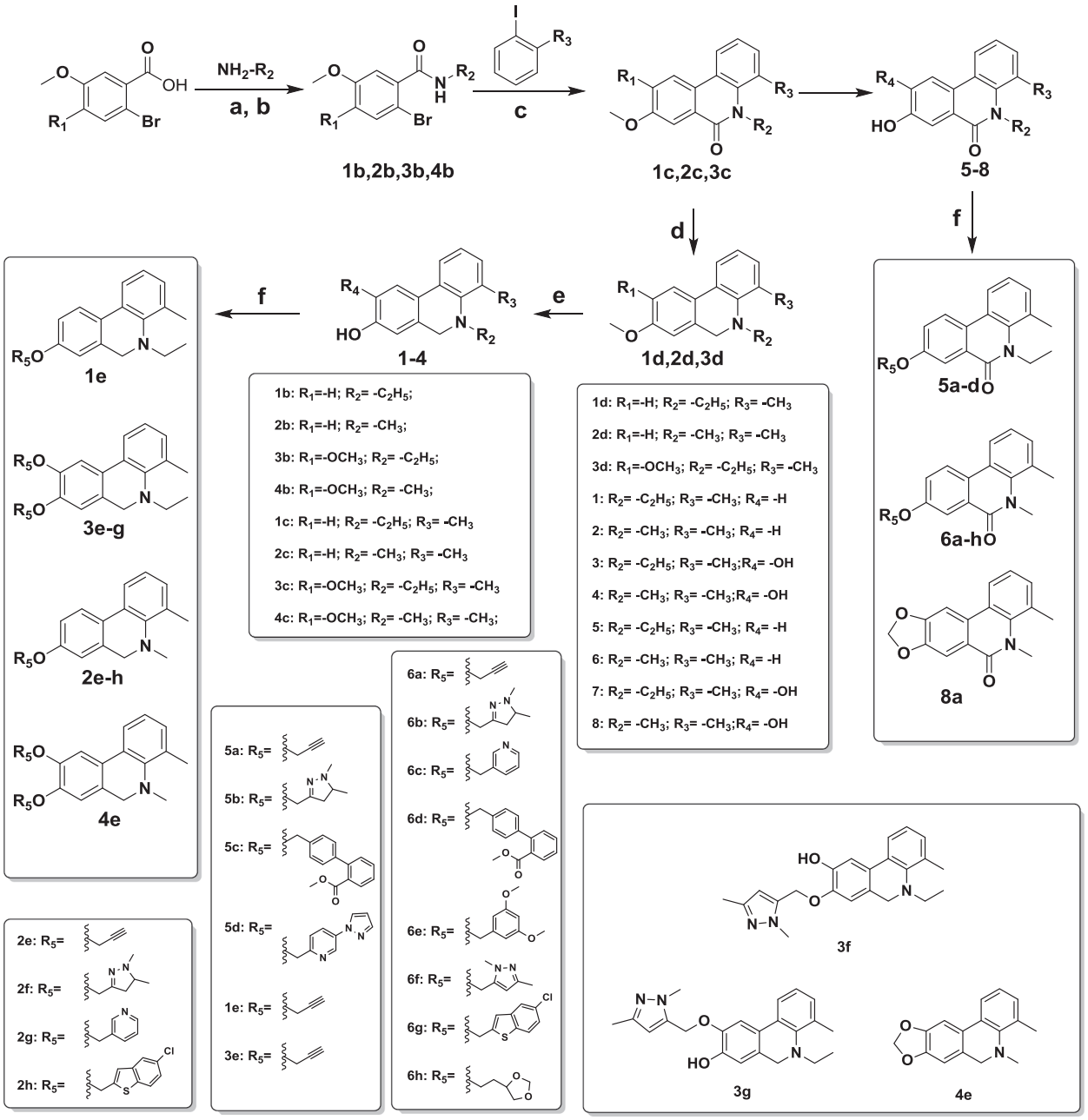
4.1.1. General experimental procedures

Melting points were measured using an X-4 apparatus (YingyuYuhua Instrument Factory, Gongyi, Henan Province, P. R. China). ESI and HRMS data were recorded using aFinnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer, respectively. NMR experiments were conducted on a Bruker AM-400, DRX-500, or Avance III 600 spectrometer using residual $CDCl_3$ and DMSO-d_6 or TMS as internal standards. Column chromatography was performed on silica gel (60–80 mesh, 200–300 mesh, 300–400 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, P. R. China). Pre-coated silica gel 60 GF254 (Merck, Darmstadt, Germany) was used for TLC analyses. Semi-preparative HPLC analyses were performed on a Hypersil Gold RP-C_{18} column (i.d. 10 × 250 mm, 5 μm, 5 mL/min) developed with CH_3CN-H_2O at room temperature (r.t.). All regular solvents and reagents were reagent grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, United States), Acros Organics (Geel, Belgium), and J&K Scientific (Beijing, P. R. China). The purities of all compounds used in biological assays exceeded 95% as determined by HPLC. HPLC was performed on an X-Bridge RP-C_{18} column (4.6 × 250 mm, 5 μm, 5 mL/min) with CH_3OH-H_2O at r.t. All reported yields are for dry compounds that required no further purification for use in subsequent reactions.

4.1.2. Synthesis of phenanthridine skeletons

2-Bromo-*N*-ethyl-5-methoxybenzamide(1b). Compound**1a** (260 mg, 1 mmol) was dissolved in DCM (10 mL), to which DMF (0.1 mL) and $SOCl_2$ (0.5 mL, 4 mmol) were added. The reaction solution was stirred for 2 h at 50 °C, and then concentrated to remove DCM. The residue was then added to a 30% solution of ethylamine in water (20 mL) at 5 °C and filtered. The cake was purified bycolumn chromatography to give **1b** as a pale yellow solid (205 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.8 Hz, 1H), 7.08 (d, J = 3.1 Hz, 1H), 6.81 (dd, J = 8.8, 3.1 Hz, 1H), 3.80 (s, 3H), 3.49 (q, J = 7.0 Hz, 2H), 1.26 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.23 (C), 158.94 (C), 138.53 (C), 134.13 (CH), 117.81 (CH), 114.63 (CH), 109.35 (C), 55.66 (CH_3), 35.06 (CH_2), 14.69 (CH_3); HREIMS m/z 257.0050 $[M]^+$ (calcd for $C_{10}H_{12}BrNO_2$, 257.0051).

4-Methyl-*N*-ethyl-8-methoxyphenanthridin-6(5*H*)-one (1c). A



Scheme 1. Design and syntheses of phenanthridinederivatives.[a].([a]Reagents and conditions: (a) $SOCl_2$, DCM, 50–80 °C, 3 h; (b) RNH_2 (30%), 5 °C, 1 h; (c) K_2CO_3, norbornene, $Pd(OAc)_2$, TFP, MeCN or DMF, 85–115 °C, 6–20 h; (d) BH_3-THF, THF, 60 °C, 2 h; (e). BBr_3, CH_2Cl_2, -78 °C, 4 h, 80%. (f). NaH, alkylating agent, CH_2Cl_2 or THF, 60–100 °C, 6–20 h.)

flask was charged under nitrogen with $Pd(OAc)_2$ (3.0 mg, 0.013 mmol), tri(2-furyl) phosphine (6.2 mg, 0.027 mmol), K_2CO_3 (72.3 mg, 0.52 mmol), the amide **1b** (0.26 mmol), a solution of norbornene (26.9 mg, 0.286 mmol) in anhydrous solvent (5.8 mL), and 1-iodo-2-methylbenzene (0.26 mmol). The reaction mixture was heated with stirring at 85 °C for 6 h and then cooled to rt. After the addition of saturated NH_4Cl (30 mL) and extraction with EtOAc (3 × 15 mL), the combined organic extracts were washed with brine (30 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave the crude product, which was purified by flash chromatography on silica gel to furnish **1c** as a white wax (55 mg, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 9.0 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.92 (d, J = 2.8 Hz, 1H), 7.32 (dd, J = 8.9, 2.8 Hz, 1H), 7.28 (d, J = 7.2 Hz, 1H), 7.20 (t, J = 7.7 Hz, 1H), 4.51 (q, J = 6.9 Hz, 2H), 3.96 (s, 3H), 2.70 (s, 3H), 1.37 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.90 (C), 159.37 (C), 137.15 (C), 133.12 (CH), 127.56 (C), 126.84 (C), 125.82 (C), 123.69 (CH), 122.74 (CH), 122.37 (CH), 121.81 (C), 120.90 (CH), 108.76 (CH), 55.64 (CH_3), 42.60 (CH_2), 23.87 (CH_3), 14.83 (CH_3); HREIMS m/z 267.1263 $[M]^+$ (calcd for $C_{17}H_{17}NO_2$, 267.1259).

4-Methyl-*N*-ethyl-8-methoxy-5,6-dihydrophenanthridine (1d). A solution of **1c** (30 mg, 0.1 mmol) in THF (5 mL) was added to BH_3-THF (0.12 mol) at 60 °C. The reaction was stirred for 2 h and then quenched using H_2O (5 mL). The mixture was then extracted with EtOAc (20 mL) twice. The organic phase was washed with brine and concentrated, and the residue was purified by column chromatography to give **1** as a colourless solid (18 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.5 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 6.6 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.90 (dd, J = 8.5, 2.6 Hz, 1H), 6.77 (d, J = 2.6 Hz, 1H), 4.10 (s, 2H), 3.86 (s, 3H), 2.70 (q, J = 7.1 Hz,

Table 1
Wnt activating efficacy of phenanthridine derivatives.

Compound	Concentration that doubled Wnt activation	Compound	Concentration that doubled Wnt activation
HLY78	5 μM	**4e**	10 μM
1	10 μM	**5**	> 20 μM
1c	> 20 μM	**5a**	NA[a]
1d	5 μM	**5b**	10 μM
1e	10 μM	**5c**	NA
2	5 μM	**5d**	> 20 μM
2c	> 20 μM	**6**	NA
2d	5 μM	**6a**	> 20 μM
2e	> 20 μM	**6b**	> 20 μM
2f	5 μM	**6c**	10 μM
2g	10 μM	**6d**	> 20 μM
2h	NA	**6e**	> 20 μM
3	10 μM	**6f**	5 μM
3c	> 20 μM	**6g**	NA
3d	10 μM	**6h**	NA
3e	10 μM	**7**	> 20 μM
3f	0.5 μM	**8**	NA
3g	1 μM	**8a**	> 20 μM
4	10 μM		

2H), 2.37 (s, 3H), 1.10 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.30 (C), 146.27 (C), 135.05 (C), 133.38 (C), 129.69 (CH), 129.38 (C), 125.87 (C), 124.31 (CH), 123.86 (CH), 120.83 (CH), 112.92 (CH), 111.92 (CH), 55.33 (CH_3), 50.25 (CH_2), 46.11 (CH_2), 17.81 (CH_3), 13.64 (CH_3); HRESIMS m/z 254.1539 $[M+H]^+$ (calcd for $C_{17}H_{20}NO$, 254.1539).

***N*-ethyl-4-methyl-5,6-dihydrophenanthridin-8-ol (1).** Compound **1d** (0.1 mmol) was dissolved in 10 mL CH_2Cl_2. The reaction solution was then cooled to -78 °C and BBr_3 (100 μL, 0.2 mmol) was added. The mixture was then stirred for 4 h after which it was diluted in 10 mL saturated $NaHCO_3$. The solution was extracted twice with CH_2Cl_2 (15 mL), and the organic layer was washed with brine, concentrated, and then purified by column chromatography using chloroform–methanol (20:1) as the eluent to yield compound**1** (19.5 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 8.3 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 7.3 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.81 (dd, J = 8.3, 2.4 Hz, 1H), 6.71 (d, J = 2.2 Hz, 1H), 4.05 (s, 2H), 2.69 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 1.08 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.29 (C), 146.18 (C), 135.33 (C), 133.38 (C), 129.71 (CH), 129.32 (C), 125.99 (C), 124.51 (CH), 123.87 (CH), 120.78 (CH), 114.42 (CH), 113.39 (CH), 50.02 (CH_2), 46.08 (CH_2), 17.79 (CH_3), 13.59 (CH_3); HRESIMS m/z240.1386 $[M+H]^+$ (calcd for $C_{16}H_{18}NO$, 240.1383).

2-Bromo-*N*-methyl-5-methoxybenzamide(2b). By referencing the synthesis method of compound **1b**, compound **2b** was synthesized through the amidation of compound **1a** in the presence of a 30% solution of methylamine (20 mL) for a yield of 72% (200 mg). m.p. 152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.8 Hz, 1H), 7.07 (d, J = 3.1 Hz, 1H), 6.81 (dd, J = 3.1, 8.8 Hz, 1H), 3.79 (s, 3H), 3.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.0 (C), 158.8 (C), 138.3 (C), 134.1 (CH), 117.8 (CH), 114.6 (CH), 109.3 (C), 55.6 (CH_3), 26.7 (CH_3); HREIMS m/z 242.9889 $[M]^+$ (calcd for $C_9H_{10}BrNO_2$, 242.9895).

4-Methyl-*N*-methyl-8-methoxyphenanthridin-6(5*H*)-one (2c). By referencing the synthesis method of compound **1c**, compound **2b** (0.1 mmol) was coupled to 1-iodo-2-methylbenzene (0.12 mol) to afford compound **2c** (68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 9.0 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 2.8 Hz, 1H), 7.31 (dd, J = 8.9, 2.8 Hz, 1H), 7.28–7.23 (m, 1H), 7.18 (d, J = 7.5 Hz, 1H), 3.95 (s, 3H), 3.81 (s, 3H), 2.66 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.99 (C), 159.42 (C), 138.5 (C), 132.77 (CH), 127.54 (C), 126.67 (C), 126.12 (C), 123.74 (CH), 122.31 (CH), 122.9 (CH), 121.38 (C), 120.50 (CH), 108.80 (CH), 55.71 (CH_3), 38.51 (CH_3), 23.63 (CH_3); HREIMS m/z 253.1102 $[M]^+$ (calcd for $C_{16}H_{15}NO_2$, 253.1103).

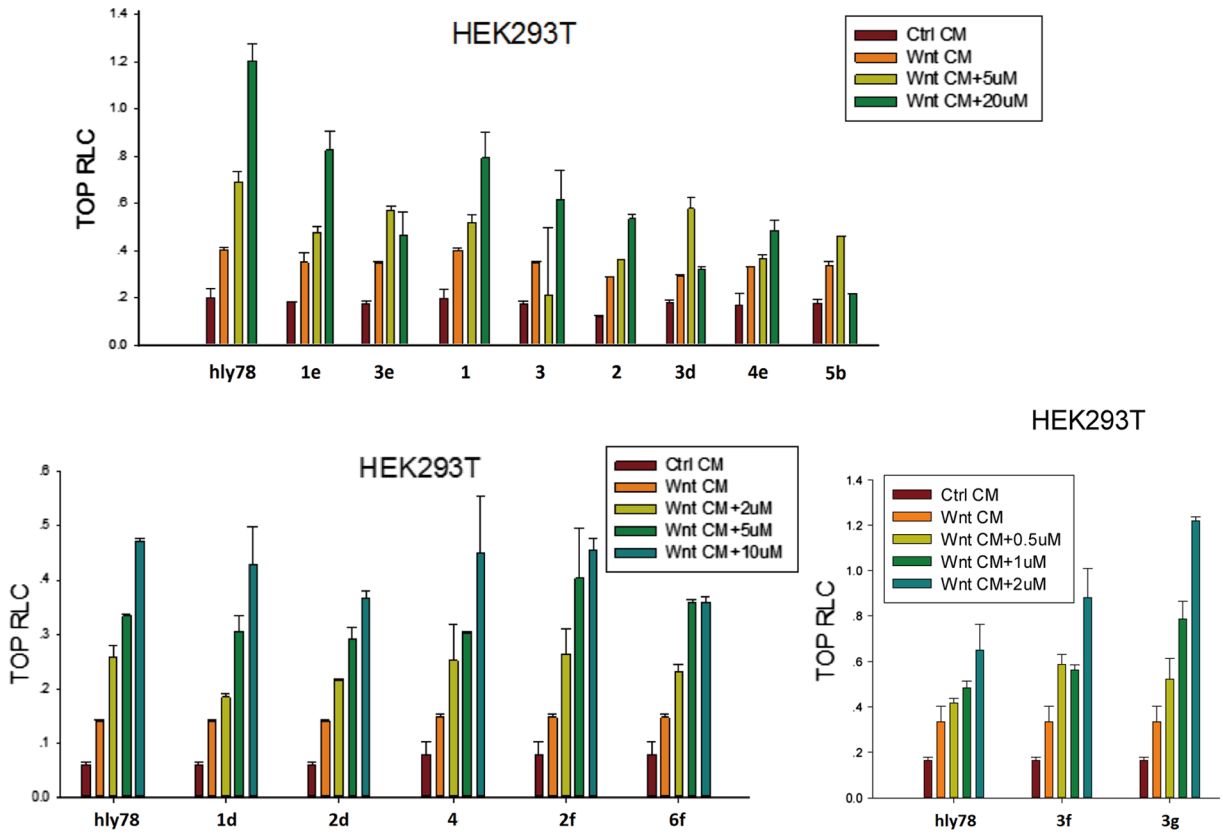


Fig. 4. The Wnt active activities of phenanthridine derivatives. Compounds **1**, **1e**, **2**, **2d**, **3**, **3d**, **3e**, **4e** and **5b**exhibit weaker activity than that of HLY78; Compounds **1d**, **2f**, **4** and **6f**show equal activity to HLY78; Compounds **3f** and **3g** exhibit stronger activity than that of HLY78.

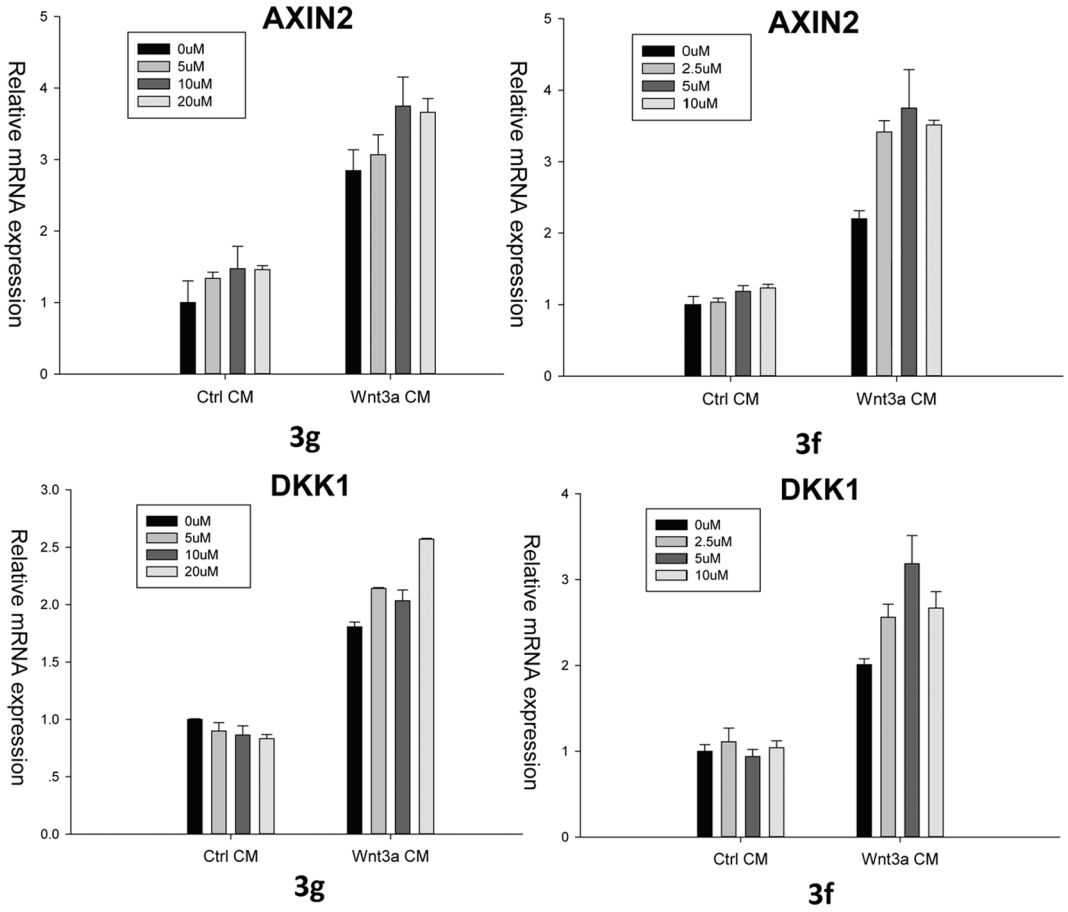


Fig. 5. Compounds **3g** and **3f**clearly activated the expression of endogenous Wnt target genes (*Axin2* and *DKK1*), and **3f** is more efficient than **3g**.

4-Methyl-*N*-methyl-8-methoxy-5,6-dihydrophenanthridine (2d). By referencing the synthesis method of compound **1**, compound **2d** was synthesized through the reduction of compound **2c** (0.1 mmol) in the presence of BH_3-THF (0.25 mmol) for a yield of 72%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 8.6 Hz, 1H), 7.61 (dd, J = 7.5, 1.5 Hz, 1H), 7.18–7.08 (m, 2H), 6.93 (dd, J = 8.5, 2.7 Hz, 1H), 6.81 (d, J = 2.6 Hz, 1H), 4.09 (s, 2H), 3.88 (s, 3H), 2.52 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.33 (C), 145.89 (C), 134.22 (C), 133.30 (C), 129.53 (CH), 125.47 (C), 125.14 (C), 124.26 (CH), 124.19 (CH), 120.93 (CH), 113.02 (CH), 112.01 (CH), 55.39 (CH_2), 55.21 (CH_3), 40.37 (CH_3), 17.50 (CH_3); HRESIMS m/z 240.1385 $[M+H]^+$ (calcd for $C_{16}H_{18}NO$, 240.1383).

2-Bromo-*N*-ethyl-4,5-dimethoxybenzamide(3b). By referencing the synthesis method of compound **1b**, compound **3b** was synthesized through the amidation of compound **3a** (1 mmol) in the presence of ethylamine (50 mL) for a yield of 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (s, 1H), 6.98 (s, 1H), 3.89 (s, 3H), 3.89 (s, 3H), 3.50 (q, J = 7.2 Hz, 2H), 1.27 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.72 (C), 150.69 (C), 148.42 (C), 129.27 (C), 115.67 (CH), 112.90 (CH), 109.70 (C), 56.29 (CH_3), 56.15 (CH_3), 35.13 (CH_2), 14.70 (CH_3); HREIMS m/z

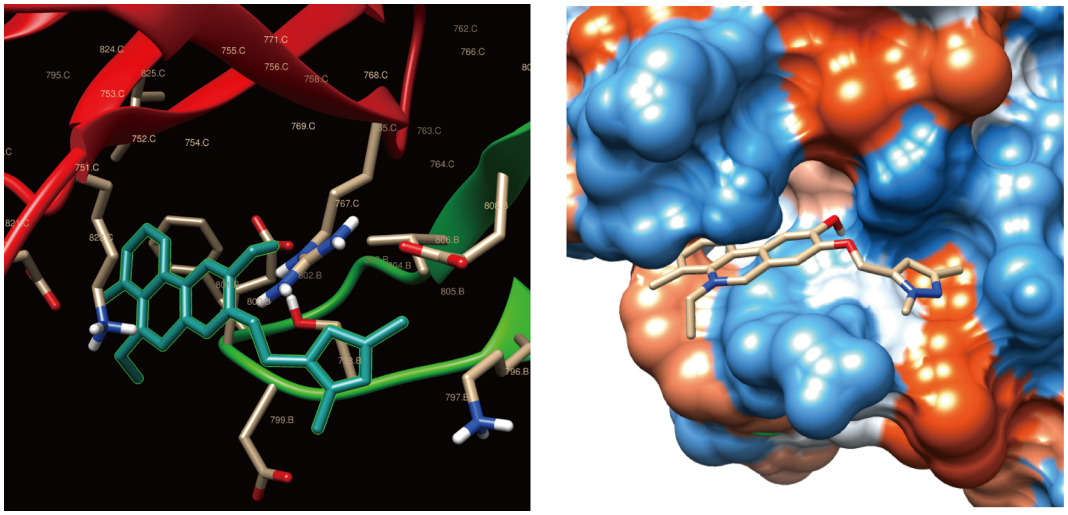


Fig. 6. Molecular dockinganalysis of compound **3f**. Compound **3f**can bind tightertoaxin than **3g**.

287.0157 $[M]^+$ (calcd for $C_{11}H_{14}BrNO_3$, 287.0157).

4-Methyl-*N*-ethyl-8,9-dimethoxyphenanthridin-6(5*H*)-one (3c). By referencing the synthesis method of compound **1c**, compound **3b** (0.1 mmol) was coupled to 1-iodo-2-methylbenzene (0.12 mmol)to afford compound **3c** (75% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 7.8 Hz, 1H), 7.87 (s, 1H), 7.52 (s, 1H), 7.26 (dd, J = 5.1, 2.1 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 4.47 (q, J = 7.0 Hz, 2H), 4.05 (s, 3H), 4.01 (s, 3H), 2.68 (s, 3H), 1.34 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.64 (C), 153.27 (C), 149.69 (C), 137.81 (C), 133.36 (CH), 128.75 (C), 125.90 (C), 122.49 (CH), 121.53 (C), 120.93 (CH), 119.64 (C), 108.75 (CH), 102.83 (CH), 56.17 (CH_3), 56.10 (CH_3), 42.45 (CH_2), 23.95 (CH_3), 14.97 (CH_3); HRESIMS m/z298.1441 $[M+H]^+$ (calcd for $C_{18}H_{20}NO_3$, 298.1438).

4-Methyl-*N*-ethyl-8,9-dimethoxy-5,6-dihydrophenanthridine (3d). By referencing the synthesis method of compound **1**, compound **3d** was synthesized through the reduction of compound **3c** (0.1 mmol) in the presence of BH_3-THF (0.3 mmol) for a yield of 65%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 6.9 Hz, 1H), 7.24 (s, 1H), 7.12 (d, J = 7.4 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.73 (s, 1H), 4.06 (s, 2H), 3.96 (s, 3H), 3.93 (s, 3H), 2.70 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 1.09 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.43 (C), 146.36 (C), 140.87 (C), 133.45 (C), 129.81 (CH), 129.38 (C), 125.95 (C), 125.47 (C), 123.75 (CH), 120.70 (CH), 109.64 (CH), 106.54 (CH), 56.12 (CH_3), 56.01 (CH_3), 49.65 (CH_2), 45.96 (CH_2), 17.83 (CH_3), 13.69 (CH_3); HRESIMS m/z284.1648 $[M+H]^+$ (calcd for $C_{18}H_{22}NO_2$, 284.1645).

2-Bromo-*N*-methyl-4,5-dimethoxybenzamide(4b). By referencing the synthesis method of compound **1b**, compound **4b** was synthesized through the amidation of compound **4a** (1 mmol) in the presence of ethylamine (50 mL) for a yield of 85%. ^{1}H NMR (500 MHz, MeOD) δ 7.13 (s, 1H), 7.00 (s, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 2.88 (s, 3H); ^{13}C NMR (125 MHz, MeOD) δ 171.29 (C), 152.14 (C), 149.82 (C), 131.56 (C), 117.11 (CH), 113.11 (CH), 111.16 (C), 56.75 (CH_3), 56.63 (CH_3), 26.73 (CH_3); HREIMS m/z 273.0000 $[M]^+$ (calcd for $C_{10}H_{12}BrNO_3$, 273.0001).

Synthesis of Compounds 2–8. By referencing the synthesis method of compound **1**, compounds**2-8**weresynthesized by the deprotection of compounds **1c**, **2c**, **3c**, **2d**, and **3d** (0.1 mmol), respectively, in the presence of BBr_3 (0.3 mmol) for a yield of 65%.

***N*-methyl-4-methyl-5,6-dihydrophenanthridin-8-ol (2).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.4 Hz, 1H), 7.57–7.52 (m, 1H), 7.14–7.01 (m, 2H), 6.83 (dd, J = 8.4, 2.5 Hz, 1H), 6.73 (d, J = 2.4 Hz, 1H), 4.00 (s, 2H), 2.45 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.76 (C), 145.82 (C), 134.52 (C), 133.37 (C), 129.60 (CH), 129.12 (C), 125.10 (C), 124.55 (CH), 124.31 (CH), 120.96 (CH), 114.66 (CH), 113.75 (CH), 55.23 (CH_2), 40.42 (CH_3), 17.59 (CH_3); HRESIMS m/z 226.1228 $[M+H]^+$ (calcd for $C_{15}H_{16}NO$, 226.1226).

***N*-ethyl-4-methyl-5,6-dihydrophenanthridin-8,9-diol (3).** 80% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 7.7 Hz, 1H), 7.24 (s, 1H), 7.11 (d, J = 7.0 Hz, 1H), 7.05 (t, J = 7.6 Hz, 1H), 6.73 (s, 1H), 3.99 (s, 2H), 2.68 (q, J = 7.1 Hz, 2H), 2.34 (s, 3H), 1.07 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.44 (C), 142.85 (C), 141.90 (C), 133.42 (C), 129.84 (CH), 129.17 (C), 126.59 (C), 126.14 (C), 123.87 (CH), 120.86 (CH), 113.57 (CH), 110.43 (CH), 49.35 (CH_2), 45.93 (CH_2), 17.78 (CH_3), 13.58 (CH_3); HRESIMS m/z256.1322 $[M+H]^+$ (calcd for $C_{16}H_{18}NO_2$, 256.1332).

***N*-methyl-4-methyl-5,6-dihydrophenanthridin-8,9-diol (4).** 80% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, J = 7.9 Hz, 1H), 7.19 (d, J = 3.1 Hz, 2H), 6.92 (d, J = 7.8 Hz, 1H), 6.65 (s, 1H), 3.88 (s, 2H), 2.42 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.27 (C), 142.60 (C), 141.80 (C), 137.16 (C), 133.32 (C), 128.32 (CH), 127.90 (C), 126.09 (C), 120.94 (CH), 120.30 (CH), 113.81 (CH), 110.16 (CH), 54.56 (CH_2), 42.98 (CH_3), 22.76 (CH_3); HREIMS m/z 241.1102 $[M]^+$ (calcd for $C_{15}H_{15}NO_2$, 241.1103).

***N*-ethyl-8-hydroxy-4-methylphenanthridin-6(5H)-one (5).** 80% yield; ^{1}H NMR (500 MHz, MeOD) δ 7.82 (d, J = 8.9 Hz, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 2.7 Hz, 1H), 6.90 (d, J = 7.4 Hz, 1H), 6.85 (dd, J = 8.8, 2.8 Hz, 1H), 6.80 (t, J = 7.7 Hz, 1H), 4.08 (q, J = 7.0 Hz, 2H), 2.28 (s, 3H), 0.90 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD) δ 165.68 (C), 158.93 (C), 137.64 (C), 134.09 (CH), 127.98 (C), 127.69 (C), 127.41 (C), 125.34 (CH), 124.43 (CH), 123.50 (C), 123.30 (CH), 122.01 (CH), 112.89 (CH), 43.74 (CH_2), 23.95 (CH_3), 15.01 (CH_3); HRESIMS m/z254.1176 $[M+H]^+$ (calcd for $C_{16}H_{16}NO_2$, 254.1176).

***N*-methyl-8-hydroxy-4-methylphenanthridin-6(5H)-one (6).** 80% yield; ^{1}H NMR (600 MHz, MeOD) δ 8.23 (d, J = 8.9 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.72 (d, J = 2.7 Hz, 1H), 7.33 (m, 1H), 7.31 (dd, J = 8.9, 2.7 Hz, 1H), 7.27–7.23 (m, 1H), 3.78 (s, 3H), 2.65 (s, 3H); ^{13}C NMR (150 MHz, MeOD) δ 165.68 (C), 158.92 (C), 138.97 (C), 133.71 (CH), 127.93 (C), 127.72 (C), 127.39 (C), 125.34 (CH), 124.52 (CH), 123.20 (CH), 122.96 (C), 121.56 (CH), 112.82 (CH), 39.04 (CH_3), 23.66 (CH_3); HRESIMS m/z 240.1017 $[M+H]^+$ (calcd for $C_{15}H_{14}NO_2$, 240.1019).

***N*-ethyl-8,9-dihydroxy-4-methylphenanthridin-6(5H)-one (7).** 80% yield; ^{1}H NMR (500 MHz, MeOD) δ 8.00 (dd, J = 6.5, 4.8 Hz, 1H), 7.69 (s, 1H), 7.62 (d, J = 2.9 Hz, 1H), 7.28 (d, J = 6.5 Hz, 1H), 7.18 (td, J = 7.7, 3.7 Hz, 1H), 4.46 (q, J = 6.9 Hz, 2H), 2.67 (s, 3H), 1.28 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, MeOD) δ 165.67 (C), 152.77 (C), 148.00 (C), 138.16 (C), 134.12 (CH), 129.97 (C), 127.37 (C), 124.15 (CH), 123.29 (C), 122.08 (CH), 119.57 (C), 113.47 (CH), 108.12 (CH), 43.47 (CH_2), 23.99 (CH_3), 15.12 (CH_3); HREIMS m/z 269.1050 $[M]^+$ (calcd for $C_{16}H_{15}NO_3$, 269.1052).

***N*-methyl-8,9-hydroxy-4-methylphenanthridin-6(5H)-one (8).** 80% yield; ^{1}H NMR (500 MHz, MeOD) δ 7.83 (dd, J = 37.5, 7.8 Hz, 1H), 7.59 (s, 1H), 7.53 (s, 1H), 7.18 (d, J = 7.3 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 3.66 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (125 MHz, MeOD) δ165.70 (C), 152.72 (C), 147.99 (C), 139.56 (C), 133.75 (CH), 129.93 (C), 127.71 (C), 124.26 (CH), 122.77 (C), 121.63 (CH), 119.26 (C), 113.38 (CH), 108.18 (CH), 38.87 (CH_3), 23.67 (CH_3); HRESIMS m/z 256.0972 $[M+H]^+$ (calcd for $C_{15}H_{14}NO_3$, 256.0968).

*4.1.3. Alkylation of compounds **1–8***

The phenanthridine skeletons**1-8** (0.1 mmol) were dissolved in dry THF (10 mL), and NaH (50 mg, 2 mmol), and 3-(bromomethyl)pyridine, 3-(chloromethyl)-1,5-dimethyl-1H-pyrazole or 5-bromomethyl-1,3-dimethyl-1H-pyrazole (1 mmol) were added. The mixture was stirred at rt. for 12 h and quenched with H_2O (50 mL) in an ice bath. The solution was evaporated to remove the THF and extracted with CH_2Cl_2 (2 × 30 mL). The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using petroleum-EtOAc as the eluent to afford about 35 mg of each phenanthridine compound.

5-ethyl-4-methyl-8-(prop-2-yn-1-yloxy)-5,6-dihydrophenanthridine (1e). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.06 (d, J = 7.0 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 6.89 (dd, J = 8.5, 2.6 Hz, 1H), 6.76 (d, J = 2.6 Hz, 1H), 4.67 (d, J = 2.4 Hz, 2H), 4.02 (s, 2H), 2.62 (q, J = 7.1 Hz, 2H), 2.48 (t, J = 2.3 Hz, 1H), 2.29 (s, 3H), 1.01 (t, J = 7.1 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.52 (C), 146.42 (C), 136.44 (C), 133.59 (C), 129.90 (CH), 129.88 (C), 124.49 (C), 124.29 (CH), 123.85 (CH), 120.90 (CH), 113.90 (CH), 112.94 (CH), 76.22 (C), 75.85 (CH), 58.88 (CH_2), 50.17 (CH_2), 46.09 (CH_2), 17.80 (CH_3), 13.62 (CH_3); HREIMS m/z 277.1463 $[M]^+$ (calcd for $C_{19}H_{19}NO$, 277.1467).

4,5-dimethyl-8-(prop-2-yn-1-yloxy)-5,6-dihydrophenanthridine (2e). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 8.6 Hz, 1H), 7.60 (dd, J = 7.5, 1.3 Hz, 1H), 7.14 (dd, J = 7.3, 0.8 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.99 (dd, J = 8.5, 2.7 Hz, 1H), 6.86 (d, J = 2.6 Hz, 1H), 4.74 (d, J = 2.4 Hz, 2H), 4.08 (s, 2H), 2.57 (t, J = 2.4 Hz, 1H), 2.50 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.38 (C), 146.09 (C), 134.38 (C), 133.44 (C), 129.85 (CH), 128.94 (C), 126.14 (C), 124.36 (CH), 124.29 (CH), 121.13 (CH), 113.91 (CH), 113.20 (CH), 78.62 (C), 75.67 (CH), 55.90 (CH_2), 55.42 (CH_2), 40.48 (CH_3), 17.60 (CH_3); HREIMS m/z 263.1309 $[M]^+$ (calcd for $C_{18}H_{17}NO$, 263.1310).

8-((1,5-dimethyl-1H-pyrazol-3-yl)methoxy)-4,5-dimethyl-5,6-

dihydrophenanthridine (2f). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.6 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.15–7.04 (m, 2H), 6.99 (dd, J = 8.5, 2.5 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.14 (s, 1H), 5.04 (s, 2H), 4.05 (s, 2H), 3.79 (s, 3H), 2.47 (s, 3H), 2.39 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.65 (C), 146.92 (C), 146.01 (C), 139.69 (C), 134.19 (C), 133.36 (C), 129.57 (CH), 129.15 (C), 125.33 (C), 124.27 (CH), 124.22 (CH), 121.01 (CH), 113.92 (CH), 113.03 (CH), 105.01 (CH), 64.22 (CH_2), 55.45 (CH_2), 40.45 (CH_3), 36.08 (CH_3), 17.58 (CH_3), 11.27 (CH_3); HRESIMS m/z 334.1920 $[M+H]^+$ (calcd for $C_{21}H_{24}N_3O$, 334.1914).

4,5-dimethyl-9-(pyridin-3-ylmethoxy)-5,6-dihydrophenanthridine (2g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, J = 4.7 Hz, 1H), 7.74 (td, J = 7.7, 1.8 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.57 (dd, J = 10.1, 4.8 Hz, 2H), 7.23 (dd, J = 11.0, 4.9 Hz, 1H), 7.16–7.04 (m, 2H), 6.98 (dd, J = 8.6, 2.7 Hz, 1H), 6.87 (d, J = 2.6 Hz, 1H), 5.26 (s, 2H), 4.05 (s, 2H), 2.47 (s, 3H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.22 (C), 157.26 (C), 149.25 (CH), 146.02 (C), 136.93 (CH), 134.40 (C), 133.41 (C), 129.73 (CH), 128.99 (C), 125.73 (C), 124.42 (CH), 124.26 (CH), 122.71 (CH), 121.34 (CH), 121.05 (CH), 113.87 (CH), 113.14 (CH), 70.68 (CH_2), 55.41 (CH_2), 40.46 (CH_3), 17.59 (CH_3); HRESIMS m/z 317.1650 $[M+H]^+$ (calcd for $C_{21}H_{21}N_2O$, 317.1648).

9-((5-chlorobenzo[*b*] thiophen-2-yl)methoxy)-4,5-dimethyl-5,6-dihydrophenanthridine (2h). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 1.8 Hz, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.64–7.55 (m, 2H), 7.37 (dd, J = 8.6, 1.6 Hz, 1H), 7.20–7.07 (m, 2H), 7.02 (dd, J = 8.5, 2.3 Hz, 1H), 6.91 (d, J = 2.3 Hz, 1H), 5.29 (s, 2H), 4.09 (s, 2H), 2.51 (s, 3H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.33 (C), 146.07 (C), 139.12 (C), 138.79 (C), 134.49 (C), 133.46 (C), 131.37 (C), 130.85 (C), 129.82 (CH), 128.98 (C), 127.24 (CH), 125.92 (C), 125.22 (CH), 124.46 (CH), 124.31 (CH), 123.90 (CH), 121.89 (CH), 121.11 (CH), 113.92 (CH), 113.08 (CH), 64.64 (CH_2), 55.46 (CH_2), 40.53 (CH_3), 17.61 (CH_3); HRESIMS m/z 406.1029 $[M+H]^+$ (calcd for $C_{24}H_{21}ClNOS$, 406.1027).

5-ethyl-4-methyl-8,9-bis(prop-2-yn-1-yloxy)-5,6-dihydrophenanthridine (3e). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 7.0 Hz, 1H), 7.44 (s, 1H), 7.14 (dd, J = 7.4, 0.7 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.90 (s, 1H), 4.82 (d, J = 2.4 Hz, 2H), 4.80 (d, J = 2.4 Hz, 2H), 4.06 (s, 2H), 2.70 (q, J = 7.1 Hz, 2H), 2.54 (q, J = 2.3 Hz, 2H), 2.36 (s, 3H), 1.08 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.32 (C), 146.92 (C), 146.47 (C), 133.46 (C), 130.13 (CH), 129.07 (C), 127.46 (C), 126.96 (C), 123.82 (CH), 120.98 (CH), 113.09 (CH), 110.64 (CH), 78.68 (C), 78.54 (C), 75.98 (CH), 75.95 (CH), 57.35 (CH_2), 57.06 (CH_2), 49.58 (CH_2), 46.00 (CH_2), 17.80 (CH_3), 13.65 (CH_3); HRESIMS m/z332.1648 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_2$, 332.1645).

8-((1,3-dimethyl-1H-pyrazol-5-yl)methoxy)-5-ethyl-4-methyl-5,6-dihydrophenanthridin-9-ol (3f). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 7.6 Hz, 1H), 7.35 (s, 1H), 7.14 (d, J = 7.2 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.81 (s, 1H), 6.13 (s, 1H), 5.06 (s, 2H), 4.05 (s, 2H), 3.84 (s, 3H), 2.70 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 1.10 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.51 (C), 146.43 (C), 145.27 (C), 144.90 (C), 137.33 (C), 133.40 (C), 130.10 (CH), 129.02 (C), 127.30 (C), 125.38 (C), 123.91 (CH), 121.17 (CH), 110.41 (CH), 110.22 (CH), 107.19 (CH), 61.42 (CH_2), 49.62 (CH_2), 45.93 (CH_2), 36.33 (CH_3), 17.78 (CH_3), 13.69 (CH_3), 13.40 (CH_3); HRESIMS m/z 364.2022 $[M+H]^+$ (calcd for $C_{22}H_{26}N_3O_2$, 364.2020).

9-((1,3-dimethyl-1H-pyrazol-5-yl)methoxy)-5-ethyl-4-methyl-5,6-dihydrophenanthridin-8-ol (3g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, J = 7.0 Hz, 1H), 7.31 (s, 1H), 7.13 (d, J = 6.8 Hz, 1H), 7.07 (t, J = 7.5 Hz, 1H), 6.81 (s, 1H), 6.14 (s, 1H), 5.10 (s, 2H), 4.02 (s, 2H), 3.84 (s, 3H), 2.69 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 2.27 (s, 3H), 1.08 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.54 (C), 146.42 (C), 145.68 (C), 144.83 (C), 137.46 (C), 133.62 (C), 129.88 (CH), 129.25 (C), 127.95 (C), 125.30 (C), 123.77 (CH), 120.54 (CH), 113.42 (CH), 107.45 (CH), 107.17 (CH), 61.63 (CH_2), 49.38 (CH_2), 45.92 (CH_2), 36.35 (CH_3), 17.81 (CH_3), 13.63 (CH_3), 13.41 (CH_3); HRESIMS m/z364.2022 $[M+H]^+$ (calcd for $C_{22}H_{26}N_3O_2$, 364.2020).

5-ethyl-4-methyl-8-(prop-2-yn-1-yloxy)phenanthridin-6(5H)-one (5a). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.12 (d, J = 9.0 Hz, 1H), 8.01 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 2.8 Hz, 1H), 7.33 (dd, J = 8.9, 2.8 Hz, 1H), 7.25 (d, J = 6.8 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 4.81 (d, J = 2.3 Hz, 2H), 4.46 (q, J = 7.0 Hz, 2H), 2.66 (s, 3H), 2.55 (t, J = 2.3 Hz, 1H), 1.34 (t, J = 7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.89 (C), 157.39 (C), 137.45 (C), 133.54 (CH), 128.44 (C), 126.92 (C), 126.01 (C), 124.04 (CH), 122.96 (CH), 122.80 (CH), 121.79 (C), 121.17 (CH), 110.33 (CH), 78.17 (C), 76.20 (CH), 56.24 (CH_2), 42.81 (CH_2), 24.05 (CH_3), 15.05 (CH_3); HRESIMS m/z292.1336 $[M+H]^+$ (calcd for $C_{19}H_{18}NO_2$, 292.1332).

8-((1,5-dimethyl-1H-pyrazol-3-yl)methoxy)-5-ethyl-4-methylphenanthridin-6(5H)-one (5b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 9.0 Hz, 1H), 8.02 (t, J = 5.0 Hz, 2H), 7.37 (dd, J = 8.9, 2.8 Hz, 1H), 7.24 (d, J = 7.3 Hz, 1H), 7.15 (t, J = 7.6 Hz, 1H), 6.15 (s, 1H), 5.13 (s, 2H), 4.48 (q, J = 6.9 Hz, 2H), 3.77 (s, 3H), 2.67 (s, 3H), 2.25 (s, 3H), 1.35 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.85 (C), 158.49 (C), 146.26 (C), 139.62 (C), 137.13 (C), 133.08 (CH), 127.63 (C), 126.73 (C), 125.75 (C), 123.63 (CH), 122.75 (CH), 122.70 (CH), 121.79 (C), 120.88 (CH), 109.94 (CH), 105.24 (CH), 64.29 (CH_2), 42.54 (CH_2), 36.06 (CH_3), 23.84 (CH_3), 14.82 (CH_3), 11.19 (CH_3); HRESIMS m/z362.1865 $[M+H]^+$ (calcd for $C_{22}H_{24}N_3O_2$, 362.1863).

4,5-dimethyl-5,6-dihydro-[1,3]dioxolo[4,5–*j*]phenanthridine (4e). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, J = 7.5, 1.2 Hz, 1H), 7.23 (s, 1H), 7.12 (d, J = 6.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 6.72 (s, 1H), 5.98 (s, 2H), 4.00 (s, 2H), 2.48 (s, 3H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.36 (C), 147.15 (C), 145.95 (C), 133.34 (C), 129.81 (CH), 129.20 (C), 126.55 (C), 126.24 (C), 124.21 (CH), 121.12 (CH), 107.19 (CH), 103.76 (CH), 100.92 (CH_2), 55.22 (CH_2), 40.08 (CH_3), 17.54 (CH_3); HRESIMS m/z 254.1175 $[M+H]^+$ (calcd for $C_{16}H_{16}NO_2$, 254.1176).

Methyl 4′-(((5-ethyl-4-methyl-6-oxo-5,6-dihydrophenanthridin-8-yl)oxy)methyl)-[1,1′-biphenyl]-2-carboxylate (5c). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, J = 9.0 Hz, 1H), 8.07 (d, J = 7.7 Hz, 1H), 8.04 (d, J = 2.8 Hz, 1H), 7.84 (dd, J = 7.8, 1.0 Hz, 1H), 7.52 (d, J = 8.2 Hz, 3H), 7.44–7.38 (m, 3H), 7.36 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 3.4 Hz, 1H), 7.20 (t, J = 7.6 Hz, 1H), 5.27 (s, 2H), 4.51 (q, J = 7.0 Hz, 2H), 3.65 (s, 3H), 2.70 (s, 3H), 1.37 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.04 (C), 163.90 (C), 158.57 (C), 142.15 (C), 141.23 (C), 137.24 (C), 135.46 (C), 133.24 (CH), 131.38 (CH), 130.80 (CH), 130.75 (C), 129.90 (CH), 128.64 (CH), 128.53 (CH), 128.47 (CH), 127.86 (C), 127.44 (CH), 127.30 (CH), 126.98 (C), 126.88 (C), 123.84 (CH), 122.90 (CH), 122.81 (CH), 121.83 (C), 120.98 (CH), 109.99 (CH), 70.10 (CH_2), 52.04 (CH_3), 42.65 (CH_2), 23.91 (CH_3), 14.87 (CH_3); HRESIMS m/z478.2023 $[M+H]^+$ (calcd for $C_{31}H_{28}NO_4$, 478.2013).

8-((5-(1H-pyrazol-1-yl)pyridin-2-yl)methoxy)-5-ethyl-4-methylphenanthridin-6(5H)-one (5d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, J = 25.7 Hz, 2H), 8.17 (d, J = 8.7 Hz, 1H), 8.06 (d, J = 7.9 Hz, 1H), 8.04–8.00 (m, 2H), 7.95 (d, J = 8.5 Hz, 1H), 7.75 (s, 1H), 7.39 (d, J = 8.9 Hz, 1H), 7.28 (dd, J = 10.2, 4.4 Hz, 1H), 7.20 (t, J = 7.3 Hz, 1H), 6.48 (d, J = 1.7 Hz, 1H), 5.24 (s, 2H), 4.51 (q, J = 6.8 Hz, 2H), 2.70 (s, 3H), 1.37 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.81 (C), 158.08 (C), 151.48 (C), 147.46 (CH), 142.20 (CH), 138.50 (CH), 137.27 (C), 133.39 (CH), 129.78 (C), 128.20 (C), 127.17 (CH), 126.87 (C), 125.94 (C), 124.00 (CH), 122.87 (CH), 122.82 (CH), 121.71 (C), 121.03 (CH), 112.32 (CH), 109.90 (CH), 107.93 (CH), 67.44 (CH_2), 42.68 (CH_2), 23.91 (CH_3), 14.85 (CH_3); HRESIMS m/z411.1818 $[M+H]^+$ (calcd for $C_{25}H_{23}N_4O_2$, 411.1816).

4,5-dimethyl-8-(prop-2-yn-1-yloxy)phenanthridin-6(5H)-one (6a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (dd, J = 8.9, 4.0 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 2.9 Hz, 1H), 7.38 (dd, J = 8.9, 2.8 Hz, 1H), 7.28 (d, J = 7.3 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 4.85 (d, J = 2.4 Hz, 2H), 3.82 (s, 3H), 2.67 (s, 3H), 2.56 (t, J = 2.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.84 (C), 157.29 (C), 138.66 (C), 133.01 (CH), 128.27 (C), 126.62 (C), 126.18 (C), 123.92 (CH), 122.99 (CH),

122.62 (CH), 121.24 (C), 120.60 (CH), 110.25 (CH), 77.99 (C), 76.01 (CH), 56.15 (CH_2), 38.49 (CH_3), 23.62 (CH_3); HRESIMS *m/z* 278.1177 $[M+H]^+$ (calcd for $C_{18}H_{16}NO_2$, 278.1176).

8-((1,5-dimethyl-1H-pyrazol-3-yl)methoxy)-4,5-dimethylphenanthridin-6(5H)-one (6b). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, J = 9.0 Hz, 1H), 8.02 (d, J = 2.8 Hz, 1H), 7.99 (d, J = 7.9 Hz, 1H), 7.38 (dd, J = 8.9, 2.9 Hz, 1H), 7.23 (d, J = 6.8 Hz, 1H), 7.17 (t, J = 7.6 Hz, 1H), 6.15 (s, 1H), 5.13 (s, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 2.64 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.85 (C), 158.47 (C), 146.19 (C), 139.54 (C), 138.42 (C), 132.64 (CH), 127.51 (C), 126.50 (C), 125.97 (C), 123.59 (CH), 122.80 (CH), 122.59 (CH), 121.29 (C), 120.39 (CH), 109.92 (CH), 105.19 (CH), 64.29 (CH_2), 38.36 (CH_3), 36.03 (CH_3), 23.51 (CH_3), 11.15 (CH_3); HRESIMS *m/z* 370.1532 $[M + Na]^+$ (calcd for $C_{21}H_{21}N_3O_2$, 370.1526).

4,5-dimethyl-9-(pyridin-3-ylmethoxy)phenanthridin-6(5H)-one (6c). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (d, J = 4.7 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 8.00 (dd, J = 9.4, 5.4 Hz, 2H), 7.71 (td, J = 7.7, 1.7 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.40 (dd, J = 8.9, 2.9 Hz, 1H), 7.23 (dd, J = 10.1, 4.8 Hz, 2H), 7.17 (t, J = 7.6 Hz, 1H), 5.32 (s, 2H), 3.78 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.73 (C), 158.12 (C), 156.46 (C), 149.37 (CH), 138.48 (C), 136.79 (CH), 132.80 (CH), 127.82 (C), 126.64 (C), 126.03 (C), 123.80 (CH), 122.86 (CH), 122.75 (CH), 122.11 (CH), 121.49 (CH), 121.19 (C), 120.45 (CH), 110.55 (CH), 70.82 (CH_2), 38.38 (CH_3), 23.52 (CH_3); HREIMS *m/z* 330.1379 $[M]^+$ (calcd for $C_{21}H_{18}N_2O_2$, 330.1368).

Methyl 4′-(((4,5-dimethyl-6-oxo-5,6-dihydrophenanthridin-8-yl)oxy)methyl)-[1,1′- biphenyl]-2-carboxylate (6d). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 (d, J = 9.0 Hz, 1H), 8.08–8.01 (m, 2H), 7.84 (dd, J = 7.7, 1.0 Hz, 1H), 7.53 (d, J = 7.8 Hz, 3H), 7.44–7.40 (m, 2H), 7.39–7.34 (m, 3H), 7.28 (d, J = 7.4 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 5.27 (s, 2H), 3.83 (s, 3H), 3.65 (s, 3H), 2.67 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.05 (C), 163.98 (C), 158.60 (C), 142.14 (C), 141.23 (C), 138.57 (C), 135.43 (C), 132.86 (CH), 131.38 (CH), 130.80 (CH), 130.75 (C), 129.90 (CH), 128.64 (2CH), 127.80 (C), 127.45 (2CH), 127.31 (CH), 126.68 (C), 126.17 (C), 123.86 (CH), 122.99 (CH), 122.81 (CH), 121.38 (C), 120.54 (CH), 110.02 (CH), 70.13 (CH_2), 52.05 (CH_3), 38.53 (CH_3), 23.63 (CH_3); HRESIMS *m/z* 464.1865 $[M+H]^+$ (calcd for $C_{30}H_{26}NO_4$, 464.1856).

8-((3,5-dimethoxybenzyl)oxy)-4,5-dimethylphenanthridin-6(5H)-one (6e). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (d, J = 9.0 Hz, 1H), 8.03 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 2.8 Hz, 1H), 7.40 (dd, J = 8.9, 2.8 Hz, 1H), 7.28 (s, 1H), 7.20 (t, J = 7.6 Hz, 1H), 6.64 (d, J = 2.2 Hz, 2H), 6.43 (t, J = 2.2 Hz, 1H), 5.16 (s, 2H), 3.82 (s, 3H), 3.81 (s, 6H), 2.67 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.96 (C), 161.04 (2C), 158.49 (C), 138.79 (C), 138.58 (C), 132.85 (CH), 127.79 (C), 126.69 (C), 126.16 (C), 123.83 (CH), 122.97 (CH), 122.66 (CH), 121.37 (C), 120.53 (CH), 110.12 (CH), 105.40 (2CH), 100.14 (CH), 70.27 (CH_2), 55.42 (2CH_3), 38.52 (CH_3), 23.62 (CH_3); HRESIMS *m/z* 390.1701 $[M+H]^+$ (calcd for $C_{24}H_{24}NO_4$, 390.1700).

9-((1,3-dimethyl-1H-pyrazol-5-yl)methoxy)-4,5-dimethylphenanthridin-6(5H)-one (6f). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J = 9.0 Hz, 1H), 7.90 (t, J = 5.0 Hz, 2H), 7.23 (dd, J = 8.9, 2.7 Hz, 1H), 7.20–7.15 (m, 1H), 7.10 (t, J = 7.6 Hz, 1H), 6.08 (s, 1H), 5.04 (s, 2H), 3.78 (s, 3H), 3.72 (s, 3H), 2.56 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.73 (C), 157.71 (C), 147.33 (C), 138.51 (C), 137.39 (C), 132.98 (CH), 128.11 (C), 126.53 (C), 126.11 (C), 123.95 (CH), 122.95 (CH), 122.58 (CH), 121.10 (C), 120.52 (CH), 109.65 (CH), 106.95 (CH), 60.65 (CH_2), 38.44 (CH_3), 36.43 (CH_3), 23.58 (CH_3), 13.40 (CH_3); HREIMS *m/z* 347.1639 $[M]^+$ (calcd for $C_{21}H_{21}N_3O_2$, 347.1634).

8-((5-chlorobenzo[*b*]thiophen-2-yl)methoxy)-4,5-dimethylphenanthridin-6(5H)-one (6g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 9.0 Hz, 1H), 8.10 (d, J = 2.8 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 2.0 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.64 (s, 1H), 7.41 (dd, J = 8.9, 2.9 Hz, 1H), 7.36 (dd, J = 8.6, 1.9 Hz, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 5.42 (s, 2H), 3.84 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.91 (C), 158.17 (C), 138.72 (C), 138.58 (C), 132.94 (CH), 130.86 (C), 130.82 (C), 128.05 (C), 127.72 (CH), 126.64 (C), 126.18 (C), 125.32 (C), 125.21 (CH), 123.98 (CH), 123.84 (CH), 123.00 (CH), 122.81 (CH), 121.89 (CH), 121.27 (C), 120.56 (CH), 109.80 (CH), 64.71 (CH_2), 38.53 (CH_3), 23.61 (CH_3); HREIMS *m/z* 419.0750 $[M]^+$ (calcd for $C_{24}H_{18}ClNO_2S$, 419.0747).

9-(2-(1,3-dioxolan-4-yl)ethoxy)-4,5-dimethylphenanthridin-6(5H)-one (6h). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (d, J = 8.9 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 2.4 Hz, 1H), 7.32 (dd, J = 8.9, 2.6 Hz, 1H), 7.27 (s, 1H), 7.20 (t, J = 7.6 Hz, 1H), 5.14 (t, J = 4.6 Hz, 1H), 4.30 (t, J = 6.5 Hz, 2H), 4.07–3.98 (m, 2H), 3.95–3.86 (m, 2H), 3.82 (s, 3H), 2.67 (s, 3H), 2.23 (q, J = 6.4 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.96 (C), 158.66 (C), 138.51 (C), 132.72 (CH), 127.51 (C), 126.64 (C), 126.10 (C), 123.67 (CH), 122.89 (CH), 122.48 (CH), 121.38 (C), 120.46 (CH), 109.66 (CH), 101.99 (CH), 64.97 (CH_2), 64.01 (CH_2), 38.49 (CH_3), 33.66 (CH_2), 29.69 (CH_2), 23.59 (CH_3); HRESIMS *m/z* 340.1548 $[M+H]^+$ (calcd for $C_{20}H_{22}NO_4$, 340.1543).

4,5-dimethyl-[1,3]dioxolo[4,5–*j*]phenanthridin-6(5H)-one (8a). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 7.9 Hz, 1H), 7.85 (s, 1H), 7.56 (s, 1H), 7.27 (dd, J = 7.4, 0.7 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 6.10 (s, 2H), 3.79 (s, 3H), 2.65 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.50 (C), 152.23 (C), 148.25 (C), 139.05 (C), 133.15 (CH), 130.85 (C), 126.13 (C), 122.74 (CH), 121.19 (C), 121.10 (C), 120.77 (CH), 106.64 (CH), 101.93 (CH_2), 100.74 (CH), 38.36 (CH_3), 23.64 (CH_3); HRESIMS *m/z* 268.0969 $[M+H]^+$ (calcd for $C_{16}H_{14}NO_3$, 268.0968).

4.2. Bioactivity assays

4.2.1. Cell culture

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated FBS in a humidified 5% CO_2/95% air (v/v) atmosphere at 37 °C.

4.2.2. Reporter gene assay

HEK293T cells were transfected using Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions. For reporter gene assays, HEK293T cells were seeded in 24-well plates. Each well was transfected with 250 ng of plasmids in total, including 20 ng of TOPFlash and 25 ng of EGFP-C1. The LacZ plasmid was added to equalize the total amount of plasmid in the wells to 250 ng. Eighteen hours after transfection, the cells were treated with Wnt3a-conditioned medium (Wnt3a CM) or control medium (Ctr CM) for an additional 6 h and were lysed using a Boehringer Mannheim Luciferase Assay Kit (200 μL/well) for luciferase assays. The fluorescence intensity emitted by green fluorescent protein (GFP) in the resultant cell lysates was first determined in a Wallac multi-counter capable of counting fluorescence and luminescence. Next, the luciferase substrate was added to the cell lysates, and the luciferase activities were determined by measuring the luminescence intensity using the same counter. The luminescence intensities were normalized against the fluorescence intensities [20].

4.2.3. Target gene assay

Cells were treated with the synthesized compounds as indicated and control or Wnt3a CM for 6 h. Total RNA was extracted with TRIzol. Additionally, purified RNA was reverse transcribed using oligo (dT) priming and the Superscript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. The gene transcripts were quantified by quantitative real-time PCR using a Quantitative SYBR green PCR kit (Takara SYBR premix Ex Taq) and ABI Quant Studio 6. Gene expression was normalized by GAPDH. The following primer pairs were used for the target genes: *Axin2*: 5′-AGGCT AGCTGAGGTGT-3′ and 5′-AGGCTTG-GATTGGAGAA-3′; *NKD1*: 5′-GTC AACCACTCCCCAACATC-3′ and 5′-AATGGTGGTAG-CAGCCAGAC-3′; *GAPDH*: 5′-AGGTCGGAGTCAACGGATTTG-3′ and 5′-TGTAAACCA-TGTAGTTGAGGTCA-3′.

4.3. Molecular docking protocol

Compounds **12** and HLY78 were docked with EPAC2 using the X-ray structure of axin (PDB code: **1WSR**) and AutoDock 4.2. The H_2O molecules and ligand within the crystal structure were removed, and polar hydrogen moieties were added using AutoDockTools. In the structures of the analogues, all bonds were rotatable except the aromatic, amide, cyano, and double bonds; the protein was treated as a rigid structure. The docking runs were performed using the standard parameters of the programmefor interactive growth and subsequent scoring.

Author contributions

D-Z Chen and B-J Yang designed and performed the chemical syntheses under the guidance of X-J Hao; J-Y Caiand J-J Guo purified all the compounds; X-LHe, HZhangand Y Zhang evaluated the Wnt activities and other bioactivities of all compoundsunder the guidance of L Lin.; D-Z Chen and X-L He wrote the manuscript with advice from all of the authors; X.H. guided all aspects of this study.

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Appendix A. Supplementary material

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