A novel and efficient route for synthesis of Taladegib

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Taladegib (LY-2940680), a small molecule Hedgehog signalling pathway inhibitor, was obtained from *N*-benzyl-4-piperidone *via* Borch reductive amination, acylation with 4-fluoro-2-(trifluoromethyl)benzoyl chloride, debenzylation, substitution with 1,4-dichlorophthalazine and Suzuki cross-coupling reaction with 1-methyl-1*H*-pyrazole-5-boronic acid. The advantages of this synthesis route were the elimination of Boc protection and deprotection and the inexpensive starting materials. Furthermore, the debenzylation reaction was achieved with simplified operational procedure using ammonium formate as hydrogen source that provided high reaction yield. This synthetic procedure was suitable for large-scale production of the compound for biological evaluation and further study.

Keywords: Taladegib (LY-2940680), Hedgehog signalling pathway, inhibitor, synthesis, *N*-benzyl-4-piperidone

The Hedgehog (Hh) signalling pathway, which belongs to one of the five cell signalling transduction pathways, regulates proliferation and differentiation during embryonic development.[1,2] Aberrant activation of the Hh signalling pathway has been associated with tumorigenesis as well as progression of cancer, such as basal cell carcinoma (BCC), medulloblastoma (MB), pancreatic and colon cancer.[3,4] Therefore, the Hh signalling pathway has been identified as a promising target for the discovery of anticancer drugs.

Many classes of Hh signalling pathway inhibitors which target the Smoothened protein (Smo), a key component protein in the Hh signalling pathway,[5] have been developed and some of them have been marketed or are currently in clinical trial including Vismodegib (GDC-0449), Sonidegib (NVP-LDE-225), Taladegib (LY-2940680), BMS-833923 (XL-139), NVP-LEQ506 and PF-04449913 (Fig. 1).[6–13] Vismodegib (GDC-0449) was approved by the FDA in 2012 for treatment of metastatic BCC,[12] and Sonidegib (NVP-LDE-225) was also approved by the FDA for treatment of locally advanced BCC last year.[15,16]

LY-2940680, also known as Taladegib, is a small molecule Hh signalling pathway inhibitor developed by Lilly, which is currently in a phase II clinical trial. It is a promising small molecule inhibitor which could enter phase III clinical trials.

The synthesis of LY-2940680 reported in a patent (WO 2010147917A1)[9] (Scheme 1) consists of a substitution reaction and a Suzuki cross-coupling reaction, Boc deprotection and an acylation reaction. The starting material 4-*N*-Boc-4-*N*-methylaminopiperidine (**1**) is expensive, and the synthesis process also requires a Boc deprotection procedure. In the synthesis of **1** reported in a US patent (US 20120071461A1)[17] (Scheme 2), hydrogen gas was used as hydrogen source. This

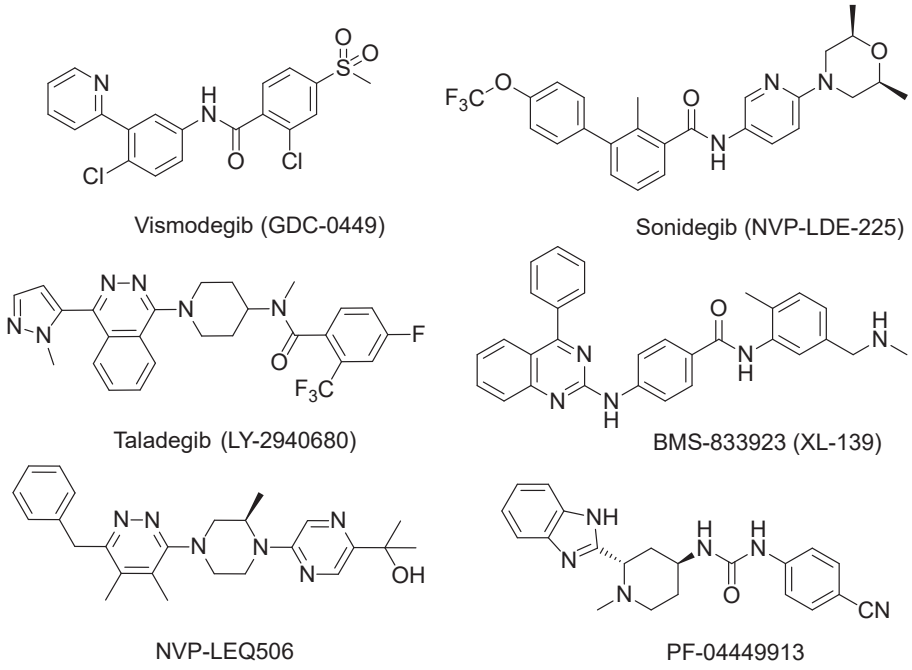

Fig. 1 Chemical structures of Hh signalling pathway inhibitors marketed or currently in clinical trials.

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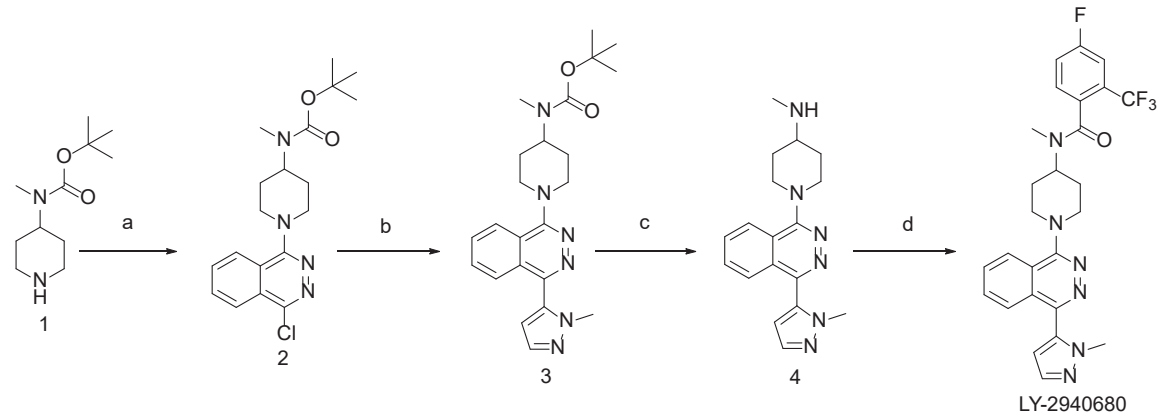
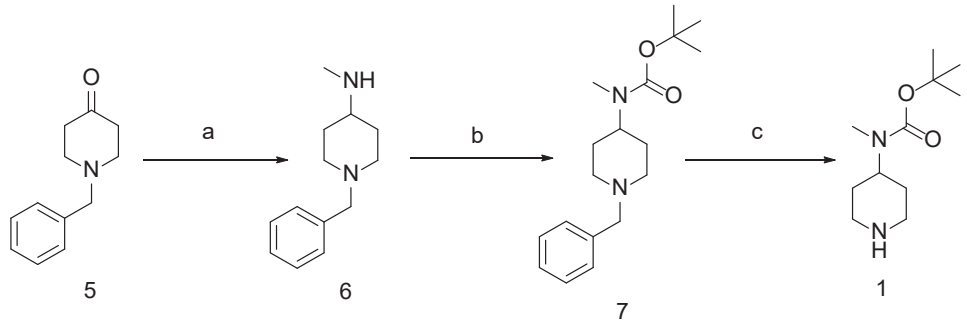
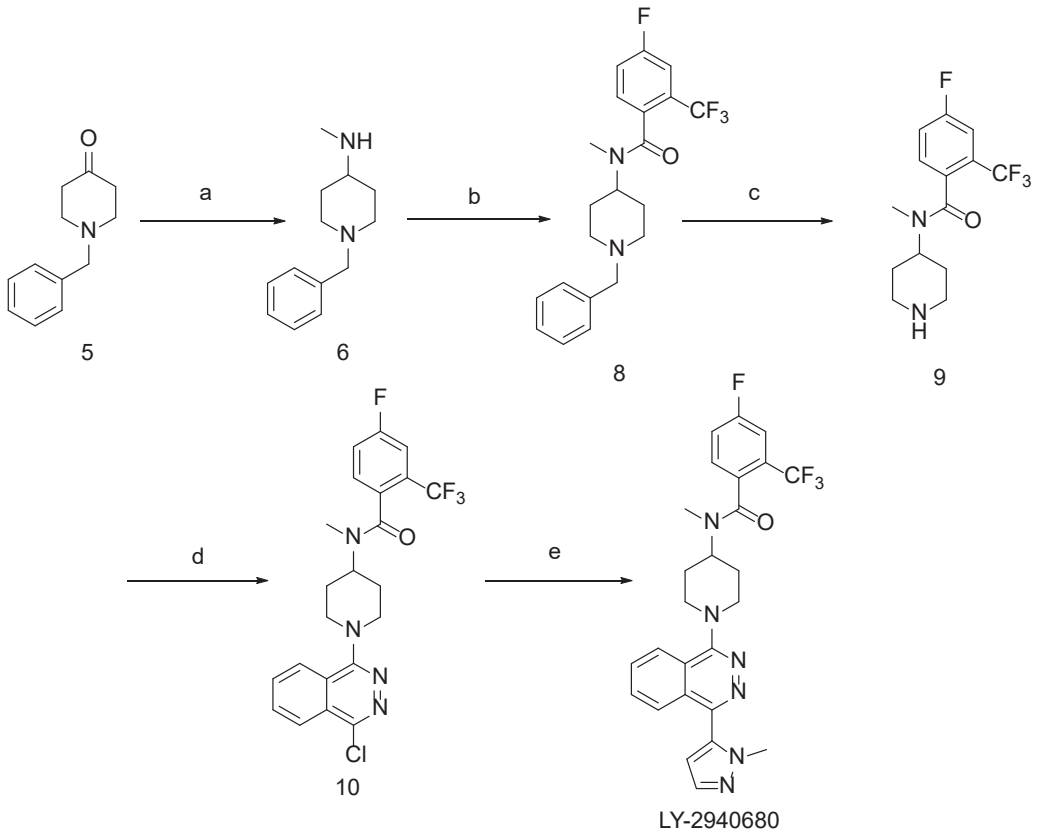


Scheme 1 Reagents and conditions: (a) 1,4-dichlorophthalazine, K_2CO_3, *N*-methylpyrrolidine, 80 °C, overnight, 37%; (b) 1-methyl-1*H*-pyrazole-5-boronic acid pinacol ester, Na_2CO_3, toluene:ethanol:H_2O = 3:1:1, N_2, tetrakis(triphenylphosphine)palladium(0), 74 °C, overnight, 70%; (c) 1 M HCl in diethyl ether, CH_2Cl_2, r.t., 2 h, 98%; (d) 4-fluoro-2-(trifluoromethyl)benzoyl chloride, TEA, CH_2Cl_2, r.t., 3 h, 86%.



Scheme 2 Reagents and conditions: (a) (i) AcOH, CH_3NH_2HCl, MeOH, r.t., 2 h; (ii) $NaCNBH_3$, r.t., 16 h, 92%; (b) Boc anhydride, TEA, CH_2Cl_2, r.t., 16 h, 60%; (c) MeOH, Ar, Pd/C, H_2, r.t., 4 h, 85%.



Scheme 3 Reagents and conditions: (a) (i) AcOH, CH_3NH_2HCl, MeOH, r.t., 2 h; (ii) $NaCNBH_3$, r.t., 16 h, 87%; (b) 4-fluoro-2-(trifluoromethyl)benzoyl chloride, TEA, CH_2Cl_2, r.t., 6 h, 94%; (c) MeOH, N_2, Pd/C, $HCOONH_4$, 50 °C, 12 h, 91%; (d) 1,4-dichlorophthalazine, K_2CO_3, *N*-methylpyrrolidine, 80 °C, 12 h, 73%; (e) 1-methyl-1*H*-pyrazole-5-boronic acid pinacol ester, Na_2CO_3, toluene:ethanol:H_2O = 3:1:1, N_2, tetrakis(triphenylphosphine)palladium(0), 74 °C, 12 h, 92%.

process involved harsh conditions and complex operating procedures, and the yield was low. We, therefore, designed a novel route (Scheme 3) for the synthesis of LY-2940680 using an economic starting material *N*-benzyl-4-piperidone and ammonium formate as hydrogen source, and eliminated the Boc protection and deprotection steps. This new synthetic route proceeded with high yields under mild conditions was suitable for large-scale production of the compound.

Results and discussion

As shown in Scheme 3, *N*-benzyl-4-piperidone (**5**) which is commercially available and cheap, was chosen as the starting material instead of 4-*N*-Boc-4-*N*-methylaminopiperidine (**1**) to reduce costs. Compound **6** was obtained by Borch reductive amination using sodium triacetoxyborohydride as a reducing agent. The key step in the synthesis of LY-2940680 involved the direct amination of compound **6** by 4-fluoro-2-(trifluoromethyl) benzoyl chloride instead of Boc protection. The key group was successfully added and played a role in protecting the amino group in this process and the yield was high (94%). Thus, the Boc deprotection step shown in Scheme 1 was eliminated.

At first the debenzylation reaction, hydrogen gas was used as hydrogen source. This process involved a complex operating procedure and gave an unsatisfactory yield (62%). With an increased amount of material, the reaction time increased and the yield decreased (Table 1). This was caused by poor contact between compound **8** and hydrogen. Therefore, ammonium formate was chosen as hydrogen source in our route (Scheme 3). The ammonium formate was soluble in methanol and in full contact with compound **8** resulting in an improvement of the yield of the debenzylation reaction to 91%. This process was suitable for large-scale synthesis. Since 4-*N*-Boc-4-*N*-methyl aminopiperidine (**1**) was not detectable under UV light, it was difficult to detect the unreacted compound **1** during the purification of the product (compound **2**) in this step (**Scheme 2**). In the new route, all the compounds contained conjugated structures and were detected under UV light so that each step of the route was easily monitored, and the reaction products were easily purified.

Compound **10** was obtained by a substitution reaction of 1,4-dichlorophthalazine with compound **9**. The resulting residue after extraction was purified directly by flash silica gel chromatography without diethyl ether extraction as reported in patent and the yield of this step was improved to 73%. Because of electronic effects, the Suzuki cross-coupling reaction in last step proceeded efficiently giving a high yield (92%).

Conclusion

A novel and efficient route for synthesis of Taladegib (LY-2940680) from the starting material *N*-benzyl-4-piperidone *via* Borch reductive amination, acylation reaction, debenzylation, substitution reaction and Suzuki cross-coupling reaction has been developed. The raw materials were easily obtained cheaply commercially, and Boc protection and deprotection were eliminated. The entire process was carried out under mild conditions and simplified operational procedures and with high yields. Since all key reactants contained conjugated structures and were visible under the UV light, each step of the route was easily monitored, and the products were purified from the reaction mixtures. In conclusion, this new synthetic procedure is economic, efficient and suitable for large-scale production of Taladegib.

Table 1 The yields under various conditions using hydrogen gas as hydrogen source

Entry	Material amount	Pd/C/mg	Reaction time/h	Yield/%
1	1.0 g	100	4	57
2	1.0 g	200	4	62
3	1.5 g	200	12	50
4	2.0 g	200	20	41

Experimental

The solvents, reagents and materials were commercially available and used without further purification unless otherwise noted. All reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (GF-254, Qindao Ocean Chemical Company, China). Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qindao Ocean Chemical Company, China). Melting points were determined on a RY-1 hot stage microscope and are uncorrected. NMR spectra were recorded on a Bruker Avance DPX-300 MHz/500 MHz instruments in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference and the chemical shifts (δ) are reported in parts per million (ppm). Mass spectra (MS) were obtained from Agilent 1100 LC/MS Spectrometry Services.

Synthesis of 1-benzyl-4-(methylamino)piperidine (**6**)

A solution of *N*-benzyl-4-piperidone (**5**, 2.00 g, 10.57 mmol, 1.0 equiv.) in MeOH (30 mL), was treated with AcOH (0.1 mL) and CH_3NH_2HCl (0.79 g, 11.63 mmol, 1.1 equiv.) and the mixture was stirred at r.t. for 2 h, $NaCNBH_3$ (1.33 g, 21.14 mmol, 2.0 equiv.) was added in portions with cooling in an ice-water bath, and the reaction stirred for 16 h at r.t. The reaction mixture was poured into sat. aqueous sodium hydrogen carbonate and extract with DCM. The organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by chromatography (DCM/2 M NH_3 in MeOH, 20:1) to give the product as an oil. Yield 1.88 g, 87%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.28 (m, 4H), 7.25–7.21 (m, 1H), 3.49 (s, 2H), 2.83 (d, J = 11.8 Hz, 2H), 2.41 (s, 3H), 2.36–2.31 (m, 1H), 2.02 (t, J = 11.5 Hz, 2H), 1.84 (d, J = 12.4 Hz, 2H), 1.40–1.33 (m, 3H); MS calcd for $C_{13}H_{20}N_2$ $[M + H]^+$: 205.1705; found: 205.1751.

Synthesis of N-(1-benzyl-piperidin-4-yl)-4-fluoro-N-methyl-2-trifluoromethyl-benzamide (**8**)

A solution of **6** (1.50 g, 7.34 mmol, 1.0 equiv.) in DCM (30 mL) TEA (2 mL) and 4-fluoro-2-(trifluoromethyl)benzoyl chloride (1.66 g, 1.0 equiv.) and then the mixture was stirred at r.t. for 6 h. After monitoring by TLC, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by flash silica gel chromatography (DCM/MeOH, 20:1) to provide the product as an oil. Yield 2.72 g, 94%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.38 (m, 1H), 7.32–7.22 (m, 7H), 4.65–4.54 (m, 1H), 3.40 (s, 2H), 3.10–2.83 (m, 2H), 2.65 (s, 3H), 2.16 (s, 2H), 1.93–1.58 (m, 4H); MS calcd for $C_{21}H_{22}F_4N_2O$ $[M + H]^+$: 395.1747; found: 395.1808.

Synthesis of N-methyl-N-piperidin-4-yl-4-fluoro-2-trifluoromethyl-benzamide (**9**)

A solution of **8** (1.50 g, 3.81 mmol, 1.0 equiv.) in MeOH (30 mL) was treated with $HCOONH_4$ (2.40 g, 38.05 mmol, 10.0 equiv.), and the mixture was purged with nitrogen. Pd/C (200 mg) was then added and heat the mixture at 50 °C for 12 h. After monitoring by TLC, the reaction mixture was cooled to room temperature, and Pd/C was filtered off. The filtrate was concentrated under reduced pressure. DCM was added to the resulting residue and then $HCOONH_4$ was filter off. The filtrate was concentrated and the product was purified by flash silica gel chromatography (DCM/2 M NH_3 in MeOH, 20:1) to give a white solid. Yield 1.05 g, 91%; m.p. 113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.39 (m, 1H), 7.32–7.29 (m, 2H), 4.73–4.63 (m, 1H), 3.21–3.05(m, 2H), 2.66 (s, 3H), 2.41–2.27 (m, 2H), 1.79–1.68 (m, 5H); MS calcd for $C_{14}H_{16}F_4N_2O$ $[M + H]^+$: 305.1277; found: 305.1239.

Synthesis of N-[1-(4-chloro-phthalazin-1-yl)-piperidin-4-yl]-4-fluoro-N-methyl- 2-trifluoromethyl-benzamide (**10**)

A solution of **9** (0.50 g, 1.64 mmol, 1.0 equiv.) in 1-methylpyrrolidine (30 mL) was treated with K_2CO_3 (0.45 g, 3.29 mmol, 2.0 equiv.) and

1,4-dichlorophthalazine (0.36 g, 1.81 mmol, 1.1 equiv.) and the mixture was stirred at 80 °C for 12 h. After monitoring by TLC, the reaction mixture was cooled to room temperature, poured into water and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography (petroleum ether/EtOAc, 1:1) to give a white solid. Yield 0.56 g, 73%; m.p. 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22–8.19 (m, 1H), 8.07–7.99 (m, 1H), 7.92–7.89 (m, 2H), 7.46–7.34 (m, 3H), 4.93–4.85 (m, 1H), 4.16–3.89 (m, 2H), 3.50–3.19 (m, 2H), 2.77 (s, 3H), 2.26–2.04 (m, 2H), 1.97–1.72 (m, 2H); MS calcd for $C_{22}H_{19}ClF_4N_4O$ $[M + H]^+$: 467.1262; found: 467.1289.

Synthesis of Taladegib (LY-2940680)

A solution of **10** (0.20 g, 0.43 mmol, 1.0 equiv.) in a mixture of toluene (18 mL), ethanol (6 mL) and H_2O (6 mL) was treated with Na_2CO_3 (0.09 g, 0.86 mmol, 2.0 equiv.) and 1-methyl-1*H*-pyrazole-5-boronic acid pinacol ester (0.10 g, 0.47 mmol, 1.1 equiv.) and the mixture was purged with nitrogen for 20 min. $Pd(PPh_3)_4$ (60 mg) was added and the mixture was stirred at 74 °C for 12 h. After monitoring by TLC, the reaction mixture was cooled to room temperature and diluted with DCM. The organic portion was washed with sat. sodium chloride solution, and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash silica gel chromatography (dichloromethane/MeOH, 30:1) to provide Taladegib as a yellow foam. Yield 0.20 g, 92%; m.p. 95 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (dd, J = 7.6, 7.7 Hz, 2H), 7.90–7.80 (m, 2H), 7.65 (d, J = 1.8 Hz, 1H), 7.47–7.28 (m, 3H), 6.59 (d, J = 1.8 Hz, 1H), 4.97–4.89 (m, 1H), 4.21–4.08 (m, 2H), 4.05 (s, 3H), 3.44–3.35 (m, 2H), 2.76 (s, 3H), 2.35–2.11(m, 2H), 2.04–1.88 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 163.8, 159.9, 147.4, 138.2, 136.7, 132.0, 131.9, 131.5, 129.4, 129.0, 128.0, 126.3, 124.6, 121.4, 119.5, 114.5, 109.1, 56.9, 51.4, 38.3, 31.8, 29.7, 28.4; MS calcd for $C_{26}H_{24}F_4N_6O$ $[M + H]^+$: 513.2026; found: 513.2018.

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