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The Chemical Development of CHIR-258

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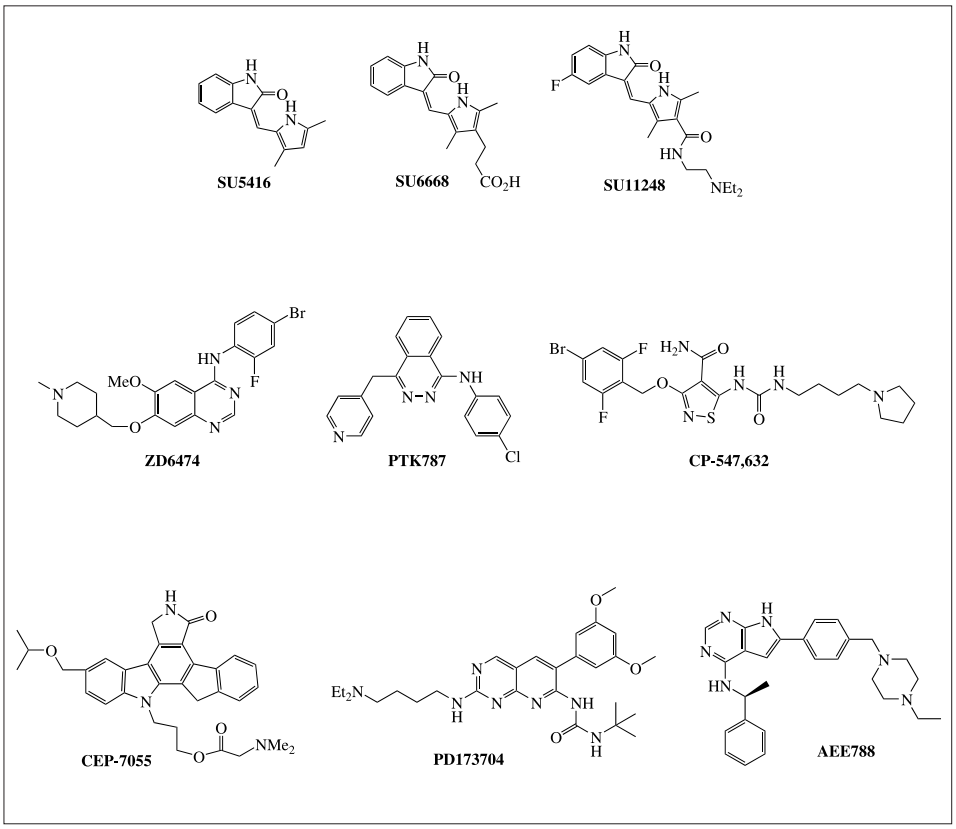
Abstract: This paper is a case history of the early stage chemical development of CHIR-258 (4-amino-5-fluoro-3-[6-(4-methyl-1-piperazinyl)-1H-benzimidazol-2-yl]-2(1H)-quinolinone, DL-lactate salt), a vascular endothelial growth factor (VEGF) kinase inhibitor for treatment of solid and hematologic cancers. The article covers various aspects of work in chemical development. In particular we discuss evaluation of the medicinal chemistry route, scale-up, salt preparation, process impurities, in-process control (IPC) method development, supply route development, technology transfer to contract manufacturing organization (CMO) and pilot plant production. Some interesting points regarding regulatory requirement for chemical development are also discussed.

Keywords: 4-Amino-5-fluoro-3-[6-(4-methyl-1-piperazinyl)-1H-benzimidazol-2-yl]-2(1H)-quinolinone · API · Chemical development · CHIR-258 · Contract manufacturing organization (CMO) · In-process control (IPC) · Kinase inhibitor · Lactate · Technology transfer · Vascular endothelial growth factor (VEGF)

Introduction

Tumor growth needs new vascular supply and therefore angiogenesis is a critical process required in the growth for most solid tumors. Vascular endothelial growth factor (VEGF) and its corresponding receptor tyrosine kinases (RTK) are believed to play specific and critical roles for blood vessel formation [1][2]. The application of VEGF kinase inhibitors is an emerging strategy for anti-angiogenic chemotherapy [3–5]. This has attracted broad interests in investigation of many small molecule VEGF kinase inhibitors [6–19] and has led to the development of several promising and orally bioavailable small molecules [20–31].

Sugen's (later Pharmacia, now Pfizer) indolinone class SU5416 (Phase III), SU6668 (Phase I) and SU11248 (Phase III) are the first few small molecule VEGF inhibitors that have gone into clinical use [20–23]. AstraZeneca's anilinoquinazoline compound ZD6474 (Phase II) is derived from Iressa™ (ZD1839), an early endothelial growth factor receptor (EGFR) tyrosine kinase inhibitor, which is still under clinical evaluation [12][24]. Novartis and Schering's anilinophthalazine compound PTK787/ZK222584 is in Phase II/III stage development and looks very promising [5][25]. Pfizer's isothiazole compound CP-547,632 has gone through Phase I successfully with advanced solid tumor patients without serious safety concerns and is currently in Phase II development [26][27]. Many other orally bioavailable small molecule VEGF kinase inhibitors are still in early stage development. Some of their structures have been published, for example, Cephalon's indolocarbazole compound CEP-7055 (Phase I) [18], Parke-Davis' (later Warner Lambert, now Pfizer)



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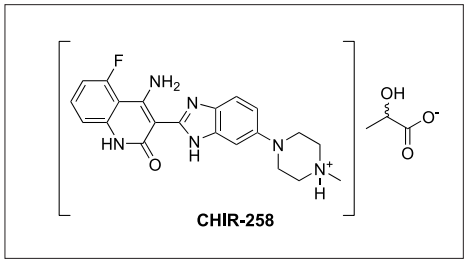
pyridopyrimidine compound PD173704 (Phase I) [28] and Novartis' pyrrolopyrimidine compound AEE788 (Phase I) [29–31]. Structures for others such as Amgen's compound AMG-706 (Phase I) have not been revealed publicly.

CHIR-258 is a novel benzimidazole quinolinone compound that inhibits receptor tyrosine kinases (RTKs) involved in solid and hematologic cancers, as well as tumor angiogenesis. It has high oral bioavailability in mice, rats and monkeys and moderate oral bioavailability in dogs. It is a potent inhibitor for class III/IV/V RTKs and is currently in Phase I clinical trials [32–38]. This article describes our on-going efforts in the chemical development of CHIR-258. It covers various aspects of the work, in particular, evaluation of the medicinal chemistry route, scale-up, salt preparation and characterization, process impurities, in-process control (IPC) method development, supply route [39] development, technology transfer to contract manufacturing organizations (CMO) and pilot plant production. Some interesting points regarding regulatory requirements for chemical development will also be discussed.



CHIR-258

Evaluation of Medicinal Chemistry Route

When a molecule is selected for development, the first stop in its development pathway is in Chemical Development with a requirement of tens to hundreds of grams of the material for scheduled animal studies. At that time, a medicinal chemistry route is provided, which typically has been used to make only a few milligrams to as much as a few grams of the material. The first activity for a process chemist is to evaluate this medicinal chemistry route and to determine its suitability for scale-up. The evaluation must first repeat the original synthesis by following the medicinal chemistry route, to identify any potential scale-up issues, and to make samples of all intermediates and the final compound for use as reference materials. During the evaluation, it is also very common to change the medicinal chemistry route for the benefit of timeline and cost. A convergent or parallel synthesis route is usually created when possible for development [40–41]. In our case, the medicinal chemistry route was a well-designed short synthesis [35] (Scheme 1), thus we first looked to optimize the existing route.

The first step in the original medicinal chemistry route was a very simple SnAr reaction between fluoronitroaniline (reagent **1**) and an amine, N-methylpiperazine. The reaction worked well, but two road blocks to scale-up were identified. The first issue was high concentration (4.5 mol/l) resulting in solidification of the reaction mixture upon addition of water during work up. The second issue was the prohibitively expensive reagent **1** ($6,000/kg). Replacement of this reagent would be required at some point to make the synthesis more economically feasible.

Step two in the original medicinal chemistry route was a one-pot two-step reaction with simple operation and good yields; however, the use of anhydrous ethanol at high catalyst loading (10% load of anhydrous 10% Pd/C), long reaction times (4 days for hydrogenation), and low volume efficiency (150 g/3 l) hindered successful scale-up. These problems were easily resolved and a robust process was developed as described in the scale-up section.

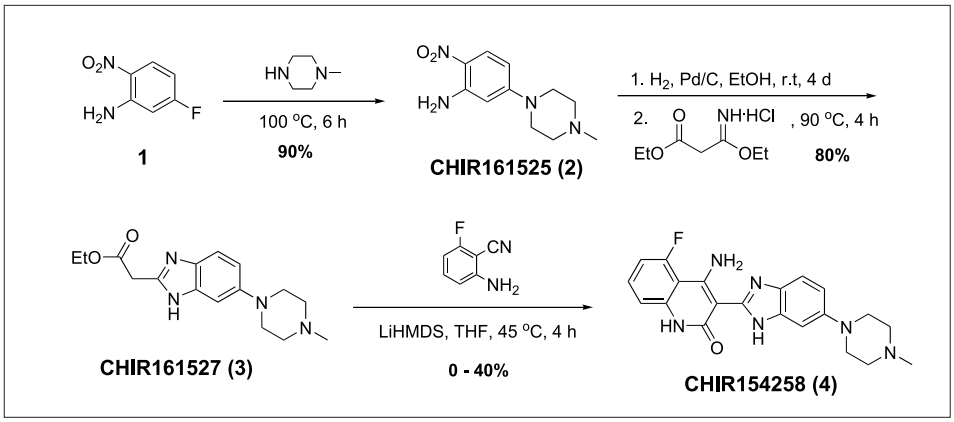
The final critical step in the original medicinal chemistry route proved to be the most difficult for scale-up. The critical issue was that the reaction was not reproducible and yields ranged from 0–40%. The isolated solid (final product) also retained THF, had an inconsistent impurity profile, and required extraction, sonication, and column purification in order to isolate it in a form pure enough for initial studies. The original route also used an excess of an expensive and overly strong base and suffered from poor solvent efficiency (0.14 mol/l). After a careful evaluation of this final step along with the previous two steps, we concluded that an alternative route was not needed for development but modifications and improvement for each step, particularly for the synthesis, isolation, and purification of the final product (CHIR154258, **4**), would be required before scaling up.

Scale-up – Development of An Initial Supply Route

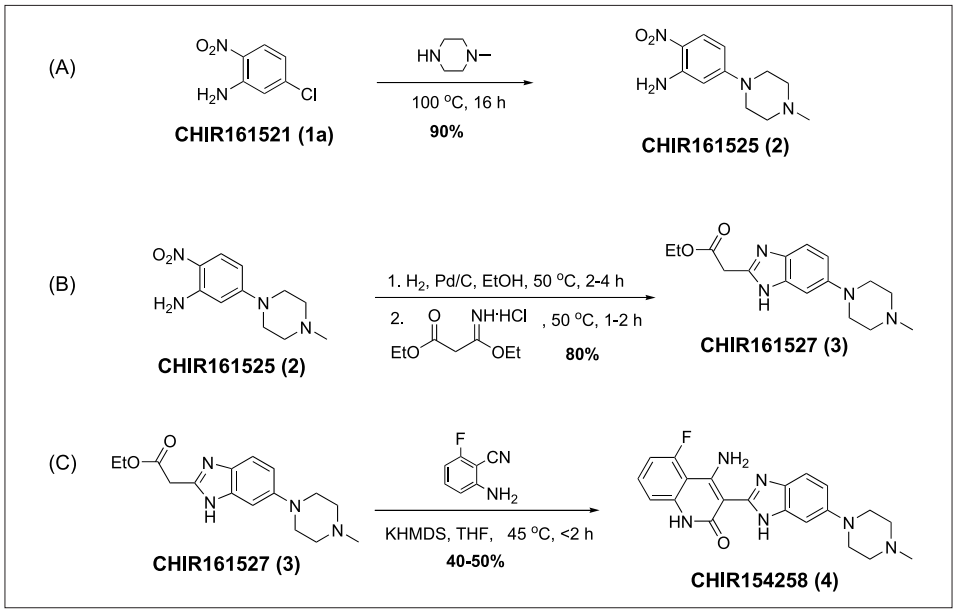
The goal of early stage process development of a new chemical entity (NCE) is to produce certain amount of active pharmaceutical ingredient (API) to supply preclinical and clinical studies. This is the so-called scale-up process that can be divided into two distinct phases. The first phase occurs in the kilo lab with a goal to develop scalable reactions that will produce up to a kilogram of API. The materials produced in the kilo lab are usually used for preclinical studies, pre-formulation development, analytical development and early stability programs. Depending on the nature of the drug development program as well as the conditions under which the API is produced, kilo lab materials can sometimes be used for early clinical studies (Phase I, safety evaluation). For example, for some oncology drug development programs, the need of API for Phase I clinical studies is often less than a kilogram. In this situation, the API could be produced in the kilo lab during early stage development time, provided that current good manufacturing practice (cGMP) is followed. The second phase of scale-up takes place in the pilot plant with a goal to develop a safe, robust and cost-effective manufacturing process that will produce hundreds of kilograms of API under cGMP for clinical studies and formulation development.

In order to supply tens to hundreds of grams of API for preclinical studies, the medicinal chemistry route must be evaluated in the kilo lab and often it is improved initially for reproducibility and scalability. In our case, based on our evaluation of the medicinal chemistry route, the first two reaction steps were quickly modified so that they could be safely implemented in the kilo lab (Scheme 2).

For the first step, a cheaper starting material chloronitroaniline, CHIR161521 (**1a**) ($700/kg), was examined and found to successfully replace reagent **1** ($6,000/kg) with acceptable reaction time (16 h) (Scheme



Scheme 1. The medicinal chemistry route



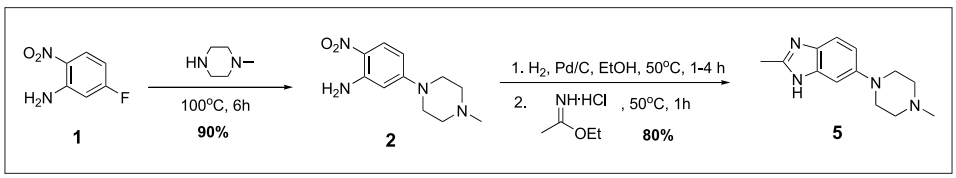
Scheme 2. Improved synthesis of compounds **2**, **3** and **4**

2A). The original reaction concentration (4.5 M) was too high and it presented a safety risk upon scale-up. More dilute concentrations were examined and 3.0 M or even less concentrated conditions were found much safer at the kilo lab scale. Additionally, the original conditions often resulted in solidification and sometimes rock formation upon cooling and addition of water. It was found, however, that when reaction was performed at the diluted concentration, a filterable slurry was obtained instead.

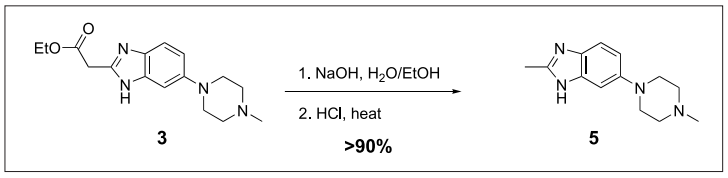
The original medicinal chemistry route for the second step required stirring in anhydrous ethanol (0.2 M) at room temperature under H_2 for four days with a catalyst load of 10 wt% anhydrous Pd/C. Although the reaction worked, some quick improvements were made for it to be amenable at kilo lab scale (Scheme 2B). We first raised the reaction temperature from room temperature to 50 °C thus reducing the reaction time from 4 d to 2–4 h. We also found that there was no significant difference in reaction time or product quality by replacing the original 10 wt% anhydrous catalyst with 7.5% (by weight) of wet catalyst (5% Pd/C, 50% H_2O) and increasing the reaction concentration to 0.43 M. The improved reaction conditions were found very efficient, while the hazardous waste generation and the raw material cost were significantly reduced.

After many failed reactions on the critical third step, our efforts first focused on changing the base. By using potassium hexamethyldisilazide (KHMDS) in place of lithium hexamethyldisilazide (LiHMDS), we found that the product precipitated from the reaction mixture thus simplifying the work up (Scheme 2C). However, it did not resolve the reproducibility issue. To understand why the reaction was not reproducible, we took a sample from a failed reaction and identified that the major product formed from the reaction was compound **5**, the decomposed benzimidazole. The identity of **5** was also confirmed by synthesizing it through the route shown in Scheme 3.

Finally, we started to gain a better understanding of this reaction. We believed that CHIR161527 (**3**) would undergo hydrolysis to form intermediate CHIR582993 (**6**) in the presence of hydroxide (resulting from the presence of residual water) and further decarboxylation upon heating. This mechanistic hunch was also confirmed by



Scheme 3. Synthesis of compound **5**



Scheme 4. Decomposition of compound **3** in the presence of a base and water

treating **3** in a hot basic water system to produce **5** (Scheme 4).

Investigation into the source of the residual water revealed that CHIR161527 (**3**) would crystallize as a monohydrate. However, attempts at isolating CHIR161527 (**3**) in an anhydrous solid form failed since further drying of the monohydrate under vacuum resulted in decomposition to a sticky oil. In order to minimize the hydrolysis/decarboxylation pathway for **3**, we needed to dry the reaction mixture prior to addition of the base. We soon found that azeotropic distillation with either ethanol or THF proved successful at reducing the water content. By minimizing the water content of the reaction mixture to <0.20%, the reaction could be successfully reproduced in 40–50% yields up to 500 g in our kilo lab, and we felt the process was robust enough for scale-up in the pilot plant.

The scale of the first two runs in the pilot plant was not significantly larger (5–10 kg) than that in the kilo lab. However, new challenges were encountered due to the difference of the facility setting between the kilo lab and the pilot plant. A much more thorough safety evaluation for each step was completed and recommended changes had to be considered before the batch record was established. Because the first two steps involved nitroaniline compounds, differential scanning calorimetry (DSC) and reaction calorimetry (RC-1) analyses were performed on the reagents and reaction mixtures. For the first step, the onset temperature T_{onset} of the reaction mixture was found at 139 °C with a thermal potential of 578 J/g. Because the reaction temperature was close to T_{onset} and the thermal potential was high, the reaction mixture was diluted with ethanol and additional N-methylpiperazine as a safety measure. For the second step, further safety evaluation confirmed that the reaction conditions developed in the kilo lab were acceptable for the pilot production.

Other challenges we experienced initially at the pilot plant were the common ones resulting from use of different equipment (such as filtration, washing and drying) than that used in the kilo lab; however, upon resolving these challenges, every pilot plant campaign provided better yield and quality of API than what we had produced in our kilo lab.

Salt Preparation and Characterization

Aggressive timelines are now a common challenge in drug development in pharmaceutical and biotech industries, especially at early stage development. As a result of the tight timeline, the form of the active phar-

maceutical ingredient (API) is usually not well evaluated during the preclinical stage. Often a free base form (many NCEs contain basic organic moieties) is used for *in vitro, in vivo,* PK and tox studies. The free base form of the API is often formulated by dissolving it in acidic water (usually hydrochloric acid) due to its poor solubility in water, and often this form is transferred to the development stage with the program. The original API of CHIR-258 was its free base. When the project started at our Chemical Development department, selecting a salt form of the final API was one of the highest priority tasks, second only to quickly establishing a scalable supply route.

Selecting a biologically and pharmaceutically acceptable salt of an NCE as its API is not a simple task and is generally achieved as a combined effort of chemical development and formulation development. As the process chemists, we first made 17 different salts for first round evaluation by formulation development. Because the goal of this evaluation was to eliminate those salts with poor solubility in water at pH 4–6, the potential candidates were quickly narrowed down to three, the malate salt (46 mg/ml), the mesylate salt (180 mg/ml) and the DL-lactate salt (>180 mg/ml). We then prepared a few lots of each of these three salts under different conditions for second round evaluation. This time the focus was on morphology, hygroscopicity and X-ray powder diffraction pattern. The mesylate salts generated under different conditions had different shapes (plates and needles) as well as different X-ray powder diffraction patterns (C5 and C6). The needle type (C6) tended to pick up significantly more moisture (>5% by weight) than the plate shape (C5). The malate salts seemed to have similar physical shapes (needle shape) but different X-ray powder diffraction patterns (C7 and C8). The malate salts were all consistently non-hygroscopic. These results provided some insights that these two salts exist as different polymorphs. On the other hand, the lactate salts generated under different conditions were consistently plate shaped and had the same X-ray powder diffraction pattern (C1). They were all consistently non-hygroscopic. On the basis of the evaluation, the lactate salt (from DL-lactic acid) was selected as the API.

Once the final API salt form was selected, our next task was to develop a scalable synthetic procedure that could be used to produce high-quality API consistently. To achieve that, we screened solvents and designed experiments to determine the correct solvent and the ratio of lactic acid to free base. Our studies were complicated by the fact that commercial lactic acid exists as a mixture of lactic acid monomers, dimers, higher polymers, and water (Fig. 1). THF, acetone, n-butanol, IPA, ethanol, methanol, and water were screened under many different conditions and combinations including various equivalents of lactic acid monomer. We eventually found out that using water as the solvent and ethanol as the anti-solvent gave the best yield and quality when the ratio of lactic acid monomer to free base was set at ≥1.4. On the basis of these conditions, a procedure [42] was established and optimized during many runs in our kilo lab. The technology was later transferred to pilot plants for API production under cGMP and was proven simple, practical, and reproducible.

A few lots of reference standard of the API were prepared and were fully characterized by UV, FT-IR, NMR, LC-MS, and elemental analysis. The reference standards were also qualified for future analytical development studies and API campaigns under cGMP by performing other analytical tests such as residual solvents, lost on drying (LOD), heavy metal, residue on ignition (ROI), and residual Pd (IPC) analyses. In preparation for future new drug application (NDA) filing at regulatory agencies (such as FDA in US and MHRA in UK), we prepared single crystals of the API and obtained the crystal structure of the API by X-ray single crystal diffraction (Fig. 2).

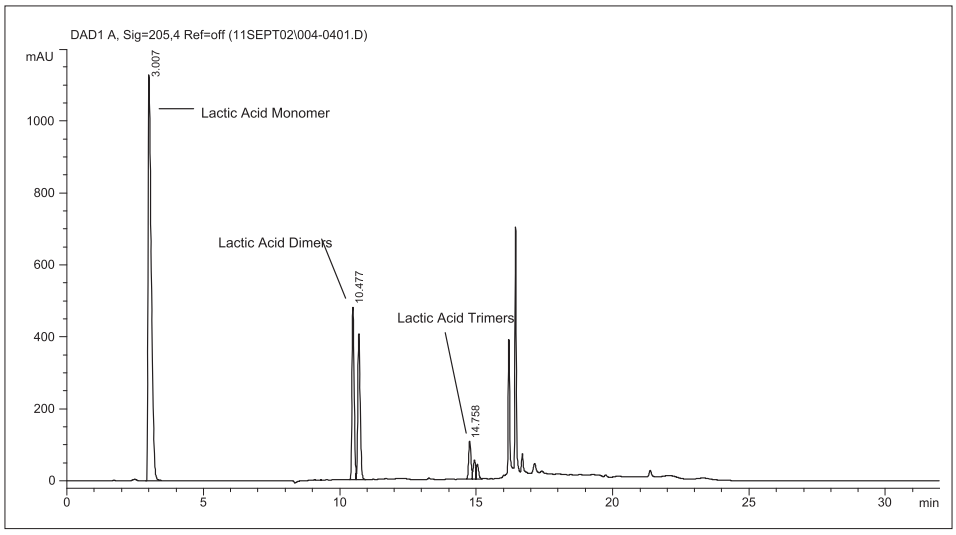


Fig. 1. A typical HPLC chromatogram of commercial DL-Lactic acid

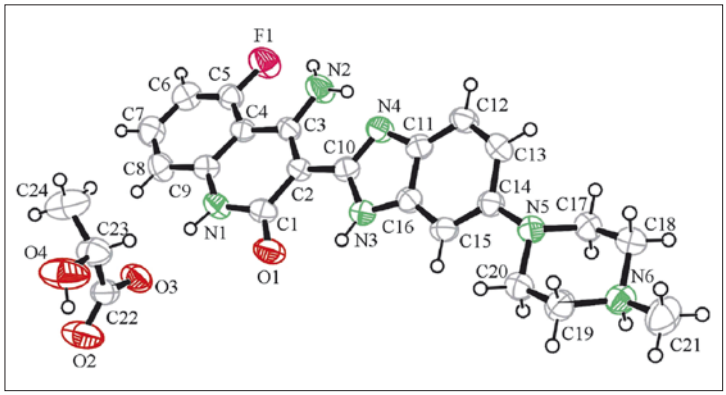


Fig. 2. ORTEP plot of CHIR-258. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

Process Impurities

As part of our development program, we identified, characterized, and independently synthesized all major process impurities. All identified impurities arise from the conversion of CHIR161527 (**3**) to CHIR154258 (**4**) (Fig. 3). Careful control of water content, temperature, and base equivalents is necessary to control the formation of these unwanted side products. Hydrolysis of starting material (CHIR161527, **3**) is the major decomposition pathway. As mentioned above, CHIR161527 (**3**) is isolated as a monohydrate, thus, removal of all waters of hydration is necessary before addition of base. Any residual water present in the basic reaction mixture quickly leads to ester hydrolysis by-product CHIR582993 (**6**). Under the reaction conditions, most of the hydrolyzed by-product decarboxylates resulting in CHIR166208 (**5**) as the major impurity. Another reaction pathway results from the reaction of ethoxide (produced during amide formation) with fluoroanthronitrile. The resulting ethyl ether **10**, can then react with CHIR161527 (**3**) to pro-

duce ethyl ether CHIR166491 (**8**). Under the basic reaction conditions, CHIR166491 (**8**) cyclizes to produce the troublesome CHIR166210 impurity (**9**). CHIR166210 (**9**) is very difficult to remove and its formation must be avoided to obtain in-spec API. By carefully controlling the temperature and equivalency of base addition, the formation of CHIR166491 (**8**) was minimized. Our studies also showed that intermediate CHIR166491 (**8**) cyclized more slowly than the desired intermediate CHIR166209 (**7**). Thus, by controlling the reaction time and temperature after the base addition, we were able to nearly eliminate its formation. A complete understanding of the reaction pathway led us to the identification of a robust process that minimized the formation of these impurities.

IPC Method Development

An in-process control (IPC) HPLC method was initially established for monitoring the completion of the first three reaction steps: formation of CHIR161525 (**2**), CHIR161527 (**3**) and CHIR154258 (**4**). The HPLC method that used a Zorbax Rx C18 column (150 × 4.6 mm, 5 μm) worked well for all three steps with different sample preparations. The method employed 0.1% TFA in water as mobile phase A and 0.1% TFA in acetonitrile as mobile phase B, a gradient elution from 5% up to 81% mobile phase B in 20 min at flow rate of 1.0 ml/min and UV wavelength at 254 nm. A few years into the development of the process, we discovered that the IPC method showed a peak drift problem when used for monitoring the third step, formation of CHIR154258 (**4**). The drift occurred between the impurity CHIR166491 (**8**) and free base CHIR154258 (**4**). The drift issue prevented an accurate calculation of peak area ratio of desired product CHIR154258 (**4**) over the unisolated intermediate CHIR166209 (**7**), the decision criteria of the reaction completion.

The actual cause of the peak drift remains unknown. Upon preliminary examination, however, a more stable bonded column Zebra SB C18 was found better suited for aggressive mobile phases applications, such as strong acidic (pH <2) mobile phase conditions as in our case over the Zebra Rx C18 column. After further development, we found that it would provide more consistent results by using less concentrated (0.05%) modifier TFA. UV wavelength was also changed to 236 nm for higher sensitivity. Gradient elution was preferred over isocratic for ensuring elution of a wider range of impurities in the reaction mixture. The improved IPC HPLC method could be used to monitor all four synthetic step reactions with excellent separation of all reagents, known impurities, intermediates, and the product (Fig. 3).

Supply Route Development

To establish a robust supply route for late-stage production at large scale, the initial supply route needed improvement and optimization. Because the reaction conditions for most steps were quite scalable and the yields were reasonable (>80%), we decided to focus on significantly improving the third step, synthesis of CHIR154258 (**4**), while modifying conditions of the other steps for consistency. Two different goals were set for the improvement of this step. One was to significantly improve the yield (from ~40% to >80%) with the existing reaction conditions and the other was to find a better and cheaper base to replace the expensive KHMDS ($214/l, 20% KHMDS solution in THF).

Upon carefully fine-tuning the existing reaction conditions (*i.e.* controlling the water content of the reaction system, correct concentration, and lower reaction temperature), the yield of this step was eventually improved to 70%–80%. Despite this improvement in yield the cost of production and the level of byproduct CHIR166210 (**9**) remained a major concern. To address it, we screened a number of milder bases. As a result, we found that *t*-BuOK was the ideal base for this reaction. A variety of *t*-BuOK solutions are commercially available. A 20% *t*-BuOK solution in THF was found very user friendly and it was also much cheaper ($24/l). In addition, by using this milder base along with temperature control (0 °C–20 °C), we could achieve higher yields (80–90%) with simpler operation while improving the quality of the product by reducing formation of the major impurity CHIR166210 (**9**) to the detection limit in most cases.

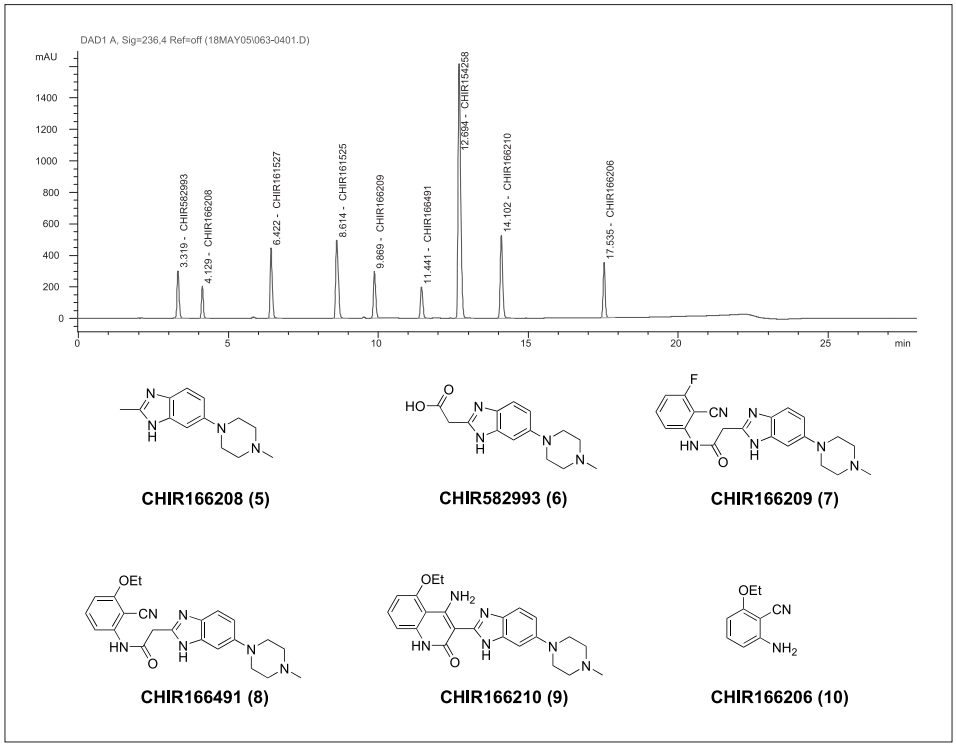


Fig. 3. The identified process impurities are well separated from intermediates and the product by an established HPLC method

Technology Transfer to CMO and Pilot Plant Production

At the beginning of the development stage, the free base CHIR154258 (**4**) was the API and our initial technology package was developed specifically for the free base form. The technology was first transferred to a Swiss API vendor to deliver a target of 5 kg of API for IND enabling studies as well as for the initial supply for Phase I studies. During the campaign of the free base at the pilot plant, the lactate salt was selected as the API, the process was quickly developed to convert the free base to lactate salt and the technology was later transferred again to the same vendor for the conversion at the pilot plant. The first batch of 5.1 kg (32% overall yield) of GMP API was made in early 2003 for the Phase I clinical supplies (powder in bottle, PIB formulation). The technology was later transferred to an US API vendor who produced another 5.4 kg (33% overall yield) of GMP API for clinic studies.

The technology was improved during 2004 and the improved technology includ-

ed all fine-tuned modifications for each step and implemented the new base (*t*-BuOK) in the third step. It was transferred to the same US vendor for the first-time delivery of 6.7 kg (54% overall yield) of GMP API. Two subsequent GMP API campaigns at 20 kg scales were also carried out at this pilot plant and 28 kg (61% overall yield) and 26 kg (59% overall yield) of API were successfully delivered for Phase II clinical capsule supplies in 2005. Currently this technology is being scaled-up to 50 kg scale at the same pilot plant.

Experimental Section

Preparation of 5-(4-Methyl-piperazin-1-yl)-2-nitroaniline (CHIR161525, 2)

5-Chloro-2-nitroaniline (**1a**, 401 g, 2.32 mol) was charged to a five-neck 12-l round-bottom flask fitted with an overhead stirrer, condenser, gas inlet, addition funnel, thermometer probe, and heating mantle. The flask was then purged with N_2. 1-Methyl-piperazine (978 g, 1.08 l, 9.76 mol) and 100% ethanol (650 ml) were added to the reaction flask with stirring. The flask was again purged with N_2, and an atmosphere of N_2 was maintained throughout the reaction. The flask was heated to an internal temperature of 97 °C (± 5 °C) and maintained at this temperature until the reaction was complete (typically about 40 h) as determined by HPLC. After the reaction was complete, heating was discontinued and the reaction mixture was cooled with stirring to an internal temperature of 20–25 °C in no less than 5 h. Water (3.15 l) was added to the mixture *via* an additional funnel over a period of 1 h while the internal temperature was maintained at 25–30 °C. The reaction mixture was then stirred for an additional hour at an internal temperature of 20–30 °C. The resulting mixture was filtered, and the flask and filter cake were sequentially washed with water (1 × 1 l), 50% ethanol (1 × 1 l), and 95% ethanol (1 × 1 l) sequentially. The golden yellow solid was transferred to a drying pan and dried in a vacuum oven at 70 °C to a constant weight. 546 g (99%) of CHIR161525 (**2**) was obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.19 (s, 3 H), 2.39 (t, 4 H, *J* = 5.1 Hz), 3.30 (t, 4 H, *J* = 4.9 Hz), 6.21 (d, 1 H, *J* = 2.6 Hz), 6.37 (dd, 1 H, *J* = 9.8 Hz, 2.6 Hz), 7.26 (s, 2 H), 7.80 (d, 1 H, *J* = 9.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.59, 46.09, 54.10, 97.45, 105.36, 122.85, 127.10, 148.28, 154.95.

Preparation of [6-(4-Methyl-piperazin-1-yl)-1H-benzimidazol-2-yl]-acetic Acid Ethyl Ester (CHIR161527, 3)

A 12-l four-neck flask was fitted with a mechanical stirrer, temperature probe, condenser, gas inlet/outlet, and heating mantle. The equipped flask was charged with CHIR161525 (**2**) (1731 g, 7.325 mol, 1.0 equiv.) and 13.8 l of 95% EtOH. The resulting suspension was purged with N_2 for 15 min. 130.8 g of 5% Pd/C (50% H_2O w/w) was subsequently added to the mixture and it was again purged with N_2 for no less than 15 min. The reaction mixture was vigorously stirred at 40–50 °C (internal temperature) while H_2 was bubbled through the mixture. The reaction is exothermic and the internal temperature was controlled by slowing the stirring and/or decreasing the H_2 flow rate. The reaction was monitored hourly for the disappearance of CHIR161525 (**2**) by HPLC. The reaction time was typically less than 6 h. After all CHIR161525 (**2**) had been consumed, the H_2 flow was stopped and the solution was purged with N_2 for 15 min. Ethyl-3-ethoxy-3-iminopropanoate hydrochloride (440.0 g, 2.25 mol) was added to the reaction mixture in one portion under N_2. The reaction was monitored by following the disappearance of the diamino compound by HPLC. The reaction time was typically 1–2 h. After the reaction was complete, the mixture was cooled to room temperature and filtered through a pad of Celite. The Celite filter pad was washed with 95 % EtOH (2 × 1.58 l), and the combined filtrates were concentrated under reduced pressure to yield a thick orange oil. The resulting oil was taken up in 4.76 l of water and cooled to 0 ºC ± 3 ºC. The resulting solution was vigorously stirred, and 20% NaOH was added at such a rate to maintain the internal temperature <5 ºC until pH = 9.0. The resulting suspension was stirred at 0 ºC ± 3 ºC for 30 min, filtered, and the filter cake was washed with H_2O (3 × 2.1 l). The collected solid was dried in a vacuum oven at 35 ºC to a constant weight to provide 1.98 kg (89.4%) of CHIR161527 (**3**) as a light-tan powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (t, 3H, *J* = 7.1 Hz), 2.22 (s, 3 H), 2.49 (m, 4 H), 3.08 (bs, 4 H), 3.89 (s, 2 H), 4.12 (q, 2 H, *J* = 7.1 Hz), 6.87–7.02 (m, 2H), 7.29–7.38 (m, 1H), 12.04 and 12.10 (s, 1H, two tautomeric forms); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.02, 35.08, 45.75, 49.85, 50.28, 55.98, 60.71, 97.41, 104.98, 110.93, 112.80, 114.09, 118.35, 128.70, 135.20, 137.13, 143.90, 146.22, 147.11, 147.46, 147.66, 168.81, 168.88.

Preparation of 4-Amino-5-fluoro-3-[6-(4-methyl-piperazin-1-yl)-1H-benzimidazol-2-yl]-1H-quinolin-2-one (CHIR154258, 4)

A 12-l four-neck jacketed flask was fitted with a mechanical stirrer, temperature probe, distilling apparatus, and gas inlet/outlet. The flask was charged with CHIR161527 (**3**) (608 g, 2.01 mol) and 2-amino-6-fluoro-benzonitrile (274 g, 2.01 mol). The reaction vessel was purged with N_2, then toluene (7.2 l) was added. The reaction mixture was heated to reach an internal temperature of 63 ± 3 ºC. The internal temperature was maintained at 63 ± 3 ºC while approximately 2.6 l of toluene was distilled from the mixture under reduced pressure. Karl Fischer analysis was used to check the water content in the mixture. If the water content was greater than 0.03 %, then another 2.6 l of toluene was added and distillation was repeated. This process was repeated until water content was less than 0.03%. After a water content of less than 0.03% was achieved, heating was discontinued, and the reaction mixture was cooled to an internal temperature of 17–19 ºC. Potassium *t*-butoxide in THF (20 % in THF, 3.39 kg, 6.04 mol) was then added under N_2 at a rate such that the internal temperature of the reaction mixture was kept below 20 ºC. After the addition of potassium *t*-butoxide was complete, the reaction mixture was stirred at an internal temperature of 17–20 ºC for 30 min. The temperature was then raised to 23–25 ºC for 1 h. The temperature was then raised again to 28–30 ºC for at least 30 min. At this point, the reaction was checked by HPLC for the consumption of the starting material (CHIR161527, **3**). If all starting material was not consumed after 2 h, another 0.05 equiv. of potassium *t*-butoxide was added. This process was continued until HPLC analysis showed complete disappearance of starting material. Upon reaction completion, 650 ml of water was added to the stirred mixture. The reaction was then warmed to an internal temperature of 50 ºC and the THF was distilled off (about 3 l by volume) from the reaction mixture under reduced pressure. Additional water (2.4 l) was then added dropwise from an additional funnel to the reaction mixture. The mixture was then cooled to room temperature and stirred for at least 1 h. Product was collected by filtration and the filter cake was washed with water 1.2 l, 70% EtOH 1.2 l, and 95 % EtOH 1.2 l sequentially. The bright-yellow solid was transferred to a drying tray and dried in a vacuum oven at 50 ºC to a constant weight to provide 674 g (85.4 %) of desired CHIR154258 (**4**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.21 (s, 3H), 2.49 (m, 4 H), 3.10 (bs, 4 H), 6.91 and 6.94 (dd, 1 H total, *J* = 8.8 Hz, 2.1 Hz and *J* = 8.8 Hz, 1.9 Hz), 7.03 (dd, 1 H, *J* = 14.0 Hz, 8.1 Hz), 7.09 and 7.22 (s, 1 H), 7.19 (d, 1H, *J* = 8.3 Hz), 7.45 and 7.52-7.56 (d & m, 1H, *J* = 8.8 Hz), 7.52–7.56 (m, 1H); 7.70–7.76 (m, 1H), 11.34 and 11.44 (s, 1H), 12.76 and 12.79 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.74, 49.76, 50.17, 54.80, 54.92, 90.65, 90.95, 98.18, 102.63 (J_{CF} = 9.0 Hz), 103.48, 107.89 (J_{CF} = 23.0 Hz), 111.79, 112.23, 113.00, 114.09, 117.12, 126.15, 131.99, 132.10, 133.65, 135.21, 139.54, 139.60, 141.88, 147.37, 147.63, 150.48, 151.28, 151.35, 151.71, 160.37 (J_{CF} = 248.0 Hz), 161.90.

Optional Purification/Rework of CHIR154258 (4)

A 3-l four-neck flask was fitted with a condenser, temperature probe, gas inlet/outlet, mechanical stirrer, and heating mantle and charged with CHIR154258 (**4**) (101.0 g, 0.26 mol). 95% Ethanol (800 ml) was charged and the mixture was heated to reflux under N_2 with stirring. A gentle reflux was maintained for 45–75 min. then the mixture was cooled to 25–30 ºC over a period of at least 2 h. The mixture was filtered, and the filter cake washed with water (3 × 250 ml). The bright yellow solid was dried *in vacuo* at 50 ºC to a constant weight to obtain 97.2 g (96.2%).

Preparation of Lactic Acid Salt of 4-Amino-5-fluoro-3-[6-(4-methyl-piperazin-1-yl)-1H-benzimidazol-2-yl]-1H-quinolin-2-one (CHIR-258)

A 3-l four-neck jacketed flask was fitted with a condenser, a temperature probe, a N_2 gas inlet, and a mechanical stirrer. CHIR154258 (**4**) (484 g, 1.23 mol) was charged to the flask, and a solution of DL-lactic acid (243.3 g, 1.72 mol of lactic acid monomer), water (339 ml), and 100% ethanol (1.21 l) was prepared and subsequently charged to the reaction flask. The reactor was purged with N_2 for at least 15 min. The reaction mixture was then heated to an internal temperature of 70±2 ºC and stirred for 15–45 min. The resulting mixture was then filtered through a 10–20 micron fritted filter and the filtrate collected in a 12-l flask. Water (48.4 ml) and 100% ethanol (484 ml) were charged to the 3-l flask and stirred to wash. The wash solution was filtered through the 10–20 micron fritted filter and collected in the 12-l flask with the first filtrate. The 12-l reactor was fitted with an internal temperature probe, reflux condenser, an addition funnel, gas inlet and outlet, and an overhead stirrer. The filtrate was stirred at a medium rate and heated to reflux under N_2. 100% ethanol (3.58 l) was added to the flask while maintaining a gentle reflux. After the addition of 100% ethanol, the reaction mixture was cooled to an internal temperature of 67±3 ºC within 30 min and held for a period of 30–60 min. At this point, the reactor was inspected for crystals. If no crystals were present, then seed crystals of CHIR-258 (484 mg) were added to the flask, and stirring continued at this temperature for another 30–60 min before again inspecting for crystals. Once crystals were present, the stirring rate was reduced and the reactor was cooled to 20±5 ºC over 3 h. The product was stirred at 20±5 ºC for up to 16 h (overnight) before filtering the reaction mixture through a 25–50 micron fritted filter. The reactor was washed with 100% EtOH (3 × 500 ml), and the filter cake rinsed with the washes. The yellow solid was transferred into a drying tray and dried in a vacuum oven at 50 °C until a constant weight was obtained. Yield 510.7 g (85.7%) of CHIR-258 as a crystalline yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (d, 3 H, J = 6.9 Hz), 2.29 (s, 3 H), 2.58 (br s, 4 H), 3.14 (br s, 4 H), 4.01 (q, 1 H, J = 6.9 Hz), 6.93 and 6.95 (d & d, 1 H, J = 10.7 Hz and J = 9.1 Hz), 7.03 (dd, 1 H, J = 14.0 Hz, 8.1 Hz), 7.11 and 7.24 (s, 1 H), 7.19 (d, 1 H, J = 8.3 Hz), 7.46 and 7.52–7.58 (d & m, 1 H, J = 8.8 Hz); 7.52–7.58 (m, 1 H), 7.71–7.77 (1 H), 11.34 and 11.43 (s & s, 1 H), 11.62, (s, 1 H), 12.74 and 12.77 (s & s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.55, 45.05, 49.38, 49.66, 54.38, 65.88, 90.98, 98.42, 102.68 (J_{CF} = 10.1 Hz), 103.72, 107.96 (J_{CF} = 23.7 Hz), 111.90, 112.26, 113.15, 114.14, 117.23, 126.40, 132.18, 132.69, 135.40, 139.65, 141.91, 147.09, 147.39, 150.62, 151.39, 151.78, 160.19 (J_{CF} = 248.1 Hz), 161.93, 176.53.

Preparation of 2-Methyl-6-(4-methyl-piperazin-1-yl)-1H-benzo[d]imidazole (CHIR166208, 5)

To a 1-l three-necked round bottom flask, equipped with overhead stirrer, condenser, thermocouple and H_2/N_2 inlet over a heating mantle, was charged CHIR161525 (**2**) (50 g, 211 mmol, 1 equiv.), 5 wt% palladium on carbon (50% water; 3.8 g, 0.9 mmol, 0.004 equiv.), and 95% ethanol (400 ml). The stirring mixture was flushed with N_2 for 5 min. The nitrogen was replaced with a steady flow of H_2 (1 atm) and the mixture was heated to 50–65 ºC until HPLC showed no remaining starting material, 2 h. Hydrogen flow was stopped and the mixture was flushed with N_2 for 10 min and ethyl acetimidate (52.6 g, 426 mmol, 2 equiv.) was charged in one portion at 50 ºC. The mixture was stirred until HPLC showed no remaining starting material, 1 h. The warm reaction mixture was filtered over a bed of Celite 545 in a fritted funnel and the Celite was washed twice with 95% ethanol (2 × 50 ml). The combined filtrate and washes were concentrated *in vacuo* to a viscous oil (45–50 ºC, 27–29 in. Hg). The oil was diluted with water (300 ml) and the pH was adjusted to 9.6 with 50 wt% NaOH, immediately forming a thick slurry. The product slurry was cooled to 0–5 ºC for 20 min, vacuum filtered, then washed twice with water (2 × 50 ml). The solids were dried *in vacuo* for 17 h (50 ºC, 29–30 mm Hg), to provide 46.6 g CHIR166208 (**5**) (95% w/w yield, 99.8 AP purity by HPLC). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3 H), 2.58 (s, 3 H), 2.62 (dd, 4 H, J = 4.8 Hz, 5.0 Hz), 3.19 (dd, 4 H, J = 4.8 Hz, 5.0 Hz), 6.95 (dd, 1 H, J = 8.8 Hz, 2.2 Hz), 7.03 (s, 1 H), 7.43 (s, 1 H), 9.41 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.2, 147.1, 112.7, 54.9, 50.1, 45.7, 14.6.

Preparation of CHIR166209 (7)

CHIR161527 (**3**) (10.0 g, 33.1 mmol), and toluene (100 ml) were charged to a 250 ml round-bottom flask. Water was removed by azeotropic distillation of solvent *in vacuo* to obtain an oil. This process was repeated twice. 2-Amino-6-fluoro-benzonitrile (4.50 g, 33.1 mmol) and toluene (100 ml) were charged to the flask and a final azeotropic distillation was performed. THF (80 ml) was charged to the resulting oil and the contents of the flask were cooled to ~0 ºC in an ice/water bath. Solid sodium *t*-butoxide (6.4 g, 66 mmol) was added in two portions and the mixture stirred for 30 min in the ice/water bath. The reaction was quenched by addition to 2 N HCl (100 ml). This solution was washed with ethyl acetate (100 ml) and the aqueous fraction was basified with saturated sodium carbonate up to pH 10. The resulting suspension was filtered, washed with water (20 ml) and dried *in vacuo* at 40 ºC to obtain 5.71 g (44.0%) of a crude solid. A portion of this crude solid (4.5 g) was further purified by heating in refluxing ethyl acetate, filtering to remove insoluble particulate, and cooling to 0 ºC to obtain a slurry. Filtration of the slurry and washing of the filter cake with ethyl acetate (2 × 20 ml) and water (20 ml) followed by drying *in vacuo* at 40 ºC yielded 2.41 g (53.6%) of purified CHIR166209 (**7**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3 H), 2.48 (t, 4 H, J = 4.4 Hz), 3.08 (t, 4 H, J = 4.2 Hz), 4.08 (s, 2 H), 6.91 (dd, 1 H, J = 8.8 Hz, 2.1 Hz), 6.98 (s, 1 H), 7.27–7.32 (m, 1 H), 7.40 (d, 1 H, J = 8.7 Hz). 7.74–7.77 (m, 1H), 11.77 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.42, 45.73, 49.97, 54.81, 94.59 (J_{CF} = 17.2 Hz), 111.73 (J_{CF} = 19.1 Hz), 111.91, 113.34, 119.49 (J_{CF} = 2.5 Hz), 135.75 (J_{CF} = 10.1 Hz), 141.78 (J_{CF} = 2.0 Hz), 147.51, 162.81 (J_{CF} = 252.6 Hz), 166.99.

Preparation of CHIR166491 (8)

CHIR161527 (**3**) (14.8 g, 49.1 mmol), and toluene (125 ml) were charged to a 250 ml round-bottom flask. Water was removed by azeotropic distillation of solvent *in vacuo* to obtain an oil. This process was repeated twice. 2-Amino-6-ethoxybenzonitrile (CHIR166206, **10**, 2.50 g, 15.4 mmol) and toluene (125 ml) were charged to the flask and a final azeotropic distillation was performed. THF (120 ml) was charged to the resulting oil and the contents of the flask were cooled to ~0 ºC in an ice/water bath. Solid sodium *t*-butoxide (9.90 g, 103 mmol) was added and the mixture stirred for 1 h in the ice/water bath. The reaction was quenched by addition to cold 2 N HCl (100 ml). The acidic solution was washed with ethyl acetate (100 ml) and then basified with saturated potassium carbonate. The precipitate thus produced was filtered and washed with water. The crude solid was

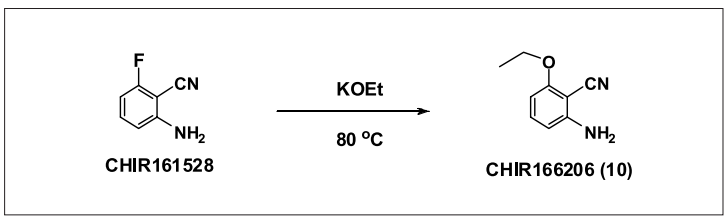
further purified by trituration in refluxing ethanol (100 ml). The purified solid was filtered, the filter cake rinsed with ethanol (25 ml) and dried *in vacuo* at 40 °C to obtain 1.74 g of CHIR166491 (**8**) (26.9% yield, based on CHIR166206). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, 3 H, *J* = 7.0 Hz)), 2.22 (s, 3 H), 2.47 (bs), 3.08 (bs, 4 H), 4.03 (s, 2 H), 4.18 (q, 2 H, *J* = 6.9 Hz), 6.89 (bs, 1 H), 6.91 (bs, 1 H). 6.99 (d, 1H, *J* = 8.5 Hz), 7.39 (bs, 1H), 7.44 (d, 1H, *J* = 8.2 Hz), 7.59 (dd, 1H, *J* = 8.4, 8.4 Hz), 11.02 (bs, 1H), 12.16 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.33, 36.45, 45.72, 49.94, 54.82, 64.66, 94.91, 97.49, 108.56, 112.86, 114.08, 115.49, 118.29, 134.72, 136.83, 141.63, 147.29, 160.78, 166.81.

Preparation of CHIR166210 (9)

To a 2-l, one-neck RB flask was charged CHIR161527 (**3**) (104.35 g, 345 mmol, 1.2 equiv.), CHIR166206 (**10**) (46.6 g, 287 mmol, 1.0 equiv.), and THF (900 ml). The suspension was concentrated *in vacuo* (45 °C, 25 mm Hg) to a final volume of 200 ml. This was repeated twice to give a clear solution. The solution was transferred to a 3-l, three-necked RB flask equipped with an overhead stirrer, condenser, thermocouple, addition funnel and N_2 adapter over a heating mantle, and diluted with THF (400 ml). A solution of 20% potassium *t*-butoxide in THF (497 g, 888 mmol, 3.0 equiv.) was added *via* addition funnel over 15 min at room temperature and the resulting slurry was heated to 69 °C and held for 3 h. The reaction mixture was cooled to 50 °C, water (470 ml) was added and the mixture was concentrated *in vacuo* to remove THF (50 °C, 26 mm Hg). The slurry was transferred to a 3-l, three-necked RB flask equipped with overhead stirrer, condenser, thermocouple, addition funnel and N_2 adapter over a heating mantle. 100% EtOH (200 ml) and water (300 ml) was added and the mixture was heated to 70 °C, held for 20 min, then cooled to 15 °C over 2.5 h. The slurry was vacuum filtered and the solids were washed with 25 % EtOH/water (200 ml). The solids were dried *in vacuo* (55 °C, 29 mm Hg)for 18 h to give 118.3 g of CHIR166210 (**9**) (82.2% wt/wt yield; 94.8 AP HPLC purity). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.50 (t, 3 H, *J* = 6.9 Hz), 2.23 (s, 3 H), 2.50 (m, 4 H), 3.10 (t, 4 H, *J* = 4.5 Hz), 4.27 (td, 2 H, *J* = 9.2, 4.5 Hz), 6.79 (d, 1 H, *J* = 8.1 Hz), 6.94 (m, 2 H), 7.22 (d, 0.6 H, *J* = 2.1 Hz), 7.06 (d, 0.4 H, *J* = 1.9 Hz), 7.41-7.54 (m, 2 H), 8.61 (t, 1 H, *J* = 5.8 Hz), 11.30 (s, 1 H), 11.42 (dd, 1 H, *J* = 4.9 Hz), 12.77 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.85, 161.80, 157.65, 157.63, 154.74, 154.36, 152.06, 151.19, 147.43, 141.97, 139.81, 139.73, 135.28, 132.57, 132.04, 131.93, 126.13, 116.89, 113.77, 112.75, 111.57, 108.65, 104.33, 103.46, 102.91, 98.20, 89.58, 89.50, 65.08, 54.93, 54.83, 50.24, 49.85, 45.76, 14.33; MS: 419.2 (M+1).

Preparation of CHIR166206 (10) (Scheme 5)

To a 2-l three-necked RB flask, equipped with overhead stirrer, condenser, thermocouple, addition funnel and N_2 adapter over a heating mantle, was added 2-amino-6-fluoro-benzonitrile (100.3 g, 0.735 mol, 1 equiv.) followed by THF (0.2 l). A 23.3 wt% solution of KOEt in ethanol (515 g, 1.07 mol, 1.45 equiv.) was added over 2 min and the suspension was heated to 77–79 °C until the reaction was complete as judged by HPLC (4 h). The reaction mixture was cooled to 50 °C, toluene (500 ml) was added, and the mixture was further cooled to 15 °C. The resulting slurry was vacuum filtered and the inorganic solids were washed with toluene (2 × 250 ml). The combined filtrate was concentrated *in vacuo* (35 °C, 29 mm Hg) to a final volume of 1 l and was washed with 3:1 saturated aqueous NH_4Cl:water (400 ml), water (400 ml), then saturated aqueous NaCl (400 ml). The combined aqueous layers were back-extracted twice with toluene (2 × 500 ml), and the combined organic layers were concentrated *in vacuo* (35 °C, 29 mm Hg) to a final volume of 300 ml. The solution was cooled to 5–10 °C and MTBE (300 ml) was added. The slurry was stirred at 5–10 °C for 1.5 h, vacuum filtered and the solid product was washed twice with cold toluene (2 × 50 ml, 5–10 °C). The cake was dried *in vacuo* (50 °C, 29 mm Hg) for 19 h to give 79.0 g CHIR166206 (**10**) as a light-yellow powder (66.3 % wt/wt yield, 97.0 AP by HPLC). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.31 (t, 3 H, *J* = 7.0 Hz), 4.04 (q, 2 H, *J* = 7.0 Hz), 5.97 (s, 2 H), 6.19 (d, 1 H, *J* = 8.0 Hz), 6.32 (dd, 1 H, *J* = 8.3 Hz, 0.5 Hz), 7.17 (t, 1 H, *J* = 8.3 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 153.0, 134.4, 115.7, 107.3, 98.3, 84.2, 63.8, 14.4.



Scheme 5.

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