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# Design, Synthesis, and Evaluation of Novel Porcupine Inhibitors Featuring a Fused 3-ring System Based on the "Reversed" Amide Scaffold

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**Abstract:** The Wnt signaling pathway is an essential signal transduction pathway which leads to the regulation of cellular processes such as proliferation, differentiation and migration. Aberrant Wnt signaling is known to have an association with multiple cancers. Porcupine is an enzyme that catalyses the addition of palmitoleate to a serine residue in Wnt proteins, a process which is required for the secretion of Wnt proteins. Here we report the synthesis and structure-activity-relationship of the novel porcupine inhibitors based on a "reversed" amide scaffold. The leading compound **53** was as potent as the clinical compound LGK974 in a cell based STF reporter gene assay. Compound **53** potently inhibited the secretion of Wnt3A, therefore was confirmed to be a porcupine inhibitor. Furthermore, compound **53** showed excellent chemical and plasma stabilities. However, the clearance of compound **53** in liver microsomal tests was moderate to high, and the solubility of compound **53** was suboptimal. Collective efforts toward further optimization of this novel tricyclic template to develop better porcupine inhibitors will be subsequently undertaken and reported in due course.

**Keywords:** Wnt signaling pathway, porcupine, antagonist, cancer therapy, scaffold hybridization

**Abbreviations:** BPO, benzoyl peroxide; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; FaSSIF, fasted state simulated intestinal fluid; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; SEM, standard error measurement; SGF, simulated gastric fluid; STF, super-top flash, TFA, trifluoroacetic acid.

## 1 Introduction

The Wnt signaling pathway plays a critical role in the regulation of cellular processes such as proliferation, differentiation and migration[1-3]. The canonical Wnt signaling pathway begins when Wnt ligands bind to the Frizzled and LRP families of cell surface receptors via the cytoplasmic protein Dishevelled (DSH), leading to an accumulation of cytoplasmic β-catenin and its translocation into the nucleus. Ultimately, β-catenin associates with the TCF/LEF family of DNA-binding proteins and activates the expression of β-catenin mediated genes downstream. In contrast, in the absence of Wnt ligand stimulation, β-catenin is phosphorylated and degraded by an intracellular β-catenin destruction complex, resulting in the inhibition of downstream gene expression[4]. Overexpression of Wnt ligands has been associated with numerous cancers[5,6]. Porcupine, a member of the membrane-bound *O*-acyltransferase family of proteins, adds palmitoleate to a serine residue in Wnt proteins - a process which is required for the secretion of Wnt proteins[7]. Porcupine inhibitors can thus block aberrant Wnt signaling and inhibit tumor growth[8]. Therefore, porcupine has emerged as a potential target for the treatment of cancer.

The IWP series of compounds (Fig. 1) identified in a high throughput screen were the first small molecule porcupine inhibitors reported by Chen et al[9]. Since then, other classes of porcupine inhibitors have also been investigated. LGK974, developed by Novartis in 2012, is a potent porcupine inhibitor which has been advanced into a phase I/II clinical trial[10,11]. Recently, Virshup and co-workers reported their work on porcupine inhibitors.[12,13] Among them, ETC-159 has been advanced into a phase I clinical trial[12] (Fig.1).

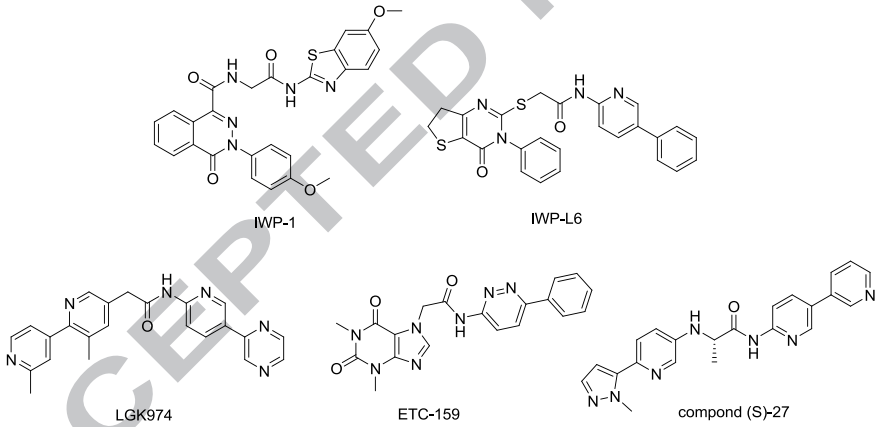



**Figure 1.** Reported porcupine inhibitors in the literature

## 2 Design

We have investigated a novel series of porcupine inhibitors by a scaffold hopping strategy from a known porcupine antagonist LGK974[14]. DC-9 was the result of optimization campaigns in a recently published Novartis patent[15]. Although the central amide bonds were reversed, both LGK974 and DC-9 showed excellent potency. Encouraged by this result, we decided to introduce the tricyclic element into the "reversed" amide porcupine inhibitor framework. Here we report the synthesis and structure-activity-relationship of the novel porcupine inhibitors based on the "reversed" amide scaffold as shown in Fig. 2.

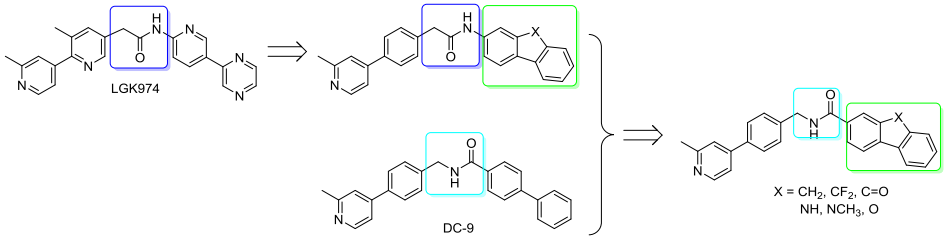
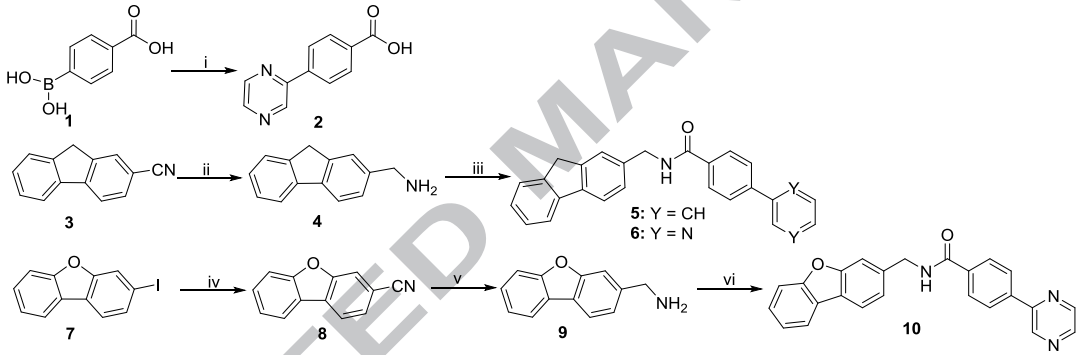



**Figure 2.** The design strategy for the novel porcupine inhibitors

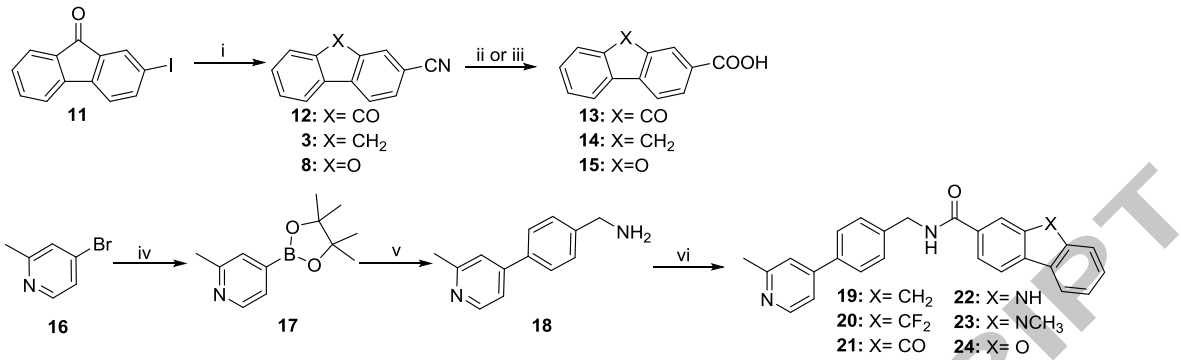
## 3 Chemistry

The synthetic route used to prepare compounds **5**, **6** and **10** is outlined in Scheme 1. The synthesis of compounds **3** and **7** was described in our previously published paper[14]. Commercially available 4-boronobenzoic acid was coupled with 2-chloropyrazine to produce aromatic acid **2**. Nitrile **3** was reduced with $H_2$ and Pd/C to give amine **4**, which was reacted with corresponding aromatic acids in the presence of HATU in DMF to give the final compounds **5** and **6**, respectively. Compound **7** was converted to nitrile **8** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Nitrile **8** was reduced with $LiAlH_4$ to give amine **9**, which was reacted with aromatic acid **2** to give the final compound **10**.



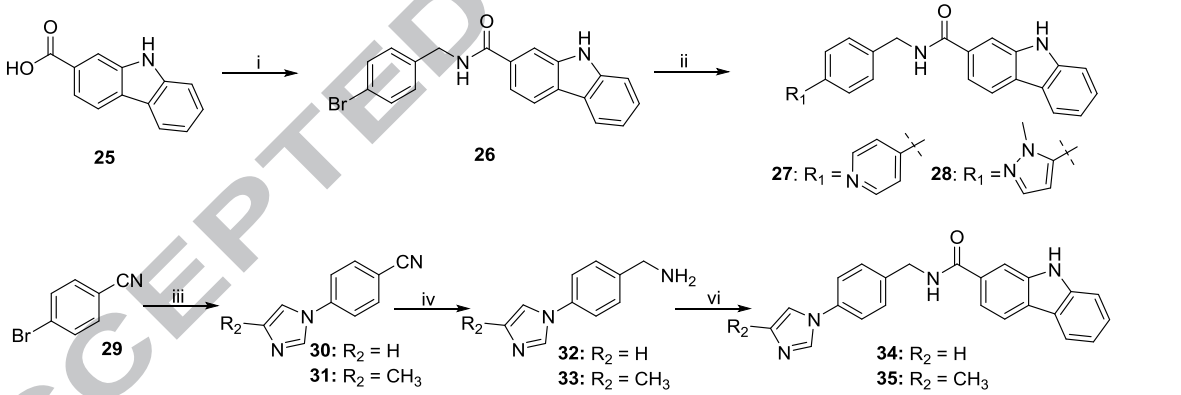

**Scheme 1**. Reagents and conditions. (i) 2-chloropyrazine, $Pd(Ph_3P)_4$, $Na_2CO_3$, $CH_3CN$, $H_2O$, 80°C, 12 h; (ii) Pd/C, 37% HCl, $H_2$, EtOH, r. t., 24 h; (iii) 4-(pyridin-3-yl)benzoic acid or **2**, HATU, DIPEA, DMF, r. t., overnight; (iv) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 90°C, 2 h; (v) $LiAlH_4$, THF, r. t., 8 h; (vi) **2**, HATU, DIPEA, DMF, r. t., overnight.

Target compounds **19-24** were prepared via the synthetic route depicted in Scheme 2. The synthesis of aryl iodide **11**, 9,9-difluoro-9*H*-fluorene-2-carboxylic acid, 9*H*-carbazole-2-carboxylic acid and 9-methyl-9*H*-carbazole-2-carboxylic acid was also described in our previously published paper[14]. Compound **11** was converted to nitrile **12** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Nitrile **12**, **3** and **8** were hydrolyzed to give carboxylic acids **13-15**, respectively. Commercially available 4-bromo-2-methylpyridine was borylated to provide aryl boronate **17**, which was coupled with (4-bromophenyl)methanamine to give intermediate **18**. Condensation of amine **18** with corresponding aromatic acids in the presence of HATU in DMF afforded the target compounds **19-24**, respectively.



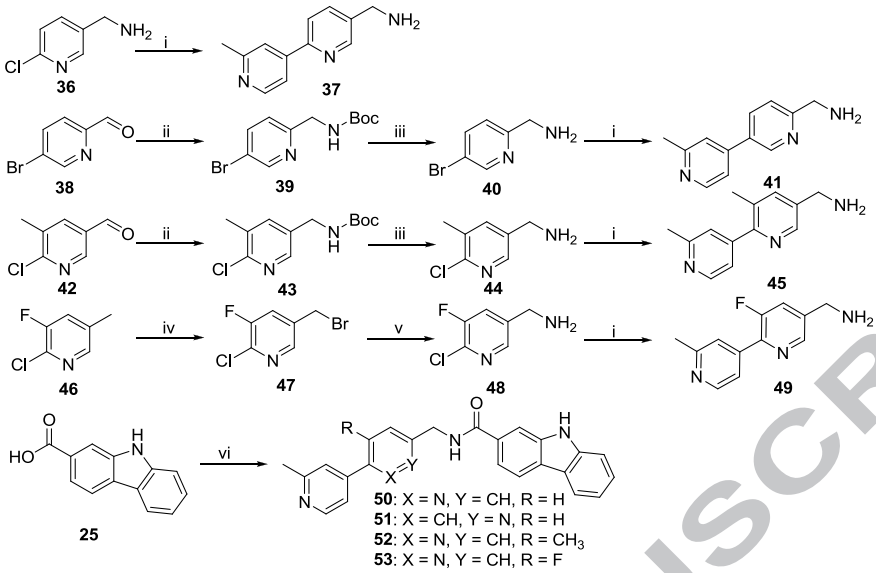

**Scheme 2**. Reagents and conditions. (i) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 90°C, 2 h; (ii) for **13** and **15**: KOH, MeOH, $H_2O$, reflux, 6 h; (iii) for **14**: 70% $H_2SO_4$, $CH_3COOH$, 100°C, 12 h; (iv) bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$, THF, 80°C, overnight; (v) (4-bromophenyl)methanamine, $Pd(dba)_2$, $K_3PO_4$, Sphos, *t*-BuOH, $H_2O$, 100°C, 12 h; (vi) HATU, DIPEA, DMF, r. t., 12 h.

Scheme 3 shows the synthetic approaches to the target compounds **27**, **28**, **34** and **35**. Compound **25** was reacted with (4-bromophenyl)methanamine to give intermediate **26**, which was coupled with corresponding aryl boronic acid to produce the desired compounds **27** and **28**, respectively. Commercially available 4-bromobenzonitrile **29** was coupled with imidazole or 4-methyl-1*H*-imidazole to give intermediates **30** and **31**, which were reduced with $LiAlH_4$ to yield amines **32** and **33**, respectively. The amide coupling was carried out by treatment of carboxylic acid **25** with aromatic amines **32** or **33** in the presence of HATU in DMF, which led to the desired compounds **34** and **35**, respectively.



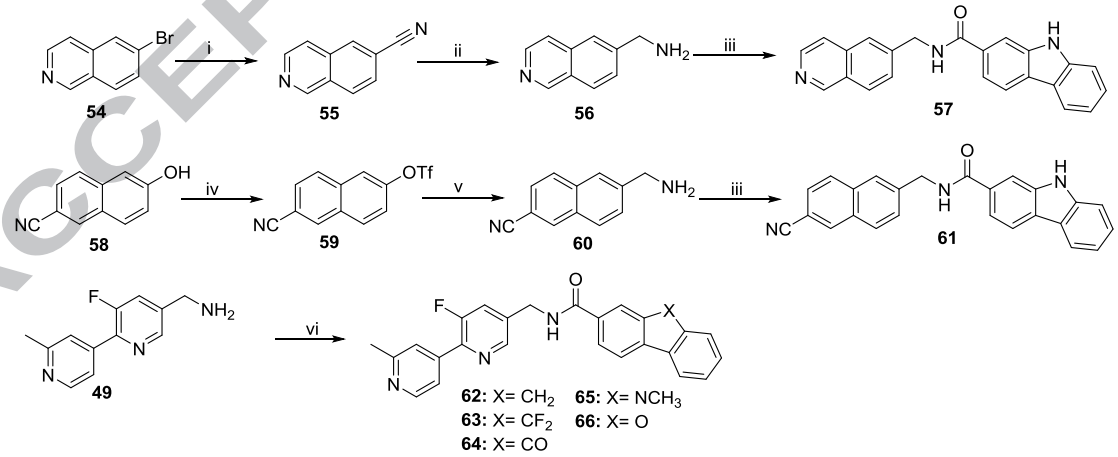

**Scheme 3**. Reagents and conditions. (i) (4-bromophenyl)methanamine, HATU, DIPEA, DMF, r. t., 12 h; (ii) pyridin-4-ylboronic acid or 1-methyl-1*H*-pyrazol-5-ylboronic acid, $Pd(dppf)Cl_2$, dppf, $K_3PO_4$, $H_2O$, dioxane, 100°C, 12 h; (iii) imidazole or 4-methyl-1H-imidazole, CuI, $Cs_2CO_3$, DMF, 130°C, 24 h; (iv) $LiAlH_4$, THF, 80°C, 2 h; (vi) **25**, HATU, DIPEA, DMF, r. t., 12 h.

The synthetic pathways used to prepare the target compounds **50-53** are summarized in Scheme 4. Suzuki coupling of commercially available 5-(aminomethyl)-2-chloropyridine **36** with aryl boronate **17** produced amine **37**. Aldehydes **38** and **42** were reacted with *tert*-butyl carbamate in the presence of TFA and $Et_3SiH$ to give compounds **39** and **43**, respectively. Removal of Boc in **39** and **43** by TFA/DCM provided amines **40** and **44**, which were coupled with aryl boronate **17** to give intermediates **41** and **45**, respectively. Bromination of 2-chloro-3-fluoro-5-methylpyridine using NBS in $CH_3CN$ provided compound **47**, which was reacted with $NaN_3$ and subsequently reduced with $PPh_3$ to give amine **48**. Compound **48** was coupled with aryl boronate **17** to give intermediate **49**. Condensation of aromatic acid **25** with corresponding amines gave the target compounds **50-53**, respectively.




**Scheme 4**. Reagents and conditions. (i) **17**, $Pd(PPh_3)_4$, $K_2CO_3$, dioxane, $H_2O$, 100°C, overnight; (ii) *tert*-butyl carbamate, TFA, $Et_3SiH$, $CH_3CN$, r. t., 24 h; (iii) TFA, DCM, r. t., 3 h; (iv) NBS, BPO, $CH_3CN$, reflux, overnight; (v) a) $NaN_3$, DMF, 50°C, overnight; b) $PPh_3$, THF, reflux, 8 h; (vi) corresponding amine, HATU, DIPEA, DMF, r. t., 12 h.

The synthesis of the target compounds **57** and **61-66** is summarized in Scheme 5. Commercially available 6-bromoisoquinoline **54** was converted to nitrile **55** in the presence of $Zn(CN)_2$ and $Pd(Ph_3P)_4$. Compound **55** was reduced with $H_2$ and Pd/C to give amine **56**, which was reacted with aromatic acid **25** to yield the target compound **57**. 6-Hydroxy-2-naphthonitrile **58** was readily converted to the triflate intermediate **59**. Compound **59** was coupled with $MeNO_2$ in the presence of $Pd(dba)_2$, $K_3PO_4$ and Xphos in dioxane and subsequently reduced by Zn in AcOH to give amine **60**, which was reacted with aromatic acid **25** to provide the target compound **61**. Condensation of amine **49** with corresponding aromatic acids gave the target compounds **62-66**, respectively.




**Scheme 5**. Reagents and conditions. (i) $Zn(CN)_2$, $Pd(Ph_3P)_4$, DMF, 90°C, 24 h; (ii) Pd/C, 37% HCl, $H_2$, EtOH, r. t., 24 h; (iii) **25**, HATU, DIPEA, DMF, r. t., overnight; (iv) $Tf_2O$, $Et_3N$, DCM, r. t., overnight; (v) a) $Pd(dba)_2$, Xphos, $K_3PO_4$, $MeNO_2$, dioxane, 80°C, 18 h; b) Zn, AcOH, 35°C, 3 h; (vi) corresponding acid, HATU, DIPEA, DMF, r. t., overnight.

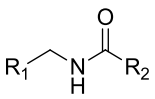
## 4. Results and Discussion

**4.1. Evaluation of pharmacological activity**

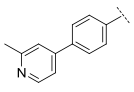
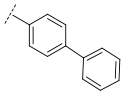
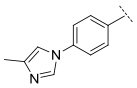
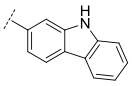
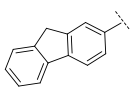
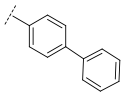
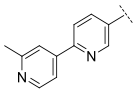
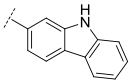
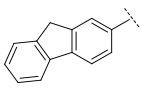
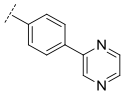
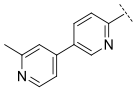
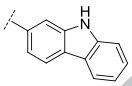
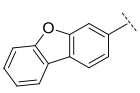
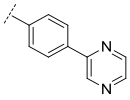
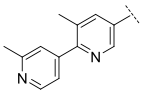
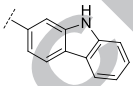
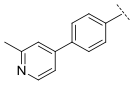
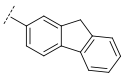
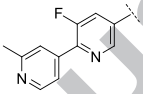
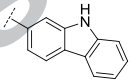
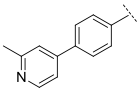
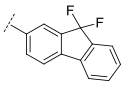
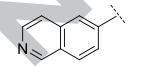
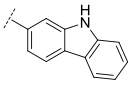
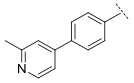
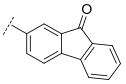
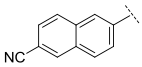
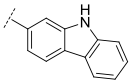
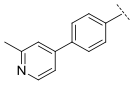
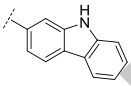
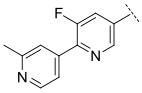
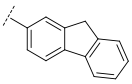
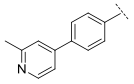
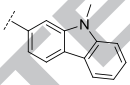
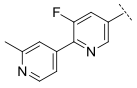
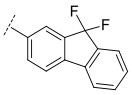
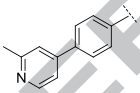
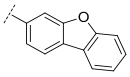
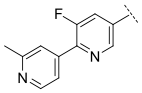
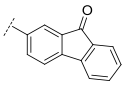
A cell based STF (super-top flash) reporter gene assay was employed to test Wnt signaling inhibition of the target compounds. We first confirmed that LGK974 was active in this assay (LGK974, 0.9 nM, Table 1), and its $IC_{50}$ number was consistent with the number reported in the literature (LGK974, 0.4 nM) [10,11]. The structure-activity relationship is summarized in Table 1.

Cyclization of the left-side rings of DC-9 led to inactive compounds, as exemplified by compounds **5** and **6**, while compound **10** was weakly active. This was consistent with recently published papers, which indicated that a hydrogen bond acceptor was needed at this region[14,16]. We thus decided to keep the key interaction by maintaining the structural element of DC-9 on the left hand side, and started to explore the tricyclic structure-activity relationship on the right hand side. When $R_1$ was the same as DC-9, the tricyclic elements fluorene, difluoro-fluorene and fluorine-9-one provided active compounds (compounds **19**, **20**, **21**; 35, 60, 85 nM, respectively). The carbazole and dibenzofuran showed increased activity (compounds **22** and **24**; 7.5 and 9.1 nM, respectively), while methylated carbazole was inactive (compound **23**, >1000 nM). The steric hindrance effect completely eliminated activity, which was consistent with the conclusion drawn by our recently published paper[14]. Among the above compounds, carbazole **22** showed the best activity. Encouraged by this result, we kept the element of compound **22** on the right side and started to explore the structure-activity relationship on the left hand side. Removal of methyl from the pyridine resulted in slightly decreased activity (compound **27**, 18 nM). When the 2-methyl-pyridine was replaced by *N*-methyl-pyrazole, imidazole and 4-methyl-imidazole, resulting in compounds **28**, **34** and **35** respectively, Wnt signaling inhibition activity was significantly decreased in all three compounds (130, 314 and 119 nM, respectively). Replacement of a carbon with a nitrogen on the internal ring of the left hand side was comparable to or slightly less potent than compound **22** (compounds **50** and **51**; 6.2 and 18 nM, respectively). Substitution on the carbon of the internal ring of the left hand side with a methyl group resulted in slightly deceased activity (compounds **52,** 10 nM); while substitution on the same position with a fluorine significantly improved potency (compound **53**, 0.5 nM). Compound **53** was twice as potent as LGK974 in the same assay. Fusion of the biphenyl to bicyclic element was not well tolerated, as demonstrated by compound **57** (207 nM), while the addition of a nitrile group restored activity (compound **61**, 13 nM). Nitrile, a versatile functional group in medicinal chemistry[17], served as a more favorable hydrogen bond acceptor in this case. Thus far, compound **53** showed the best activity among all of the synthesized compounds. Finally, we kept the left side element of compound **53** and explored the tricyclic structure-activity relationship on the right hand side. Although the fluorene-9-one and *N*-methyl-carbazole showed only moderate activity (compounds **64** and **65**; 43 and 175 nM, respectively), the tricyclic elements fluorene, difluoro-fluorene and dibenzofuran provided much more active compounds (**62**, **63**, **66**; 9.2, 16, 3.1 nM, respectively), In summary, through extensive SAR studies, numerous active compounds (eg. compounds **22**, **50**, **53**, **66)** were achieved, among these, the most promising compound **53** was as potent as LGK974 in our assay.

**Table 1.** SAR of designed compounds




| No. | $R_1$ | $R_2$ | $IC_{50}$(nM) ± SEM[a] | No. | $R_1$ | $R_2$ | $IC_{50}$ (nM) ± SEM |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **DC-9** |  |  | 1.0 ± 0.2 | **35** |  |  | 119 ± 13 |
| **5** |  |  | > 1000 | **50** |  |  | 6.2 ± 2.4 |
| **6** |  |  | > 1000 | **51** |  |  | 18 ± 0.5 |
| **10** |  |  | 701 | **52** |  |  | 10 ± 0 |
| **19** |  |  | 35 ± 10 | **53** |  |  | 0.5 ± 0.2 |
| **20** |  |  | 60 ± 16 | **57** |  |  | 207 ± 21 |
| **21** |  |  | 85 ± 2 | **61** |  |  | 13 ± 5 |
| **22** |  |  | 7.5 ± 4.5 | **62** |  |  | 9.2 ± 3.8 |
| **23** |  |  | > 1000 | **63** |  |  | 16 ± 1.5 |
| **24** |  |  | 9.1 ± 0.3 | **64** |  |  | 44 ± 14 |
| **27** |  |  | 18 ± 4 | **65** |  |  | 175 ± 66 |
| **28** |  |  | 130 ± 52 | **66** |  |  | 3.1 ± 1.1 |
| **34** |  |  | 314 ± 91 | **LGK 974**[b] | | | 0.9 ± 0.1 |

[a] Inhibition of luminescence signaling in a Wnt generating L Wnt3A cells and Wnt responding HEK293 cells co-cultured system. Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).

[b] LGK974 was run as standard in each assay. Data are expressed as geometric mean values of four runs ± the standard error measurement (SEM).

The enzyme porcupine, a member of the membrane-bound *O*-acyltransferase family of proteins, catalyzes the palmitoylation of Wnt proteins. This process is essential for their secretion and activity. Without this crucial palmitoylation, Wnt proteins cannot be secreted outside of cells. We thus performed a second assay to confirm the target of the new compounds was indeed porcupine[14,18,19]. HEK293T cells were transfected with pLinbin-Wnt3A plasmid or vehicle control. The HEK293T cells were then treated with or without compounds. Western Blot was used after 48 hours to analyze both the cell lysis and culture medium. We found that both compounds **22** and **53**, as well as LGK974 all potently inhibited Wnt3A secretion into cell culture medium, while the above compounds did not affect the amount of Wnt3A inside of HEK293T cells. These results suggested that the new compounds were indeed porcupine inhibitors (Fig. 3).

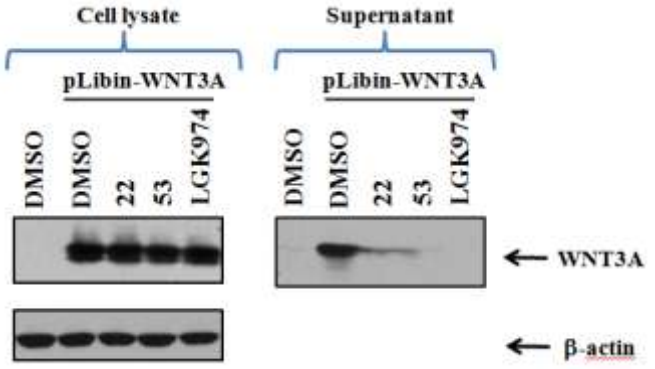



**Figure 3.** The Wnt secretion assay. Compound concentration was at 0.1 μM

**4.2. Evaluation of chemical stability, rat plasma stability, liver microsomal stability**

The majority of reported Porcupine inhibitors contain an amide group as part of their chemical structure. Similarly, an amide group was also present in our novel template. Amide groups have been reported as potentially unstable and may be hydrolyzed in saline, plasma and under the treatment of liver microsomal enzymes[9]. We thus evaluated the stability of representative compounds **22** and **53**. The stability of these compounds was tested in simulated gastric fluid (SGF), rat plasma and under the treatment of liver microsomal enzymes. Compounds **22** and **53** both showed good stability in SGF after 24 hours and were both stable after 8 hours in rat plasma. However, despite compounds **22** and **53** demonstrating moderate clearance under the treatment of human liver microsomes (59 and 57 mL/min/kg, respectively) and rat liver microsomes (7 and 24 mL/min/kg, respectively), compounds **22** and **53** exhibited high clearance when treated with mouse microsomes (141 and 109 mL/min/kg, respectively). This was in contrast to LGK974, which demonstrated excellent metabolic stability cross all species (Table 2). These results indicate that further optimization might be needed to improve the metabolic stability, and therefore in vivo bioavailability in mouse of the current compounds.

**Table 2**. Chemical stability, plasma stability and metabolic stability of compounds **22** and **53**

| Compd | % Remaining in SGF, 24 h[a] | % Remaining in rat plasma, 8 h[b] | $Cl_{int}$ (mL/min/kg)[b] | | | $t_{1/2}$ (min) | | |
|---|---|---|---|---|---|---|---|---|
| | | | HLM[c] | RLM[d] | MLM[e] | HLM | RLM | MLM |
| **22** | 100% | 100% | 59 | 7 | 141 | 30 | 277 | 43 |
| **53** | 100% | 100% | 57 | 24 | 109 | 31 | 80 | 55 |
| **LGK974** | 66% | 100% | 2 | 3 | 22 | 561 | 770 | 277 |

[a] All compounds were tested at 200 μM concentration in simulated gastric fluid (SGF) at 40°C for 24 h.

[b] All compounds were tested at 1 μM concentration.
[c] HLM = human liver microsomes.
[d] RLM = rat liver microsomes.
[e] MLM = mouse liver microsomes.

### 4.3. Evaluation of CYP inhibition and solubility

The leading compounds **22** and **53** were subjected to the standard CYP inhibition test, and they showed weak or no CYP inhibition at concentration of 10 μM. Unfortunately, the solubility of compounds **22** and **53** was poor (Table 3). The poor solubility in combination with the suboptimal metabolic stability prevented us from testing these compounds in vivo. Collective efforts toward further optimization of this novel tricyclic template to develop better porcupine inhibitors will be subsequently undertaken.

**Table 3**. CYP inhibition profiles and solubility of compounds **22** and **53**

| Compd | CYP inhibition (%) [a] | | | | | Solubility (μM) [b] |
|---|---|---|---|---|---|---|
| | 3A4 | 2D6 | 1A2 | 2C9 | 2C19 | PH 6.5 |
| **22** | 7 | 9 | 64 | -22 | 8 | 0.16 |
| **53** | 69 | 37 | 73 | 31 | 59 | 1.24 |

[a] These two compounds were tested at 10 μM concentration.
[b] 4 mg/mL suspension of each tested compound (HCl salt) in FaSSIF (pH 6.5) was shaken for 1 h, then equilibrated overnight at room temperature. Concentrations of the supernatants after centrifugation were determined by LCMS/MS detection.

## 5. Conclusion

We have designed and synthesized a series of Wnt compounds based on the "reversed" amide scaffold. This novel scaffold provided active compounds (e.g. compounds **22**, **50**, **53** and **66**; 7.5, 6.2, 0.5 and 3.1 nM, respectively). The leading compound **53** was as potent as the clinical compound LGK974 (0.9 nM). Compound **53** was confirmed to be an inhibitor of Porcupine via a cell based secretion assay and, furthermore, showed excellent chemical and plasma stabilities. However, the clearance of compound **53** in liver microsomal tests was moderate to high, and the solubility of compound **53** was suboptimal. Subsequent efforts to further optimize this novel tricyclic template to improve liver microsomal stability, solubility and develop better porcupine inhibitors will be undertaken and reported in due course.

## 6. Experimental section

### 6.1. Chemistry

Analytical thin layer chromatography was performed on silica gel HSGF254 pre-coated plates to monitor the general reaction progress. Final compounds were purified by column chromatography with silica gel 100-200 mesh. $^{1}$H NMR and $^{13}$C NMR were performed on 300 MHz (Varian) and 400 MHz (Varian) spectrometers. Chemical shifts were given in ppm using tetramethylsilane as internal standard. Mass spectra were performed on an Agilent 1100 LC/MSD Trap SL version Mass Spectrometer. HRMS analysis was obtained using an Agilent 6540 UHD Accurate-Mass Q-TOF LC/MS.

#### 6.1.1. 4-(Pyrazin-2-yl)benzoic acid (2)

To a suspension of 4-boronobenzoic acid (664 mg, 4 mmol), 2-chloropyrazine (458 mg, 4 mmol) and

$Pd(PPh_3)_4$ (230 mg, 0.2 mmol) in $CH_3CN$ (20 mL) and $H_2O$ (20 mL) was added $Na_2CO_3$ (848 mg, 8 mmol). The reaction mixture was stirred at 80°C under $N_2$ for 12 h. After cooling to room temperature, the mixture was filtered. The filtrate was washed with DCM (20 mL x 3). The pH was adjusted to 4 with 1 N HCl, and then the mixture was filtered to give the desired product (647 mg, 80%) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.33 (d, $J$ = 0.8 Hz, 1H), 8.81-8.74 (m, 1H), 8.68 (d, $J$ = 2.4 Hz, 1H), 8.27 (d, $J$ = 8.4 Hz, 2H), 8.08 (d, $J$ = 8.4 Hz, 2H). ESI-MS (m/z): 201.0 $[M-H]^-$.

**6.1.2. (9*H*-Fluoren-2-yl)methanamine (4)**

To a solution of 9*H*-fluorene-2-carbonitrile (100 mg, 0.5 mmol) in ethanol (10 mL) was added Pd/C (20 mg) and 37% HCl (0.1 mL), and the reaction mixture was stirred at room temperature for 24 h under $H_2$. Saturated aqueous $Na_2CO_3$ (10 mL) was added. The mixture was filtered, and the filtrate was extracted with DCM (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$ and concentrated to give the desired product (40 mg, 39%) as a light yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.80-7.72 (m, 2H), 7.56-7.50 (m, 2H), 7.39-7.35 (m, 1H), 7.33-7.27 (m, 2H), 3.95 (s, 2H), 3.89 (s, 2H).

**6.1.3. General procedure for the synthesis of compounds 5 and 6**

To a solution of **4** (1 eq.) and corresponding acid (1 eq.) in DMF (1.5 mL) were added HATU (1 eq.) and DIPEA (5 eq.). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography to give the desired products **5** and **6**.

**6.1.3.1. *N*-((9*H*-Fluoren-2-yl)methyl)-[1,1'-biphenyl]-4-carboxamide (5).** White solid (yield: 83%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.15 (t, $J$ = 6.0 Hz, 1H), 8.02 (d, $J$ = 8.4 Hz, 2H), 7.86 (dd, $J$ = 7.2, 5.2 Hz, 2H), 7.79 (d, $J$ = 8.0 Hz, 2H), 7.74 (d, $J$ = 7.6 Hz, 2H), 7.57 (d, $J$ = 9.2 Hz, 2H), 7.52-7.46 (m, 2H), 7.44-7.34 (m, 3H), 7.32-7.27 (m, 1H), 4.59 (d, $J$ = 6.0 Hz, 2H), 3.91 (s, 2H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 165.7, 143.1, 142.9, 142.7, 140.8, 139.8, 139.1, 138.4, 138.3, 133.1, 128.9, 127.9, 126.7, 126.6, 126.4, 126.0, 125.0, 124.0, 119.7, 119.6, 42.8, 36.2. ESI-MS (m/z): 375.9 $[M+H]^+$.

**6.1.3.2. *N*-((9*H*-Fluoren-2-yl)methyl)-4-(pyrazin-2-yl)benzamide (6).** Off white solid (yield: 35%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.67 (s, 1H), 8.56 (s, 1H), 8.12 (d, $J$ = 8.0 Hz, 2H), 7.97 (d, $J$ = 8.0 Hz, 2H), 7.83-7.75 (m, 2H), 7.61-7.54 (m, 2H), 7.43-7.36 (m, 2H), 7.34-7.29 (m, 1H), 6.50 (s, 1H), 4.76 (d, $J$ = 5.2 Hz, 2H), 3.91 (s, 2H). ESI-MS (m/z): 378.0 $[M+H]^+$.

**6.1.4. Dibenzo[b,d]furan-3-carbonitrile (8)**

To a suspension of 3-iododibenzo[b,d]furan (500 mg, 1.7 mmol) and $Pd(PPh_3)_4$ (196 mg, 0.17 mmol) in DMF (15 mL) was added $Zn(CN)_2$ (117 mg, 1.0 mmol), and the reaction mixture was stirred at 90°C under $N_2$ for 2 h. After cooling to room temperature, $H_2O$ (40 mL) was added and the mixture was extracted with ethyl acetate (40 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by silica gel column chromatography (petroleum ether/ethyl acetate = 50/1), **8** was obtained as a white solid (310 mg, 85%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, $J$ = 12.4, 8.0 Hz, 2H), 7.88 (s, 1H), 7.64 (d, $J$ = 8.4 Hz, 2H), 7.60-7.55 (m, 1H), 7.45-7.40 (m, 1H).

**6.1.5. Dibenzo[b,d]furan-3-ylmethanamine (9)**

To a solution of **8** (130 mg, 0.67 mmol) in THF (3 mL) was added $LiAlH_4$ (77 mg, 2.02 mmol) at 0°C. The mixture was stirred at room temperature for 8 h. Aqueous NaOH (2 N, 20 mL) was added and the mixture was extracted with DCM (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$ and concentrated to give a white solid (80 mg, 60%), which was used directly in the next step without further purification.

**6.1.6. *N*-(Dibenzo[b,d]furan-3-ylmethyl)-4-(pyrazin-2-yl)benzamide (10)**

To a solution of **9** (50 mg, 0.25 mmol) and **2** (50 mg, 0.25 mmol) in DMF (2 mL) were added HATU (95 mg, 0.25 mmol) and DIPEA (161 mg, 1.25 mmol). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (dichloromethane/methanol = 100/1), **11** was obtained as a white solid (15 mg, 15%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.38-9.28 (m, 2H), 8.77 (s, 1H), 8.69-8.65 (m, 1H), 8.27 (d, $J$ = 7.6 Hz, 2H), 8.15-8.06 (m, 4H), 7.71-7.65 (m, 2H), 7.53-7.47 (m, 1H), 7.43-7.36 (m, 2H), 4.69 (d, $J$ = 6.0 Hz, 2H). ESI-MS (m/z): 380.0 $[M+H]^+$.

**6.1.7. 9-Oxo-9*H*-fluorene-2-carbonitrile (12)**

To a suspension of 2-iodo-9*H*-fluoren-9-one (**11**) (1.2 g, 3.92 mmol) and $Pd(PPh_3)_4$ (452 mg, 0.39 mmol) in DMF (15 mL) was added $Zn(CN)_2$ (275 mg, 2.35 mmol), and the reaction mixture was stirred at 90°C under $N_2$ for 2 h. After cooling to room temperature, $H_2O$ (40 mL) was added and the mixture was extracted with ethyl acetate (40 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 50/1), **12** was obtained as a yellow solid (800 mg, 93%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.80 (d, $J$ = 7.6 Hz, 1H), 7.75 (d, $J$ = 7.6 Hz, 1H), 7.68-7.56 (m, 3H), 7.46-7.40 (m, 1H).

**6.1.8. 9-Oxo-9*H*-fluorene-2-carboxylic acid (13)**

To a solution of **12** (200 mg, 0.98 mmol) in $CH_3OH$ (7 mL) and $H_2O$ (7 mL) was added KOH (1.6 g, 29.4 mmol), and the reaction mixture was stirred at 110°C for 6 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The pH was adjusted to 3 with 1 N HCl, and then the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated to give the desired product (200 mg, 91%) as a yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.30 (s, 1H), 8.19 (d, $J$ = 7.6 Hz, 1H), 8.03 (s, 1H), 7.97-9.89 (m, 2H), 7.72-7.64 (m, 2H), 7.50-7.44 (m, 1H).

**6.1.9. 9*H*-Fluorene-2-carboxylic acid (14)**

To a solution of **3** (170 mg, 0.89 mmol) in $CH_3COOH$ (6 mL) was added 70% $H_2SO_4$ (6 mL), and the reaction mixture was stirred at 100°C for 12 h. After cooling to room temperature, the mixture was diluted with $H_2O$ (30 mL). The pH was adjusted to 9 with $Na_2CO_3$, and then the mixture was washed with DCM (30 mL). The pH of the aqueous layer was adjusted to 3 with 1 N HCl, and then the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated to give the desired product (80 mg, 43%) as a white solid. $^1$H NMR (400 MHz,

DMSO-$d_6$) δ 12.87 (s, 1H), 8.15(s, 1H), 8.02-7.98 (m, 3H), 7.64 (d, *J* = 6.8 Hz, 1H), 7.45-7.38 (m, 2H), 4.01 (s, 2H).

**6.1.10. Dibenzo[b,d]furan-3-carboxylic acid (15)**

To a solution of **8** (160 mg, 0.83 mmol) in $CH_3OH$ (6.5 mL) and $H_2O$ (6.5 mL) was added KOH (1.4 g, 24.8 mmol), and the reaction mixture was stirred at 110°C for 6 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The pH was adjusted to 3 with 1 N HCl, and then the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated to give the desired product (160 mg, 91%) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.19 (s, 1H), 8.30-8.16 (m, 3H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.65-7.58 (m, 1H), 7.49-7.43 (m, 1H).

**6.1.11. (4-(2-Methylpyridin-4-yl)phenyl)methanamine (17)**

To a suspension of 4-bromo-2-methylpyridine (2.0 g, 11.6 mmol), Pd(dppf)$Cl_2$ (169 mg, 0.23 mmol) and KOAc (2.27 g, 23.2 mmol) in THF (40 mL) was added bis(pinacolato)diboron (3.18 g, 12.5 mmol). The mixture was stirred at 80°C under $N_2$ overnight. After cooling to room temperature, the mixture was diluted with DCM (250 mL) and then filtered. The filtrate was concentrated to give a black oil (5.1 g, crude), which was used directly in the next step without further purification.

**6.1.12. (4-(2-Methylpyridin-4-yl)phenyl)methanamine (18)**

To a suspension of **17** (5.1 g, crude), (4-bromophenyl)methanamine (1.8 g, 9.68 mmol) and Pd(dba)$_2$ (557 mg, 0.97 mmol) in *t*-BuOH (40 mL) and $H_2O$ (10 mL) were added $K_3PO_4$ (4.1 g, 19.4 mmol) and Xphos (397 mg, 0.97 mmol). The mixture was stirred at 100°C under $N_2$ for 12 h. After cooling to room temperature, the mixture was evaporated and the residue was purified by silica gel column chromatography (dichloromethane/methanol = 10/1) to give the desired product (800 mg, 42%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 5.2 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.37 (s, 1H), 7.31 (d, *J* = 5.2 Hz, 1H), 3.94 (s, 2H), 2.62 (s, 3H).

**6.1.13. General procedure for the synthesis of compounds 19-24**

To a solution of **18** (1 eq.) and corresponding acid (1 eq.) in DMF (1.5 mL) were added HATU (1 eq.) and DIPEA (5 eq.). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography to give the desired products **19-24**.

**6.1.13.1. *N*-(4-(2-Methylpyridin-4-yl)benzyl)-9*H*-fluorene-2-carboxamide (19).** White solid (yield: 22%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.16 (t, *J* = 6.0 Hz, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.14 (s, 1H), 8.03-7.94 (m, 3H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.57 (s, 1H), 7.51-7.46 (d, *J* = 8.0 Hz, 3H), 7.45-7.35 (m, 2H), 4.56 (d, *J* = 6.0 Hz, 2H), 4.00 (s, 2H), 2.52 (s, 3H). ESI-MS (m/z): 391.0 $[M+H]^+$.

**6.1.13.2. 9,9-Difluoro-*N*-(4-(2-methylpyridin-4-yl)benzyl)-9*H*-fluorene-2-carboxamide (20).** White solid (yield: 39%).$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.31 (t, *J* = 6.0 Hz, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.23 (s, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.76 (d, *J* =

8.0 Hz, 3H), 7.67-7.61 (m, 1H), 7.57 (s, 1H), 7.52-7.46 (m, 4H), 4.56 (d, *J* = 6.0 Hz, 2H), 2.51 (s, 3H). ESI-MS (m/z): 427.0 $[M+H]^+$.

**6.1.13.3. *N*-(4-(2-methylpyridin-4-yl)benzyl)-9-oxo-9*H*-fluorene-2-carboxamide (21).** Light yellow solid (yield: 44%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.31 (t, *J* = 5.6 Hz, 1H), 8.47 (d, *J* = 5.2 Hz, 1H), 8.20-8.14 (m, 2H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.70-7.64 (m, 2H), 7.57 (s, 1H), 7.50-7.42 (m, 4H), 4.55 (d, *J* = 5.7 Hz, 2H), 2.52 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 192.5, 164.9, 158.5, 149.5, 147.0, 146.4, 143.1, 140.6, 135.9, 135.6, 135.0, 134.7, 133.9, 133.4, 130.2, 128.1, 126.8, 124.2, 122.4, 121.9, 121.2, 120.3, 118.3, 42.5, 24.2. HRMS (ESI): calcd for $C_{27}H_{21}N_2O_2$ $[M+H]^+$ 405.1598, found 405.1597.

**6.1.13.4. *N*-(4-(2-Methylpyridin-4-yl)benzyl)-9*H*-carbazole-2-carboxamide (22).** White solid (yield: 62%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.49 (s, 1H), 9.22-9.15 (m, 1H), 8.48 (d, *J* = 5.6 Hz, 1H), 8.22-8.15 (m, 2H), 8.05 (s, 1H), 7.79-7.72 (m, 3H), 7.57 (s, 1H), 7.55-7.42 (m, 5H), 7.22-7.16 (m, 1H), 4.58 (d, *J* = 6.0 Hz, 2H), 2.52 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 166.9, 158.5, 149.5, 147.1, 141.1, 140.8, 139.2, 135.8, 131.4, 128.0, 126.7, 126.5, 124.7, 121.8, 120.8, 120.3, 119.8, 118.9, 118.3, 117.6, 111.2, 110.4, 42.5, 24.2. HRMS (ESI): calcd for $C_{26}H_{22}N_3O$ $[M+H]^+$ 392.1757, found 392.1756.

**6.1.13.5. 9-Methyl-*N*-(4-(2-methylpyridin-4-yl)benzyl)-9*H*-carbazole-2-carboxamide (23).** White solid (yield: 62%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.20 (t, *J* = 6.0 Hz, 1H), 8.48 (d, *J* = 5.2 Hz, 1H), 8.27-8.16 (m, 3H), 7.81-7.75 (m, 3H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.58-7.48 (m, 5H), 7.27-7.22 (m, 1H), 4.61 (d, *J* = 6.0 Hz, 2H), 3.94 (s, 3H), 2.52 (s, 3H). ESI-MS (m/z): 406.2 $[M+H]^+$.

**6.1.13.6. *N*-(4-(2-Methylpyridin-4-yl)benzyl)dibenzo[b,d]furan-3-carboxamide (24).** Light yellow solid (yield: 22%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.30 (t, *J* = 6.0 Hz, 1H), 8.48 (d, *J* = 5.2 Hz, 1H), 8.30-8.19 (m, 3H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 3H), 7.63-7.56 (m, 2H), 7.52-7.42 (m, 4H), 4.59 (d, *J* = 6.0 Hz, 2H), 2.52 (s, 3H). ESI-MS (m/z): 393.0 $[M+H]^+$.

**6.1.14. *N*-(4-Bromobenzyl)-9*H*-carbazole-2-carboxamide (26)**

To a solution of 9*H*-carbazole-2-carboxylic acid (211 mg, 1.0 mmol) and (4-bromophenyl)methanamine (186 mg, 1.0 mmol) in DMF (2 mL) were added HATU (380 mg, 1.0 mmol) and DIPEA (645 mg, 5.0 mmol). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography (dichloromethane/methanol = 50/1) to give the desired product (285 mg, 75%) as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.47 (s, 1H), 9.14 (t, *J* = 6.0 Hz, 1H), 8.21-8.15 (m, 2H), 8.03 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.56-7.49 (m, 3H), 7.47-7.41 (m, 1H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.22-7.16 (m, 1H), 4.49 (d, *J* = 5.6 Hz, 2H).

**6.1.15. General procedure for the synthesis of compounds 27** and **28**

To a suspension of **26** (1 eq.), corresponding aryl boronate (1.2 eq.), $Pd(dppf)Cl_2$ (0.05 eq.) and dppf (0.05 eq.) in 1,4-dioxane (2 mL) was added the solution of $K_3PO_4$ (2 eq.) in water (0.5 mL). The mixture was stirred at 100°C under $N_2$ for 12 h. After cooling to room temperature, the mixture was concentrated under reduced pressure and purified by silica gel column chromatography

(dichloromethane/methanol) to give the desired products **27** and **28**.

**6.1.15.1. *N*-(4-(Pyridin-4-yl)benzyl)-9*H*-carbazole-2-carboxamide (27).** Off white solid (yield: 46%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.18 (d, *J* = 6.0 Hz, 1H), 8.67 (s, 1H), 8.23-8.13 (m, 2H), 8.05 (s, 1H), 7.84-7.68 (m, 5H), 7.58-7.38 (m, 5H), 7.22-7.14 (m, 1H), 4.58 (s, 2H). ESI-MS (m/z): 378.0 $[M+H]^+$.

**6.1.15.2. *N*-(4-(1-Methyl-1*H*-pyrazol-5-yl)benzyl)-9*H*-carbazole-2-carboxamide (28).** Light yellow solid (yield: 35%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.18 (t, *J* = 6.0 Hz, 1H), 8.22-8.16 (m, 2H), 8.05 (s, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.55-7.41 (m, 7H), 7.22-7.17 (m, 1H), 6.37 (d, *J* = 1.6 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 2H), 3.84 (s, 3H). HRMS (ESI): calcd for $C_{24}H_{21}N_4O$ $[M+H]^+$ 381.1710, found 381.1707.

**6.1.16. General procedure for the synthesis of compounds 30** and **31**

To a suspension of 4-bromobenzonitrile (1 eq.), CuI (0.2 eq.) in DMF (10 mL) were added $Cs_2CO_3$ (2 eq.) and imidazole (1.4 eq.). The mixture was stirred at 130°C under $N_2$ for 24 h. After cooling to room temperature, $H_2O$ (50 mL) was added and the mixture was extracted with ethyl acetate (50 mL x 3). The combined organic layers were washed with brine (50 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography (dichloromethane/methanol) to give the desired products **30** and **31**.

**6.1.16.1. 4-(1*H*-Imidazol-1-yl)benzonitrile (30).** White solid (yield: 80%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.34 (s, 1H), 7.26 (s, 1H).

**6.1.16.2. 4-(4-Methyl-1*H*-imidazol-1-yl)benzonitrile (31).** White solid (yield: 85%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.81-7.73 (m, 2H), 7.53-7.44 (m, 2H), 7.05 (s, 1H), 2.30 (s, 3H).

**6.1.17. General procedure for the synthesis of compounds 32** and **33**

To a solution of lithium aluminum hydride (4 eq.) in tetrahydrofuran (5 mL) was added a solution of **30** or **31** (1 eq.) in tetrahydrofuran (2 mL). The reaction was refluxed at 80°C for 2 h under $N_2$. After cooling to room temperature, excess $Na_2SO_4 \cdot 10H_2O$ was added. The mixture was filtered and the filtrate was concentrated to give the desired products **32** and **33**, which was used directly in the next step without further purification.

**6.1.18. General procedure for the synthesis of compounds 34** and **35**

To a solution of **25** (1 eq.) and corresponding amine (1 eq.) in DMF (1.5 mL) were added HATU (1 eq.) and DIPEA (5 eq.). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography to give the desired products **34** and **35**.

**6.1.18.1. *N*-(4-(1*H*-Imidazol-1-yl)benzyl)-9*H*-carbazole-2-carboxamide (34).** White solid (yield: 85%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.18 (t, *J* = 5.6 Hz, 1H), 8.33 (s, 1H), 8.22-8.16 (m, 2H), 8.04 (s, 1H), 7.78-7.69 (m, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.55-7.48 (m, 3H), 7.47-7.41 (m,

1H), 7.22-7.17 (m, 1H), 7.15 (s, 1H), 4.56 (d, *J* = 5.6 Hz, 2H). ESI-MS (m/z): 367.0 $[M+H]^+$.

**6.1.18.2. *N*-(4-(4-Methyl-1*H*-imidazol-1-yl)benzyl)-9*H*-carbazole-2-carboxamide (35).** White solid (yield: 86%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.17 (t, *J* = 5.6 Hz, 1H), 8.28 (s, 1H), 8.21-8.14 (m, 2H), 8.03 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.60-7.40 (m, 7H), 7.23-7.16 (m, 1H), 4.55 (d, *J* = 6.0 Hz, 2H), 2.17 (s, 3H). ESI-MS (m/z): 381.0 $[M+H]^+$.

**6.1.19. (2'-Methyl-[2,4'-bipyridin]-5-yl)methanamine (37)**

To a suspension of (6-chloropyridin-3-yl)methanamine (500 mg, 3.50 mmol), **17** (1.68 g, 50%, 3.85 mmol) and $Pd(PPh_3)_4$ (202 mg, 0.175 mmol) in dioxane/$H_2O$ (4 mL/1 mL) was added $K_2CO_3$ (1.93 g, 14.0 mmol). The mixture was stirred at 100°C under $N_2$ overnight. After cooling to room temperature, $H_2O$ (30 mL) was added and the mixture was extracted with DCM (50 mL x 6). The combined organic layers were dried and concentrated to give the desired product **37** (620 mg, 89%) as a brown solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.60 (d, *J* = 5.2 Hz, 1H), 7.84-7.75 (m, 3H), 7.66 (d, *J* = 4.8 Hz, 1H), 3.98 (s, 2H), 2.65 (s, 3H).

**6.1.20. *tert*-Butyl ((5-bromopyridin-2-yl)methyl)carbamate (39)**

To a solution of 5-bromopicolinaldehyde (1.00 g, 5.38 mmol) and *tert*-butyl carbamate (1.27 g, 10.8 mmol) in $CH_3CN$ (50 mL) were added TFA (1.84 g, 16.1 mmol) and $Et_3SiH$ (6.24 g, 53.8 mmol). After stirring at room temperature for 24 h, the mixture was quenched with saturated aqueous $Na_2CO_3$ (30 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic layers were washed with brine (80 mL x 3), and dried over $Na_2SO_4$. After concentration and purification by silica gel column chromatography (petroleum ether/ethyl acetate = 5/1), **39** was obtained as a colorless oil (1.4 g, 90%).

**6.1.21. (5-bromopyridin-2-yl)methanamine (40)**

To a solution of **39** (1.40 g, 5.88 mmol) in DCM (10 mL) was added TFA (10 mL). After stirring at room temperature for 3 h, excess saturated aqueous $Na_2CO_3$ was added slowly. The mixture was extracted with DCM (100 mL x 3). The combined organic layers were dried over $Na_2SO_4$ and concentrated to give the desired product **40** (880 mg, 80%) as a yellow oil, which was used directly in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 3.94 (s, 2H).

**6.1.22. (2'-Methyl-3,4'-bipyridin-6-yl)methanamine (41)**

To a suspension of **40** (800 mg, 4.28 mmol), **17** (2.04 g, 50%, 4.71 mmol) and $Pd(PPh_3)_4$ (247 mg, 0.21 mmol) in dioxane/$H_2O$ (20 mL/4 mL) was added $K_2CO_3$ (1.77 g, 12.8 mmol). The mixture was stirred at 100°C under $N_2$ overnight. After cooling to room temperature, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (dichloromethane/methanol = 50/1-20/1), **41** was obtained as a brown solid (300 mg, 35%).

**6.1.23. *tert*-Butyl (6-chloro-5-methylpyridin-3-yl)methylcarbamate (43)**

To a solution of 6-chloro-5-methylnicotinaldehyde (1.00 g, 6.41 mmol) and *tert*-butyl carbamate (1.50 g, 12.8 mmol) in $CH_3CN$ (50 mL) were added TFA (2.20 g, 19.3 mmol) and $Et_3SiH$ (7.40 g, 63.8

mmol). After stirring at room temperature for 24 h, the mixture was quenched with saturated aqueous $Na_2CO_3$ (30 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic layers were washed with brine (80 mL x 3), and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 5/1), **43** was obtained as a colorless oil (1.6 g, 97%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.51 (s, 1H), 4.96 (br s, 1H), 4.28 (d, *J* = 4.8 Hz, 1H), 2.38 (s, 3H).

**6.1.24. (6-chloro-5-methylpyridin-3-yl)methanamine (44)**

To a solution of **43** (1.60 g, 6.23 mmol) in DCM (10 mL) was added TFA (10 mL). After stirring at room temperature for 3 h, excess saturated aqueous $Na_2CO_3$ was added slowly. The mixture was extracted with DCM (30 mL x 6). The combined organic layers were dried over $Na_2SO_4$ and concentrated to give the desired product **44** (810 mg, 83%) as a yellow oil, which was used directly in the next step without further purification.

**6.1.25. (2',3-dimethyl-2,4'-bipyridin-5-yl)methanamine (45)**

To a suspension of **44** (780 mg, 4.97 mmol), **17** (2.37 g, 50%, 5.46 mmol) and $Pd(PPh_3)_4$ (287 mg, 0.25 mmol) in dioxane/$H_2O$ (20 mL/4 mL) was added $K_2CO_3$ (2.74 g, 19.8 mmol). The mixture was stirred at 100°C under $N_2$ overnight. After cooling to room temperature, $H_2O$ (30 mL) was added and the mixture was extracted with DCM (50 mL x 6). The combined organic layers were dried and concentrated. After purification by column chromatography (dichloromethane/methanol = 50/1-20/1), **45** was obtained as a brown oil (794 mg, 75%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 5.2 Hz, 1H), 8.48 (s, 1H), 7.61 (s, 1H), 7.32 (s, 1H), 7.23 (d, *J* = 4.8 Hz, 1H), 3.94 (s, 2H), 2.62 (s, 3H), 2.37 (s, 3H).

**6.1.26. 5-(Bromomethyl)-2-chloro-3-fluoropyridine (47)**

To a solution of 2-chloro-3-fluoro-5-methylpyridine (5 g, 34.3 mmol) and NBS (18.3 g, 103 mmol) in $CH_3CN$ (100 mL) was added BPO (829 mg, 3.43 mmol). The mixture was refluxed overnight. After cooling to room temperature, $H_2O$ (200 mL) was added and the mixture was extracted with ethyl acetate (200 mL x 3). The combined organic layers were washed with brine (100 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 100/1), **47** was obtained as a yellow oil (7.45 g, 97%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 1.2 Hz, 1H), 7.56-7.53 (m, 1H), 4.45 (s, 2H).

**6.1.27. (6-Chloro-5-fluoropyridin-3-yl)methanamine (48)**

To a solution of **47** (7.45 g, 33.1 mmol) in DMF (50 mL) was added $NaN_3$ (8.61 g, 132 mmol) at 0°C. The mixture was stirred at 50°C overnight. After cooling to room temperature, $H_2O$ (100 mL) was added and the mixture was extracted with ethyl acetate (100 mL x 3). The combined organic layers were washed with brine (100 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 50/1), a colorless oil (5.6 g, 90%) was obtained. To a solution of the above oil in THF (50 mL) was added $PPh_3$. The mixture was refluxed and $H_2O$ (10 mL) was added slowly. The mixture was refluxed for another 8 h. After cooling to room temperature, the mixture was diluted with ethyl acetate (100 mL) and extracted with 0.2 N HCl (100 mL x 2). The combined aqueous layers were washed with ethyl acetate (100 mL). The pH was adjusted to 9 with 2 N NaOH, and then the mixture was extracted with ethyl acetate (200 mL x 3). The combined organic layers were dried over $Na_2SO_4$ and concentrated. The residue was purified by silica

gel column chromatography (dichloromethane/methanol = 10/1) to give the desired product (2.57 g, 53%) as a brown oil. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.23 (s, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 3.75 (s, 2H).

**6.1.28. (3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methanamine (49)**

To a suspension of **48** (2.36 g, 14.6 mmol), **17** (7.0 g, 50%, 16.1 mmol) and $Pd(PPh_3)_4$ (847 mg, 0.73 mmol) in dioxane/$H_2O$ (40 mL/10 mL) was added $K_2CO_3$ (8.09 g, 58.6 mmol). The mixture was stirred at 100°C under $N_2$ overnight. After cooling to room temperature, $H_2O$ (30 mL) was added and the mixture was extracted with DCM (100 mL x 6). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (dichloromethane/methanol/ ammonium hydroxide = 50/1/0.01-20/1/0.01), **49** was obtained as a brown solid (2.5 g, 79%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 4.8 Hz, 1H), 8.50 (s, 1H), 7.77 (s, 1H), 7.69 (d, *J* = 4.0 Hz, 1H), 7.58 (d, *J* = 12.0 Hz, 1H), 4.01 (s, 2H), 2.65 (s, 3H).

**6.1.29. General procedure for the synthesis of compounds 50-53**

To a solution of **25** (1 eq.) and corresponding amine (1 eq.) in DMF (1.5 mL) were added HATU (1 eq.) and DIPEA (5 eq.). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by column chromatography to give the desired products **50-53**.

**6.1.29.1. *N*-((2'-Methyl-[2,4'-bipyridin]-5-yl)methyl)-9*H*-carbazole-2-carboxamide (50).** Light yellow solid (yield: 69%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.23 (t, *J* = 5.2 Hz, 1H), 8.72 (s, 1H), 8.53 (d, *J* = 4.8 Hz, 1H), 8.22-8.14 (m, 2H), 8.08-8.01 (m, 2H), 7.91 (s, 2H), 7.82 (d, *J* = 4.4 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.48-7.40 (m, 1H), 7.22-7.16 (m, 1H), 4.60 (d, *J* = 5.2 Hz, 2H), 2.54 (s, 3H). ESI-MS (m/z): 393.0 $[M+H]^+$.

**6.1.29.2. *N*-((2'-Methyl-[3,4'-bipyridin]-6-yl)methyl)-9*H*-carbazole-2-carboxamide (51).** White solid (yield: 65%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.50 (s, 1H), 9.25 (t, *J* = 5.6 Hz, 1H), 8.96 (s, 1H), 8.53 (d, *J* = 5.2 Hz, 1H), 8.25-8.15 (m, 3H), 8.08 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.67 (s, 1H), 7.59-7.41 (m, 4H), 7.23-7.17 (m, 1H), 4.68 (d, *J* = 6.0 Hz, 2H), 2.54 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 167.1, 159.9, 158.7, 149.6, 147.1, 144.5, 140.8, 139.2, 135.0, 131.3, 126.5, 126.1, 124.8, 121.8, 121.2, 120.9, 120.5, 119.9, 118.9, 118.5, 117.7, 111.3, 110.5, 44.8, 24.2. HRMS (ESI): calcd for $C_{25}H_{21}N_4O$ $[M+H]^+$ 393.1710, found 393.1705.

**6.1.29.3. *N*-((2',3-Dimethyl-[2,4'-bipyridin]-5-yl)methyl)-9*H*-carbazole-2-carboxamide (52).** White solid (yield: 44%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.48 (s, 1H), 9.19 (t, *J* = 6.0 Hz, 1H), 8.52 (d, *J* = 5.2 Hz, 2H), 8.21-8.15 (m, 2H), 8.04 (s, 1H), 7.72 (d, *J* = 4.8 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.41 (s, 1H), 7.35 (d, *J* = 4.0 Hz, 1H), 7.22-7.16 (m, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 2.53 (s, 3H), 2.34 (s, 3H). ESI-MS (m/z): 407.0 $[M+H]^+$.

**6.1.29.4. *N*-((3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)-9*H*-carbazole-2-carboxamide (53).** White solid (yield: 84%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.50 (s, 1H), 9.25 (t, *J* = 5.2 Hz, 1H), 8.63 (s, 1H), 8.58 (d, *J* = 5.2 Hz, 1H), 8.19 (dd, *J* = 10.8, 8.8 Hz, 2H), 8.04 (s, 1H), 7.84 (d, *J* = 12.0 Hz,

1H), 7.77-7.70 (m, 2H), 7.68 (d, *J* = 5.2 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.47-7.41(m, 1H), 7.21-7.17 (m, 1H), 4.64 (d, *J* = 5.6 Hz, 2H), 2.55 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 167.3, 158.5, 157.6 (d, *J* = 260.2 Hz), 149.5, 145.3 (d, *J* = 4.2 Hz), 142.2 (d, *J* = 5.6 Hz), 140.9, 140.8 (d, *J* = 10.2 Hz), 139.2, 138.9 (d, *J* = 3.7 Hz), 131.1, 126.6, 124.9, 123.9 (d, *J* = 20.7 Hz), 121.8, 121.7 (d, *J* = 7.1 Hz), 120.9, 119.9, 119.8 (d, *J* = 6.4 Hz), 119.0, 117.7, 111.3, 110.5, 40.3, 24.3. HRMS (ESI): calcd for $C_{25}H_{20}FN_4O$ $[M+H]^+$ 411.1616, found 411.1614.

**6.1.30. Isoquinoline-6-carbonitrile (55)**

To a suspension of 6-bromoisoquinoline (624 mg, 3.0 mmol) and $Pd(PPh_3)_4$ (173 mg, 0.15 mmol) in DMF (6 mL) was added $Zn(CN)_2$ (210 mg, 1.8 mmol), and the reaction mixture was stirred at 90°C under $N_2$ for 24 h. After cooling to room temperature, $H_2O$ (40 mL) was added and the mixture was extracted with ethyl acetate (40 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 3/1), **55** was obtained as a white solid (360 mg, 78%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 8.70 (d, *J* = 5.6 Hz, 1H), 8.25 (s, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 5.6 Hz, 1H).

**6.1.31. Isoquinolin-6-ylmethanamine (56)**

To a solution of **55** (20 mg, 0.13 mmol) in ethanol (2 mL) were added Pd/C (4 mg) and 37%HCl (0.05 mL), and the reaction mixture was stirred at room temperature under $H_2$ for 24 h. Saturated aqueous $Na_2CO_3$ (10 mL) was added. The mixture was filtered, and the filtrate was extracted with DCM (30 mL x 3). The combined organic layers were dried over $Na_2SO_4$ and concentrated to give the desired product (20 mg, 97%) as a light yellow solid, which was used directly in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.60 (d, *J* = 5.6 Hz, 1H), 8.27 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 5.6 Hz, 1H), 4.00 (s, 2H).

**6.1.32. *N*-(Isoquinolin-6-ylmethyl)-9*H*-carbazole-2-carboxamide (57)**

To a solution of **25** (26 mg, 0.13 mmol) and **56** (20 mg, 0.13 mmol) in DMF (2 mL) were added HATU (48 mg, 0.13 mmol) and DIPEA (84 mg, 0.65 mmol). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (dichloromethane/methanol = 50/1), **57** was obtained as a light yellow solid (8 mg, 18%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.49 (s, 1H), 9.28 (s, 2H), 8.47 (d, *J* = 5.6 Hz, 1H), 8.19 (dd, *J* = 10.8, 8.4 Hz, 2H), 8.11 (d, *J* = 8.4 Hz, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 7.82 (d, *J* = 5.6 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.47-7.41 (m, 1H), 7.22-7.16 (m, 1H), 4.74 (d, *J* = 5.6 Hz, 2H). ESI-MS (m/z): 352.0 $[M+H]^+$.

**6.1.33. 6-Cyanonaphthalen-2-yl trifluoromethanesulfonate (59)**

To a solution of 6-hydroxy-2-naphthonitrile (1.0 g, 5.92 mmol) and $Et_3N$ (896 mg, 8.88 mmol) in DCM (1.5 mL) was added $Tf_2O$ (2.5 g, 8.88 mmol). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and

purification by column chromatography (petroleum ether/ethyl acetate = 30/1), **59** was obtained as a white solid (1.65 g, 93%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 1.6 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.52 (dd, *J* = 9.2, 2.4 Hz, 1H).

**6.1.34. 6-(Aminomethyl)-2-naphthonitrile (60)**

To a suspension of **59** (1.35 g, 4.49 mmol), $Pd(dba)_2$ (114 mg, 0.20 mmol) and $K_3PO_4$ (1.27 g, 5.99 mmol) in dioxane (25 mL) were added XPhos (143 mg, 0.30 mmol) and $MeNO_2$ (4 mL). The mixture was stirred at 80°C under $N_2$ for 18 h. After cooling to room temperature, $CH_3COOH$ (8 mL) and Zn powder (2.93 g, 45 mmol) were added. The mixture was stirred at 35°C for 3 h and filtered to remove residual Zn powder. The filtrate was diluted with water (30 mL) and washed with ethyl acetate (30 mL). The pH was adjusted to 10 with 1 N NaOH, and then the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3), and dried over $Na_2SO_4$. After concentration and purification by column chromatography (petroleum ether/ethyl acetate = 2/1), **60** was obtained as a light yellow solid (280 mg, 34%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.93-7.83 (m, 3H), 7.63-7.55 (m, 2H), 4.10 (s, 2H).

**6.1.35. *N*-((6-Cyanonaphthalen-2-yl)methyl)-9*H*-carbazole-2-carboxamide (61)**

To a solution of **25** (53 mg, 0.25 mmol) and **60** (46 mg, 0.25 mmol) in DMF (2 mL) were added HATU (95 mg, 0.25 mmol) and DIPEA (161 mg, 1.25 mmol). After stirring at room temperature for 12 h, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration and purification by column chromatography (dichloromethane/methanol = 50/1), **61** was obtained as a light yellow solid (12 mg, 13%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.47 (s, 1H), 9.28 (t, *J* = 5.6 Hz, 1H), 8.53 (s, 1H), 8.22-8.14 (m, 2H), 8.10-8.02 (m, 3H), 7.95 (s, 1H), 7.77-7.66 (m, 3H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.45-7.43 (m, 1H), 7.22-7.16 (m, 1H), 4.72 (d, *J* = 5.6 Hz, 2H). ESI-MS (m/z): 376.1 $[M+H]^+$.

**6.1.36. General procedure for the synthesis of compounds 62-66**

To a solution of **49** (1 eq.) and corresponding acid (1 eq.) in DMF (1.5 mL) were added HATU (1 eq.) and DIPEA (5 eq.). After stirring at room temperature overnight, $H_2O$ (30 mL) was added and the mixture was extracted with ethyl acetate (30 mL x 3). The combined organic layers were washed with brine (30 mL x 3) and dried over $Na_2SO_4$. After concentration, the residue was purified by silica gel column chromatography to give the desired products **62-66**.

**6.1.36.1. *N*-((3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)-9*H*-fluorene-2-carboxamide (62).** White solid (yield: 71%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 5.2 Hz, 1H), 8.57 (s, 1H), 8.03 (s, 1H), 7.83 (s, 3H), 7.75 (s, 1H), 7.67 (d, *J* = 4.8 Hz, 1H), 7.64-7.55 (m, 2H), 7.45-7.35 (m, 2H), 6.76 (t, *J* = 6.0 Hz, 1H), 4.77 (d, *J* = 6.0 Hz, 2H), 3.95 (s, 2H), 2.64 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 166.7, 158.4, 157.5 (d, *J* = 259.8 Hz), 149.4, 145.2 (d, *J* = 4.3 Hz), 144.1, 144.0, 143.0, 142.2 (d, *J* = 5.5 Hz), 140.7 (d, *J* = 9.9 Hz), 140.2, 138.7 (d, *J* = 3.6 Hz), 132.2, 127.7, 127.0, 126.4, 125.3, 124.2, 123.9 (d, *J* = 20.7 Hz), 121.7 (d, *J* = 5.4 Hz), 120.8, 119.8, 119.7, 40.3, 36.4, 24.2. HRMS (ESI): calcd for $C_{26}H_{21}FN_3O$ $[M+H]^+$ 410.1663, found 410.1662.

**6.1.36.2.**

**9,9-Difluoro-*N*-((3-fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)-9*H*-fluorene-2-carboxamide (63).** White solid (yield: 70%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 5.6 Hz, 1H), 8.56 (s, 1H), 8.03-7.99 (m, 2H), 7.76 (s, 1H), 7.70-7.58 (m, 5H), 7.55-7.49 (m, 1H), 7.45-7.39 (m, 1H), 6.75 (t, *J* = 5.6 Hz, 1H), 4.77 (d, *J* = 6.0 Hz, 2H), 2.64 (s, 3H). HRMS (ESI): calcd for $C_{26}H_{19}F_3N_3O$ $[M+H]^+$ 446.1475, found 446.1473.

**6.1.36.3. *N*-((3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)-9-oxo-9*H*-fluorene-2-carboxamide (64).** Yellow solid (yield: 50%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 5.2 Hz, 1H), 8.56 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 7.99 (s, 1H), 7.75 (s, 1H), 7.68 (d, *J* = 6.8 Hz, 2H), 7.65-7.51 (m, 4H), 7.40-7.34 (m, 1H), 6.89 (t, *J* = 5.6 Hz, 1H), 4.76 (d, *J* = 5.6 Hz, 2H), 2.64 (s, 3H). HRMS (ESI): calcd for $C_{26}H_{19}FN_3O_2$ $[M+H]^+$ 424.1456, found 424.1453.

**6.1.36.4. *N*-((3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)-9-methyl-9*H*-carbazole-2-carboxamide (65).** White solid (yield: 64%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.27 (t, *J* = 5.6 Hz, 1H), 8.64 (s, 1H), 8.58 (d, *J* = 5.2 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.22 (d, *J* = 7.6 Hz, 1H), 8.17 (s, 1H), 7.86 (d, *J* = 12.0 Hz, 1H), 7.80-7.74 (m, 2H), 7.70-7.62 (m, 2H), 7.56-7.50 (m, 1H), 7.27-7.21 (m, 1H), 4.66 (d, *J* = 5.6 Hz, 2H), 3.94 (s, 3H), 2.55 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$) δ 167.1, 158.5, 157.5 (d, *J* = 259.8 Hz), 149.4, 145.3 (d, *J* = 4.2 Hz), 142.2 (d, *J* = 5.5 Hz), 141.7, 140.8 (d, *J* = 10.3 Hz), 140.1, 138.8 (d, *J* = 3.7 Hz), 131.1, 126.7, 124.4, 123.9 (d, *J* = 20.5 Hz), 121.7 (d, *J* = 5.4 Hz), 121.3, 120.9, 120.0, 119.7 (d, *J* = 6.2 Hz), 119.2, 117.9, 109.5, 108.6, 40.3, 29.1, 24.2. HRMS (ESI): calcd for $C_{26}H_{22}FN_4O$ $[M+H]^+$ 425.1772, found 425.1769.

**6.1.36.5. *N*-((3-Fluoro-2'-methyl-[2,4'-bipyridin]-5-yl)methyl)dibenzo[b,d]furan-3-carboxamide (66).** White solid (yield: 37%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.36 (t, *J* = 5.6 Hz, 1H), 8.63 (s, 1H), 8.58 (d, *J* = 5.6 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.24-8.20 (m, 2H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 12.4 Hz, 1H), 7.80-7.74 (m, 2H), 7.68 (d, *J* = 4.8 Hz, 1H), 7.63-7.57 (m, 1H), 7.49-7.41 (m, 1H), 4.65 (d, *J* = 5.6 Hz, 2H), 2.55 (s, 3H). HRMS (ESI): calcd for $C_{25}H_{19}FN_3O_2$ $[M+H]^+$ 412.1456, found 412.1453.

### 6.2. In vitro biological assays

Super-top flash (STF) reporter gene assay and Wnt secretion assay had been reported in our published papers[14, 18].

### 6.3. Chemical stability, plasma stability and metabolic stability test

The methods of chemical stability, plasma stability and metabolic stability test had been reported in our published papers[14, 18].

### 6.4. CYP inhibition assays

The experimental procedures for the CYP inhibition assays had been reported in our published papers[18].

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**Supplementary data**

Supplementary data related to this article can be found at

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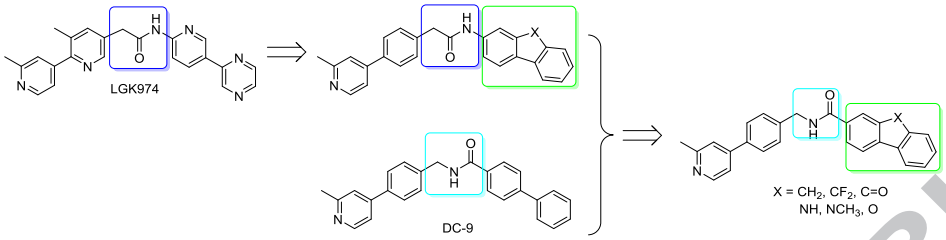


# Design, Synthesis, and Evaluation of Novel Porcupine Inhibitors Featuring a Fused 3-ring System Based on the "Reversed" Amide Scaffold

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