Process Development and Scale-Up for the Preparation of the 1-Methyl-quinazoline-2,4-dione Wnt Inhibitor SEN461

ARTICLE *in* ORGANIC PROCESS RESEARCH & DEVELOPMENT · JULY 2013

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Process Development and Scale-Up for the Preparation of the 1-Methyl-quinazoline-2,4-dione Wnt Inhibitor SEN461

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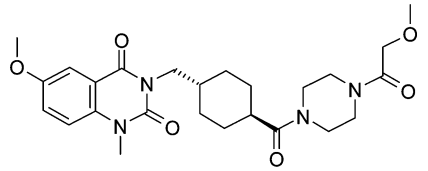
Supporting Information

ABSTRACT: A practical and scalable route to the Wnt inhibitor SEN461 **1** is described herein. The optimized route consists of nine chemical steps. The intermediates are solids and were isolated by filtrations. Critical reactions steps in the medicinal chemistry route were modified for an initial scale-up process, and as a result, we developed a synthetic procedure for the preparation of multihundred gram quantities of the final product. A further process development for the phase 1 clinical batch campaign is reported.

INTRODUCTION

The Wnt signaling pathway is known to have a fundamental role in tissue development, differentiation and homeostasis; this is achieved by regulating a specific pool of genes that strictly control temporal and spatial regulation of cell growth, movement and cell survival. Chronic activation of the canonical Wnt pathway promotes uncontrolled cell growth and survival, and can consequently drive cancer formation in a range of tissues.[1−3] In the past decade a number of studies demonstrated that up-regulation of the Wnt pathway is present in various types of cancer (e.g., lung, breast, pancreatic, gastric, colorectal cancers, medulloblastoma, glioblastoma, hepatocellular carcinoma).[4] For this reason targeting the canonical Wnt pathway can be a promising strategy in the identification and development of new anticancer agents. To date at least three Wnt-mediated intracellular signaling pathways have been identified:[5,6] the calcium-mediated and planar polarity pathways[8] and the canonical Wnt-β-catenin pathway. Siena Biotech has set up a drug discovery research program to identify new inhibitors of the β-catenin-dependent Wnt pathway for the treatment of glioblastoma multiforme.[7] After a screening campaign, a primary hit series was discovered; medicinal chemistry modifications and optimization of the original structures led to the identification of an advanced lead compound, SEN461 shown in Figure 1.



1
SEN461

Figure 1. Chemical structure of the Wnt inhibitor SEN461.

RESULTS AND DISCUSSIONS

MedChem Synthesis. The first synthesis of SEN461 (Scheme 1), carried out in our Medicinal Chemistry laboratories, delivered gram quantities of SEN461 to support in vitro and preliminary in vivo testing.

The synthesis, although long with nine steps, is convergent, but it had some important major drawbacks which necessitated its redesign: (a) the anthranilic acid **2** starting material is quite expensive; (b) the esterification of **4** with sulfuric acid and subsequent free basing gave **5** contaminated with 5−10% of its dimer **a** (Figure 2), which was difficult to separate in this stage and in the following stages; (c) the acylpiperazine **12** proved to be unstable, giving piperazine hydrochloride as a decomposition product and possibly contributing to the poor yield of the coupling step to give amide **13**; (d) the acid **8** and its corresponding lithium salt have extremely poor solubilities and consequently, were difficult to handle; (e) N-methylation in the last step was relatively low-yielding and required chromatographic purification.

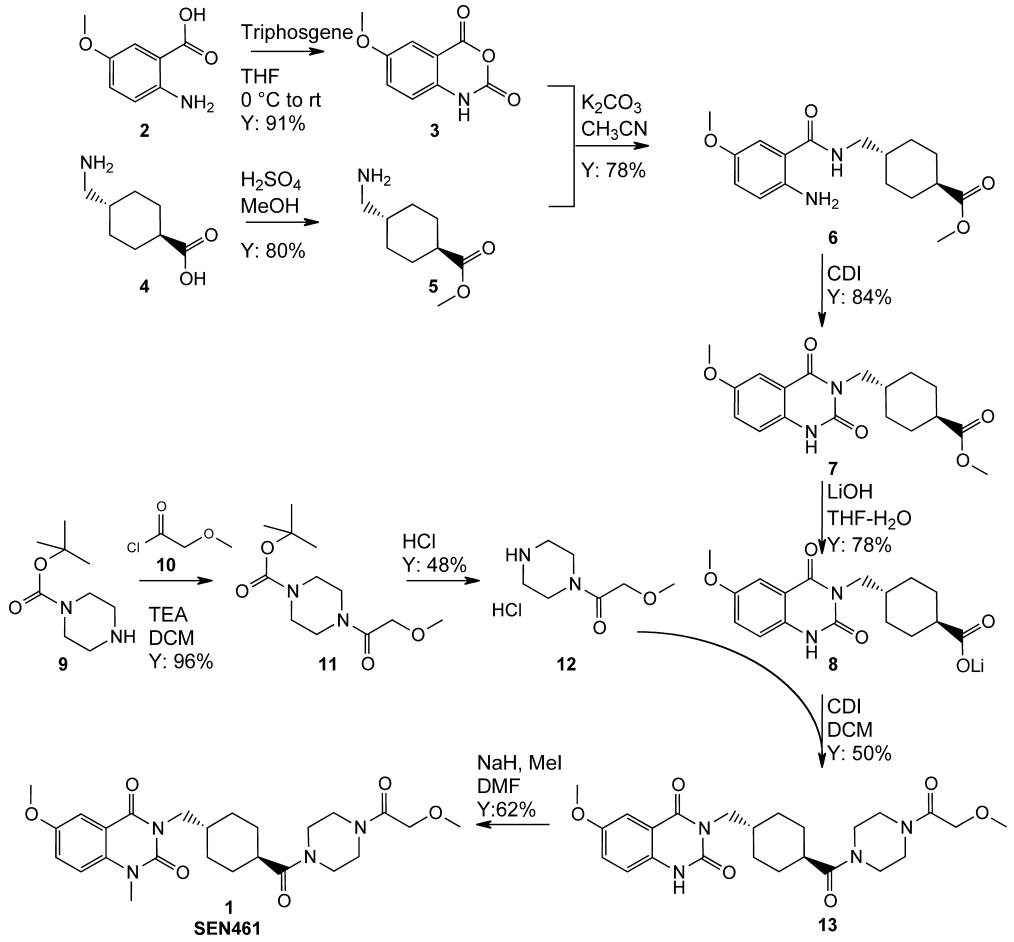
Redesign of the Synthesis. The alternative sequence shown in Scheme 2 was designed, preserving the ring-closure step to intermediate **7** but starting from the significantly less expensive nitrobenzoic acid **14**.[9] Although the sequence features the same number of steps and is more linear, it overcomes a number of problems of the original route, namely the use of the toxic and corrosive triphosgene and the use of the unstable acylpiperazine **12**. Introducing the N-Me group early on (**7** to **16**) in the synthesis avoids the relatively low-yielding final step of the original procedure, as well as renders the intermediates, especially the acid **17**, more soluble and more easy to handle.

Initial Scale-Up: 50 and 500 g Campaigns. Two campaigns were carried out to synthesise SEN461 batches for preclinical studies. The first campaign furnished 50 g of the desired compound and included some route scouting and reaction optimization work, which is described in detail in the corresponding section. For the second campaign, which

Received: June 3, 2013

   dx.doi.org/10.1021/op400145w | Org. Process Res. Dev. XXXX, XXX, XXX−XXX

Scheme 1. MedChem Route to compound 1



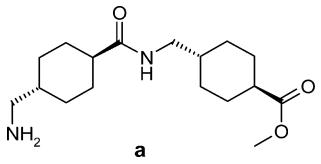


Figure 2. Dimeric impurity **a** monitored in the MedChem synthesis of **5**.

furnished 500 g of final product as well as several 100s of grams of various intermediates, the synthetic route remained the same. Most of the reactions were carried out in a 10 L jacketed glass reactor, and some adjustments to reduce solvent volumes and to facilitate working with the reactor were made.

Stage 1. Studies directed toward the multihundred gram synthesis of **5** began by exploring the feasibility of employing thionyl chloride in methanol as solvent. During the 50 g campaign, the aminoester **5** hydrochloride salt was prepared in high yield (>98%) by adding 2.0 equiv of $SOCl_2$ to a suspension of amino acid **4** in MeOH (10 vol), followed by evaporation of the solvent. In the 500 g campaign, the reaction was run in the same solvent but more concentrated than previously (3 vol), and the product was isolated by precipitation with MTBE rather than evaporation to dryness. The reaction was carried out in two main batches in 5 L round-bottom flasks affording **5** in 98% yield.

Stage 2. Next, the coupling of acid **14** with amino ester **5** was examined.[10] Even if the activation of acid **14** with CDI led to complete conversion to imidazolide, however the reaction with the amino ester **5** was very slow and gave only 30% conversion after 5 days in DCM at different temperatures (T_{int} = 20 or 40 °C). Activation with thionyl chloride or oxalyl chloride followed by addition of **5** and triethylamine afforded the desired amide **15** in 18 h and higher yields (86–90%). For the scale-up oxalyl chloride was chosen as activating agent because the reaction profile was slightly cleaner. In contrast to the 50 g campaign, the acid chloride solution was not completely evaporated in the 500 g campaign, but was partially concentrated and then added to the suspension of the aminoester **5** hydrochloride salt and the triethylamine. The desired product **15** was recovered in 77% yield (instead of 87% as in the former campaign) and 98% HPLC purity after crystallization from iPrOAc.

Stage 3. The method of choice for reducing the aromatic nitro group of **15** is catalytic hydrogenation as there are no incompatible functional groups present. As the hydrogenation with H_2 gas was not yet available at the time of initial scale-up, we chose to examine transfer hydrogenation conditions (anhydrous Pd–C 10 wt %, NH_4COOH, EtOH as solvent). In order to obtain a preliminary evaluation of the process thermal profile, two reaction mixtures (respectively with 2.5 and 1.5 wt % of Pd–C) were warmed from 20 to 60 °C (T_{int}) in 30 min. In the first experiment gas evolution (CO_2 and NH_3) started from 25 °C internal temperature, while in the second the onset was around 35 °C, possibly due to the lower catalyst loading. In any case, the reaction mixtures were warmed until

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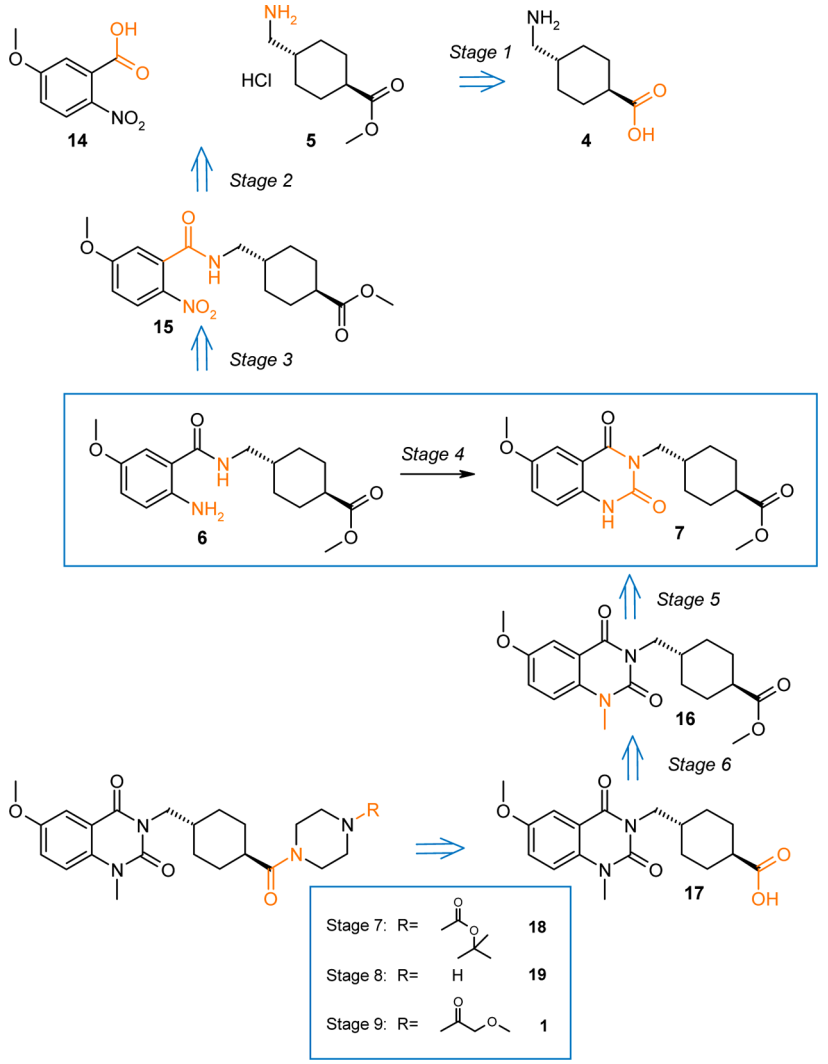
Scheme 2. Redesigned synthesis of compound 1



no gas evolved anymore. In the workup we included an aqueous wash to completely remove residual NH_4COOH: it allowed, during the 50 g campaign, to isolate a product with a superior NMR purity (95% instead of 90%) in 94% yield. In the 500 g campaign the transfer hydrogenation was replaced with hydrogenation using H_2 gas, thus eliminating the need to remove residual salts by aqueous washes. Having only a 2 L hydrogenation jacketed reactor available, the reaction had to be performed in several batches. Although the starting material was poorly soluble in EtOH, the reaction proceeded rapidly within 1 h to the product **6** which was highly soluble in EtOH. This reduction is exothermal,[11] and with no external cooling applied, a temperature increase from 20 to 55 °C over 30 min was detected using a thermometric probe. The desired intermediate **6** was isolated in 91% yield and 99% HPLC purity.

Stage 4. Both CDI as well as triphosgene can be used to close the ring as demonstrated on similar systems in the Medicinal Chemistry group (Scheme 1). The former was favored for scale-up due to its low toxicity and cost. We observed that the absence of ammonium formate in the starting material **6** was crucial for a clean ring-closure step: among the side products, two major impurities **b** and **c** (Figure 3) were identified (3−5% a/a by LC−MS).

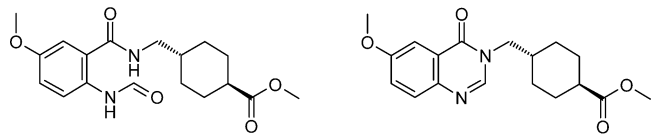


Figure 3. Major impurities **b** and **c** identified in the presence of ammonium formate.

The reaction initiates already at 15 °C, as shown by the product precipitating as a white solid and causing a moderate exotherm from 15 to 25 °C after complete CDI addition. For isolation on multihundred grams scale, the white precipitate is simply filtered and dried under vacuum giving **7** in high purity (HPLC > 95%) and 88% yield. For practicality (hygroscopicity of CDI) as well as to better control the exothermic reaction, the order of addition was changed on kilogram scale. A solution of the amide **6** was added to the CDI suspension, rather than adding the solid CDI to the amide solution. The desired 1-H-quinazoline-2,4-dione **7** was isolated with a slightly improved yield (92%).

Stage 5. Together with introducing the N-methyl group early on in the synthesis, the hazardous combination of NaH/DMF[12] was replaced with K_2CO_3/DMF,[13] affording the

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intermediate **16** in 94% yield and excellent purity (>99% by HPLC). In the kg-scale run, another 0.2 equiv of MeI (1.5 equiv instead of 1.3 equiv) had to be added to ensure complete conversion. In previous trials, we observed complete conversion with 1.3 equiv of the alkylating agent using the same batches of reactants. We hypothesize that some MeI might have been lost when charging the MeI/DMF solution to the addition funnel of the pilot plant under reduced pressure.

Stage 6. The hydrolysis of methyl ester **16** was performed by addition of lithium hydroxide using a mixture THF/H_2O as solvent and heating at 60 °C (T_{int}). The rate of conversion of the starting material was monitored by HPLC, and reaction was completed in 2 h. In order to obtain the product as free acid the organic solvent was evaporated, and the acid **17** was precipitated by addition of HCl 1 N. Following this workup procedure, the filtration of the product was very slow. Instead acidifying the reaction mixture at 45 °C (rather than room temperature) and adding 1 volume of acetone gave a precipitate that was much easier to filter. The residual water could not be completely removed (KF: 6−8% w/w) from the product by drying in a vacuum oven.

Stage 7. CDI and oxalyl chloride were investigated as activating agents for the coupling with the Boc-piperazine. The activation with oxalyl chloride was quite slow and did not go to completion in 18 h. CDI furnished complete activation of the acid in 1 h (the presence of residual water in acid **17** required an overcharge of the coupling agent to achieve full conversion to intermediate imidazolide), while the subsequent coupling with Boc-piperazine was slightly slower and was left to react overnight for convenience. When the reaction mixture was cooled to room temperature during the 50 g campaign, a precipitate started to form and was filtered. The solid obtained was free of imidazole and Boc-piperazine, but contained 5% (by HPLC) of the acid **17**, which was removed by basic aqueous washes. In the 500 g campaign, with no residual acid left, the basic washes could be eliminated and the workup was significantly simplified.

Stage 8. The Boc deprotection reaction was initially performed using HCl 4 N in dioxane[14] in analogy to the reaction in Scheme 1. This procedure had some issues: (a) the reaction rate was very slow, requiring further additions of HCl as well as heating; (b) the suspension that formed after some hours was difficult to stir. We then turned our attention to investigating the use of TFA for the Boc-deprotection.[15] Some trials were done by varying the percentage of TFA in DCM (Table 1, entries 1−4): the best conditions ensuring a complete and rapid deprotection were found when TFA 3.2 M in DCM at 50 °C (T_{bath}) was used. On multihundred-gram scale (Table 1, entry 5) it was possible to reduce the concentration of TFA by ca. 25% with respect to earlier work, maintaining a fast and complete reaction.

Stage 9. The final step of the intermediate **19** with 2-methoxy acetyl chloride was carried out in DCM using triethyl amine as base. The reaction was fast, showing complete conversion (by HPLC) already after 30 min. The workup was done by simply washing the reaction mixture with H_2O and $NaHCO_3$ solution, and the final product **1** was purified by crystallisation from EtOAc, obtaining SEN461 in high yield (90%) and purity (HPLC purity >99%).

Process Development. Although the aforementioned enabling methodology yielded excellent quality product in very good yield, the following issues were identified by the process development investigation:[16]

Table 1. Optimisation of the TFA concentration and process campaign experiments: stage 8

entry	TFA	conversion/reaction time at 50 °C (T_{bath})	yield (%)
1[a]	0.7 M	0.3%/1.5 h (20 °C)	–
		1.5%/1 h (50 °C)	
2[a]	1.7 M	56%/1 h	–
		72%/2 h	
		99%/18 h	
3[a]	2.1 M	95%/1 h	–
		98%/1.5 h	
		99% 18 h	
4[b]	3.2 M (*50 g campaign*)	100%/1 h	98
5[c]	2.4 M (*500 g campaign*)	100%/3 h	80

[a]Scale of experiment: 2 g of **18**. [b]Scale of experiment: 100 g of **18**. [c]Scale of experiment: 0.7 kg of **18**

(1) the process employed thionyl chloride, a hazardous reagent;
(2) the presence of impurity **d** (Figure 6) was monitored in the amide **15** formation;
(3) the N-methylation appeared not to be reproducible, even using an excess of methyl iodide;
(4) stage 6 displayed complications in the purification and isolation of **17** as free acid;
(5) the contamination by inorganics and water of acid **17** severely lowered the yield of the Boc-piperazine **18** formation step;
(6) the use of TFA in the Boc-deprotection stage raised an issue with plant pipes compatibility.

Thionyl Chloride: Occasion of Concern. The excess of thionyl chloride employed in the 500 g campaign was greater than effectively required. The use of this reactant is always occasion of concern especially in pilot-plant campaigns: its intrinsic acidity leads to corrosion of pilot-plant pipes. Moreover, the addition of this reagent is associated to a strong exothermal effect, evolution of toxic gases such as sulfur dioxide and potentially, in the case of $SOCl_2$/MeOH system, methyl chloride. It was observed that the reaction mass tends to get very dense and even up to a jelly consistency at about half of the addition of thionyl chloride, compromising the mixing efficiency, and consequently, the heat transfer capacity. Dilution with 4 vol of solvent together with a minor charge of thionyl chloride (1.3 equiv vs 2 equiv) solved the problem, affording in the manufacturing campaign the product with 97% yield and desired purity.

A calorimetric measurement of the reaction was performed[17] (Figures 4 and 5) and confirmed the following: release of heat and gas was controlled by the rate of addition of thionyl chloride; the peak of heat evolution was observed during the first 25% of thionyl chloride charge; average gas evolution (3L/min·kg) was constant throughout the addition. Consequently, during the manufacturing campaign on 8.9 kg of acid **4** in MeOH (4 vol), $SOCl_2$ (5 L) was charged in 50 min, maintaining the temperature below 35 °C.

Stage 2: An Oxalyl Chloride Byproduct. Changes versus the initial scale-up procedure concerned mainly the adjustment of equivalents of nitrobenzoic acid **14** in order to minimize the formation of a new impurity **d** identified by NMR and HPLC−MS analyses (Figure 6) throughout the process development optimisation.

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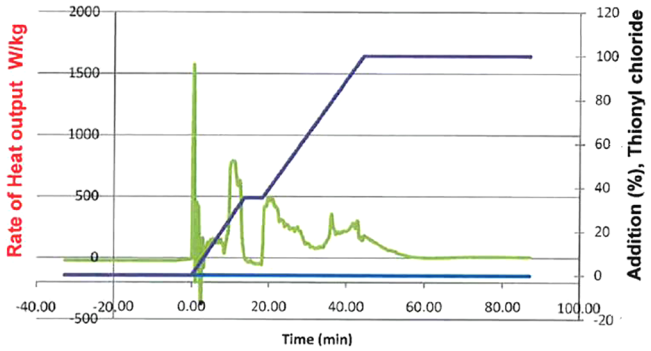


Figure 4. Specific heat of reaction (green line) as function of $SOCl_2$ addition (violet line).

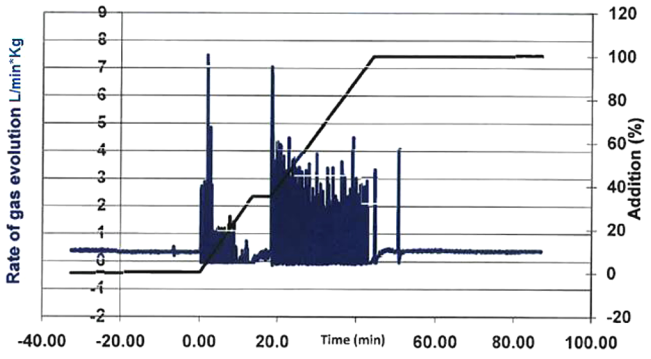
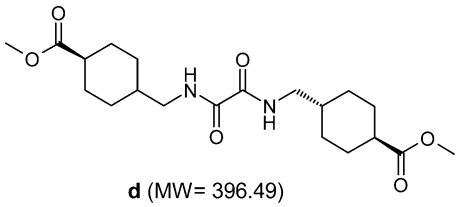


Figure 5. Gas evolution (blue line) as function of $SOCl_2$ addition (black line).



d (MW= 396.49)

Figure 6. Impurity **d**.

When an excess of oxalyl chloride (1.3 equiv) was used to activate the starting acid, 8% of **d** was detected by HPLC. Instead, using the same excess of **14** (1.2−1.3 equiv) ensured a complete consumption of oxalyl chloride without the impurity generation.[18] Moreover, the residual nitrobenzoic acid **14** could be eliminated by crystallization with i-PrOAc. However, employing the latter conditions, we experienced difficulties in the demo batch experiment: the incomplete conversion (90%) of the acid **14** was observed, also using 1.5 equiv of oxalyl chloride. The cause was identified in the unexpected high water content (5 wt % by KF analysis) of the batch of nitrobenzoic acid supplied for the plant campaign. Due to the urgency to deliver final product **1** and since the demo batch experiment produced the required ester **5** with the expected yield (89%) and purity (99% by HPLC), the excess of oxalyl chloride was used also in the manufacturing campaign without a further investigation.

A Not Reproducible N-Methylation. After a few familiarization trials, the methylation of **7** appeared not to be reproducible. An incomplete conversion was monitored (87−93% a/a by HPLC) even using an excess of MeI (from 1.3 to 1.7 equiv). To drive the conversion of starting material **7**, the amount of K_2CO_3 was increased from 1.3 to 2.0 equiv, and their suspension in DMF was stirred 1 h at 50 °C before the addition of MeI as a solution in the same solvent (after cooling to 30 °C). The procedure was successfully applied to the demo batch experiment, although a high inorganic content (up to 19 wt %, potassium salt byproducts) was measured in the product isolated by precipitation from DMF/water. To overcome the issue in the plant campaign, it was decided to increase from 5 to 10 vol the amount of water added during the precipitation, obtaining the desired intermediate **16** with low inorganics content (<0.5 wt %) in 95% yield.

Improving Isolation and Purification of Acid ***17****.* Considerable issues following the initial workup procedure resulted from the very slow filtration of product **17** since the collected solid had a particle size down to 1 μm. Some difficulties were also encountered in removing water (KF: 4 wt %) and inorganics (sulfated ashes: 18 wt %). Fortunately, the acid **17** particle size was significantly improved if precipitation occurred from THF/water, avoiding the solvent distillation. Moreover, acetone washes (instead of water) on filter allowed an easier drying in the oven (KF: 0.5−1.0 wt %).[19] The manufacturing batch of acid **17** was isolated with 96% yield, 99% HPLC purity, and <0.1 wt % inorganics content.

Stage 7. The coupling reaction failed to reach completion when the starting material **17** was contaminated by inorganics and water (70% final conversion), even if a clean product **18** was recovered after filtration (0.1% a/a of residual acid **17** was observed by HPLC) in 67% yield. Employing the inorganics-free batch of starting acid, the reaction went as expected. Moreover, thanks to the low water content, the amount of charged CDI could be lowered from 2.0 to 1.5 equiv, minimizing the homocoupling of *N*-Boc-piperazine and affording intermediate **18** in 91% yield, high purity (HPLC 99%) and suitable characteristics to be chosen as starting material for the GMP stages (stages 8 and 9) of the manufacturing campaign.

TFA and Plant Pipes Compatibility. A procedure with an excess of TFA (almost 10 equiv) was deemed problematic because of serious compatibility problems with the plant apparatus and a complex workup procedure. Deprotection with HCl 5 N in IPA and the subsequent hydrochloride salt precipitation was favored on the basis of previous experience with analogous compounds. DCM was selected as cosolvent in order to ensure an efficient stirring during the salt formation. Small-scale trials and demo batch production were conducted without problems. However, during the cGMP campaign, filtration rate of **19** as hydrochloride salt was unexpectedly slow (see Plant Manufacturing Campaign section). A further lab experiment demonstrated that the filtration rate was associated with the unfavorable ratio between wet cake height and filter diameter.

Plant Manufacturing Campaign. The reported synthetic route (Scheme 3) was followed to synthesize SEN461 in a nine-stage procedure.

Non-GMP Stages: Pilot-Plant Scale-Up. Stages 1−7 were carried out in a single run using 100−200 L Hastelloy vessels. Isolation of solid intermediates was performed by pressure/vacuum filtration using a 60 cm Hastelloy filter. Drying was carried out in tray dryer oven. No relevant deviations from the planned procedure were observed, and intermediate **18** was isolated in 65% overall yield starting from nitrobenzoic acid **14**.

GMP Stages: Kilolab Scale-Up. Stage 8 was performed in multiple batches, employing a 20 L glass reactor and a

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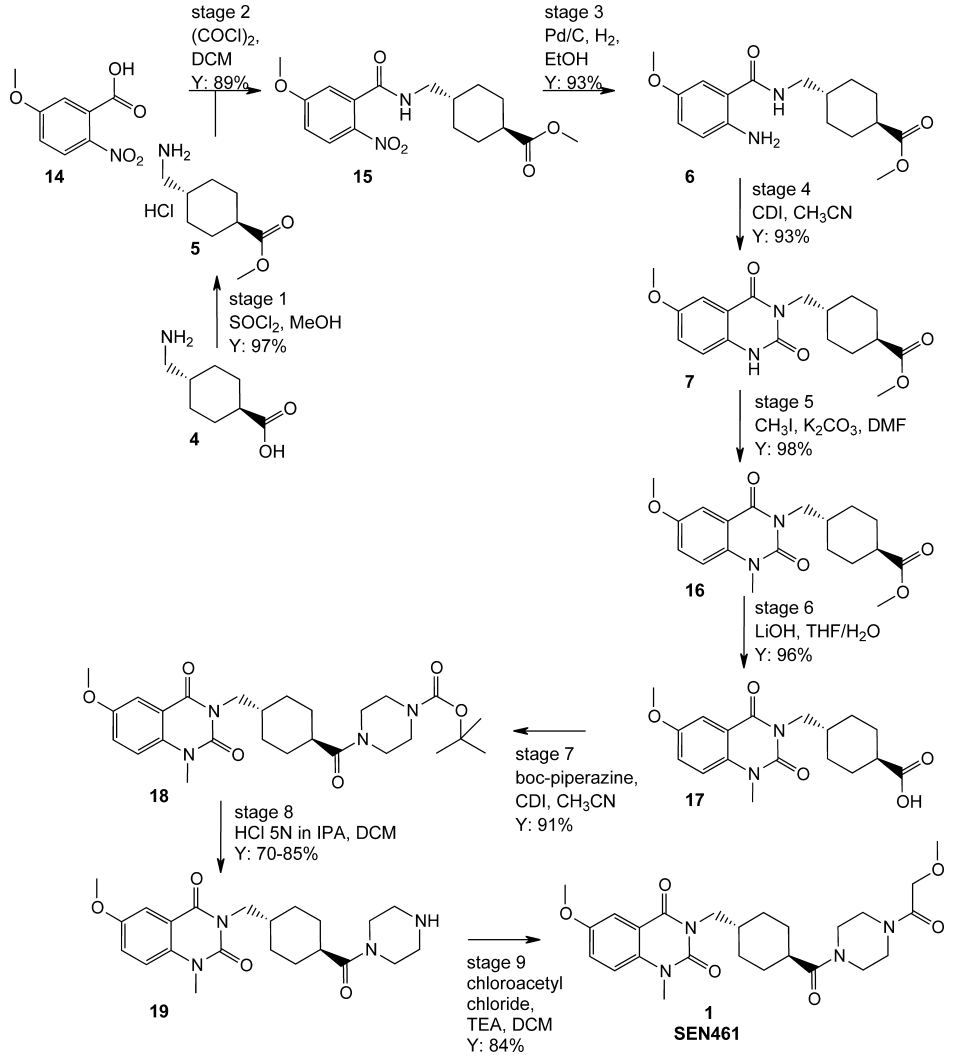
Scheme 3. Pilot-plant campaign synthetic route



Hastelloy filter dryer. During the first run of stage 8 a very slow filtration rate was observed. Trying to mitigate the impact of the slow filtration, in view of the following runs, the input weight was decreased, and the solvent composition was slightly modified to decrease solvent viscosity.[20] The first batch of SEN461 **1** (1.798 kg) was produced with 42% overall yield starting from acid **14**; the second batch (3.581 kg) was produced with 38% overall yield in the GMP synthesis.

Isolation and Identification of SEN461 Process Impurity. An unexpected impurity was detected at low level (0.2% a/a by HPLC analysis) in the manufacturing API batch of SEN461. In a preliminary investigation by LC−MS the APCI mass spectrum contained m/z 913.6 as main peak. Analyzing the MS spectra of the impurity and by a comparison with the fragmentation pattern of SEN461, it seemed that the two molecules were structurally related. The unknown compound was isolated by preparative LC, and an NMR study was performed with the aim to identify its molecular structure. The inspection of mono- and bidimensional NMR spectra allowed excluding the high-molecular weight impurity as some type of polymeric contamination of the batch. Thanks to the comparison with the proton spectrum of **1**, the structure of the impurity was determined to be a sort of dimer of SEN461 (compound **e**, Figure 7), probably formed with the contribution of dichloromethane used in the last step of the process.[21]

■ CONCLUSIONS

Initial scale-up and process optimization to the Wnt inhibitor SEN461 **1** is described herein. The optimized route consists of nine chemical steps. The current nine-step linear route is

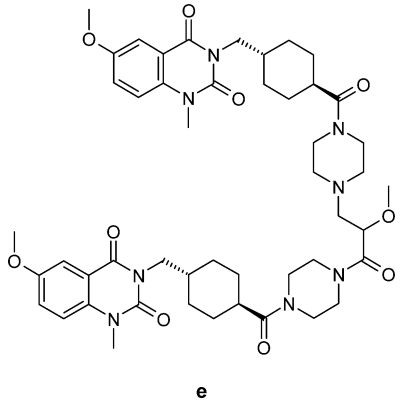


Figure 7. Elucidated structure of the SEN461 process impurity **e**.

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characterized by high yields (overall yield in the 50 g campaign: 44%; in the 500 g campaign: 38%); solid intermediates throughout the synthesis, easy purification of intermediates and final product and absence of chromatography; no highly hazardous reagents; robust reactions with low thermal hazards; no specialized equipment required and temperature range reactions between 0 and 80 °C; availability of starting material **14** from various suppliers. Critical reaction steps in the medicinal chemistry route were modified for an initial scale-up process, and as a result, a synthetic procedure for preparation of multihundred gram quantities of the final product was developed. A further process development and the clinical batch manufacturing campaign are also described: a linear and efficient access to the key GMP-intermediate **18** was developed, significantly simplifying the synthesis, reducing cost, and maintaining high yields (overall yield in plant campaign: 42%).

EXPERIMENTAL SECTION

The reported yields are corrected for purity and water/solvent content of the products. Generally, the reactions were monitored by HPLC, and purities/conversions quoted refer to HPLC area % at 215 nm. HPLC method: Waters Symmetry C18 3.5 μm 4.6 mm × 75 mm column, flow rate 1.0 mL/min, mobile phase A: 10 mM aq K_2HPO_4 buffer or 0.1% aq formic acid; mobile phase B: acetonitrile or acetonitrile/0.1% formic acid; gradient (13 min): 95:5 A/B to 10:90 A/B over 10 min, 2 min at 10:90 A/B, then 10:90 A/B to 95:5 A/B over 1 min.

UPLC-MS analyses were run using a Acquity Waters UPLC equipped with a Waters SQD (ES ionization) and Waters Acquity PDA detector. UPLC method: BEH C18 1,7 μm, 2,1 × 50 mm column, flow rate 0.6 mL/min, mobile phase A: 0.1% aq formic acid; mobile phase B: 0.1% formic acid acetonitrile; gradient (3 min): 95:5 A/B to 100:0 A/B over 2 min, 40 s at 100:0 A/B, 100:0 A/B to 95:5 A/B over 10 s, then 10 s at 95:5 A/B. Retention times were expressed in minutes. Temperature: 40 °C. UV Detection at 215 and 254 nm. ESI^+ detection in the 80−1000 m/z range.

Synthesis of 4-Aminomethyl-cyclohexanecarboxylic Acid Methyl Ester (5). 4-Aminomethyl-cyclohexanecarboxylic acid **4** (0.6 kg, 3.8 mol, 1 equiv) was charged to a 5 L four-necked round-bottom flask under a blanket of nitrogen, with 1 N aq NaOH trap for scrubbing the off-gases. Methanol (2.0 L) was added, cooling at 10 °C internal temperature with an ice bath. The mixture was stirred for 10 min.

Thionyl chloride (559 mL, 7.6 mol, 2 equiv) was added dropwise over 2.5 h, maintaining the temperature between 10 and 20 °C. After complete addition, the white suspension was warmed to 25 °C in 1 h, and HPLC indicated complete conversion.

Then the methanol was partially removed by distillation under reduced pressure (1.1 L at p = 320 mbar, T_{int} = 42 °C). The resulting mixture was cooled to 30 °C, and MTBE (2.0 L) was added over 15 min, giving the precipitation of a white solid. The suspension was cooled to −5 °C under mechanical stirring for 1.2 h and then filtered on a Buchner funnel, followed by washing with MTBE (500 mL).

The final product **5** hydrochloride salt was dried in the drying oven (p = 20 mbar, T = 20 °C, 16 h) to give 772 g of a white solid. Yield: 98%.

UPLC−MS: t_R= 0.53 min, m/z = 172 $[M + 1]^+$.

HRMS calcd for $C_9H_{18}NO_2$ $[M + 1]^+$ 172.13376, found 172.13378.

HPLC: t_R= n.d., not UV responsive intermediate.

^1H NMR (400 MHz DMSO-d_6): δ 7.83 (s, broad, 3H), 3.57 (s, 3H), 2.61 (d, J = 6.9 Hz, 2H), 2.24 (m, 1H), 1.90 (m, 2H), 1.79 (m, 2H), 1.50 (m, 1H), 1.27 (m, 2H), 0.97 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 175.9, 52.2, 44.7, 42.6, 35.5, 29.4, 28.5.

Synthesis of 4-[(5-Methoxy-2-nitro-benzoylamino)-methyl]-cyclohexanecarboxylic Acid Methyl Ester (15). 5-Methoxy-2-nitro-benzoic acid **14** (0.4 kg, 2.0 mol, 1 equiv) was charged to a 5 L four-necked round-bottom flask under a blanket of nitrogen, with 1 N aq NaOH trap for scrubbing the off-gases. Dichloromethane (3.2 L) and DMF (4.3 mL) were respectively added, cooling at 15 °C internal temperature with an ice bath. The mixture was stirred for 10 min.

Oxalyl chloride (186 mL, 2.2 mol, 1.1 equiv) was added dropwise over 45 min. An endotherm was observed during the addition (ΔT = −5 °C, from 15 to 10 °C). Gas evolution was completed in 3.5 h, and then an HPLC check confirmed the complete activation.

Part of the dichloromethane was removed by distillation under reduced pressure (1.0 L at p = 200 mbar, T_{int} = 30 °C). The resulting mixture was kept standing at room temperature under nitrogen atmosphere for 18 h and then was added dropwise in 2 h to a suspension of the 4-aminomethyl-cyclohexanecarboxylic acid methyl ester **5** (414 g, 2.0 mol, 1 equiv) and TEA (830 mL, 6.0 mol, 3 equiv) in dichloro-methane (1.6 L) at 10 °C. An exotherm was observed during the addition (ΔT = +10 °C, from 10 to 20 °C). A sample was checked by HPLC 15 min after the addition and a complete conversion was observed.

The reaction mixture was combined with the batch of the second run (0.4 kg scale) in a 10 L jacketed reactor and washed with: water (2 × 3.0 L); HCl 2 M (1 × 3.0 L); water (1 × 3.0 L). ^{i}PrOAc (6.3 L) was added dropwise in 15 min to the separated organic phase, and the formation of a pale-brown precipitate was observed. The suspension was heated to T_{int} = 75 °C for 40 min and then cooled to T_{int} = 0 °C for 1h. After filtration on a Buchner funnel and drying until constant weight in oven (p = 20 mbar, T_{oven} = 20 °C, 8 h), 1.085 kg of 4-[(5-methoxy-2-nitro-benzoylamino)-methyl]-cyclohexanecarboxylic acid methyl ester **15** as an off-white solid was recovered. Yield: 77%.

UPLC−MS: t_R = 1.23 min, m/z = 351 $[M + 1]^+$.

HRMS calcd for $C_{17}H_{23}N_2O_6$ $[M + 1]^+$ 351.15565, found 351.15566.

HPLC: t_R = 6.48 min, purity 98.7%.

^1H NMR (400 MHz $CDCl_3$): δ 8.06 (d, J = 9.1 Hz, 1H), 6.91 (dd, J = 9.1 Hz, 2.8 Hz, 1H), 6.84 (d, J = 2.8 Hz, 1H), 5.73 (m, 1H), 3.84 (s, 3H), 3.60 (s, 3H), 3.26 (t, J = 6.5 Hz, 2H), 2.20 (m, 1H), 1.96 (m, 2H), 1.86 (m, 2H), 1.56 (m, 1H), 1.48 (m, 2H), 0.99 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_d): δ 176.2, 166.2, 163.7, 140.0, 136.6, 127.5, 115.3, 114.9, 57.1, 52.0, 45.9, 43.0, 37.3, 30.0, 29.0.

Synthesis of 4-[(2-Amino-5-methoxy-benzoylamino)-methyl]-cyclohexanecarboxylic Acid Methyl Ester (6). A 2 L glass pressure reaction vessel (Buchi Ecoclave) was charged at 20 °C under a nitrogen blanket with a suspension of Pd/C 10 wt % (anhydrous, 5.0 g) in ethanol (10 mL) followed by a suspension of 4-[(5-Methoxy-2-nitro-benzoylamino)-methyl]-cyclohexanecarboxylic acid methyl ester **15** (200 g, 0.57 mol, 1 equiv) in ethanol (1.0 L). The resulting mixture was vigorously stirred under hydrogen pressure (initial H_2 pressure: 75 psi)

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until complete reduction of the starting nitro derivative **15** was observed by HPLC (theoretical absorption of H_2 achieved after 1 h). The reaction mixture was then cooled to 20 °C under nitrogen atmosphere, combined with the other 5 identical reaction batches and filtered on a Buchner funnel through a pad of powered cellulose to remove the catalyst, washing with ethanol (2 × 250 mL). Mother liquors were transferred to the 10 L jacketed reactor and concentrated under reduced pressure (from 9 to 4.5 L; p = 200 mbar, T_{int} = 45 °C); after cooling to 5 °C, water (4.5 L) was added dropwise in 25 min. The resulting suspension was stirred at 8−10 °C for 1 h and filtered on a Buchner funnel. The solid obtained was washed with EtOH/H_2O 1:1 (3 × 500 mL) and dried in a vacuum oven (T_{oven} = 50 °C, p = 10 mbar, 18 h). Finally 1.01 kg of **6** as a pale-orange solid was obtained. Yield: 91%.

UPLC−MS: t_R= 1.01 min, m/z = 321 $[M + 1]^+$.

HRMS calcd for $C_{17}H_{25}N_2O_4$ $[M + 1]^+$ 321.18146, found 321.18144.

HPLC: t_R = 4.44 min, purity 99.3%.

^{1}H NMR (400 MHz DMSO-d_6): δ 8.20 (t, J = 5.4 Hz, 1H), 7.03 (d, J = 2.8 Hz, 1H), 6.80 (dd, J = 8.8 Hz, 2.8 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 5.89 (s, 2H), 3.66 (s, 3H), 3.55 (s, 3H), 3.03 (t, J = 6.2 Hz, 2H), 2.23 (m, 1H), 1.89 (m, 2H), 1.75 (m, 2H), 1.47 (m, 1H), 1.26 (m, 2H), 0.96 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 176.2, 169.2, 150.0, 144.3, 119.7, 118.2, 116.1, 112.9, 56.3, 52.0, 44.9, 43.0, 37.6, 30.2, 29.0.

Synthesis of 4-(6-Methoxy-2,4-dioxo-1*H*-quinazolin-3-ylmethyl)-cyclohexanecarboxylic Acid Methyl Ester (7). To a suspension of 1,1-carbonyldiimidazole (859 g, 5.3 mol, 1.5 equiv) in acetonitrile (2.5 L) in a 10 L jacketed reactor, a solution of 4-[(2-amino-5-methoxy-benzoyl-amino)-methyl]-cyclohexanecarboxylic acid methyl ester **6** (1.13 kg, 3.5 mol, 1 equiv) in acetonitrile (7.5 L) was added over 1 h at room temperature and nitrogen atmosphere. An exotherm was observed (ΔT = +16 °C; from 11 to 27 °C). The reaction suspension was heated to 55 °C (T_{int}), and after 2 h the conversion was completed by HPLC analysis.

The suspension was cooled and kept at +5 °C for 40 min and then filtered on a Buchner funnel, washing with acetonitrile (1 × 1.0 L). The product was dried by suction on the filter to give 1.12 kg of **7** as a white solid. Yield: 92%.

UPLC−MS: ,t_R= 1.28 min, m/z = 347 $[M + 1]^+$.

HPLC: t_R= 6.93 min, purity 99.1%.

HRMS calcd for $C_{18}H_{23}N_2O_5$ $[M + 1]^+$ 347.16073, found 347.16072.

^{1}H NMR (400 MHz DMSO-d_6): δ 7.34 (d, J = 2.5 Hz, 1H), 7.28 (dd, J = 8.8 Hz, 2.5 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 3.76 (m, 5H), 3.55 (s, 3H), 2.24 (m, 1H), 1.86 (m, 2H), 1.65 (m, 3H), 1.22 (m, 2H), 1.04 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 176.0, 162.7, 155.3, 150.7, 134.2, 124.6, 117.4, 114.8, 108.9, 56.2, 52.0, 46.1, 42.8, 36.1, 30.0, 28.8.

Synthesis of 4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-ylmethyl)-cyclohexanecarboxylic Acid Methyl Ester (16). 4-(6-Methoxy-2,4-dioxo-1H-quinazolin-3-ylmethyl)-cyclohexanecarboxylic acid methyl ester **7** (925 g, 2.7 mol, 1 equiv), potassium carbonate (480 g, 3.5 mol, 1.3 equiv), and DMF (1.0 L) were respectively charged to a 10 L jacketed reactor at room temperature under nitrogen flux. The suspension was stirred for 1 h at 20 °C. No exotherm and no gas evolution were observed. Then a solution of methyl iodide (200 mL, 3.2 mol, 1.2 equiv) in DMF (1.0 L) was added over 15 min. A mild exotherm was observed during the addition (ΔT = +8 °C; from 20 to 28 °C). After 3 h another portion of MeI was added (35 mL, 0.6 mol, 0.2 equiv), driving the reaction to completion (by HPLC) after overnight stirring at room temperature.

The suspension was cooled to +10 °C, and water (5.0 L) was added over 40 min. The mixture was stirred at this temperature for 30 min and then filtered on a Buchner funnel, followed by washing with water (3 × 1.0 L). The product was dried in an oven (T_{oven} = 50 °C, p = 20 mbar) until constant weight, affording 903 g of **16** as a white solid. Yield: 94%.

UPLC−MS: t_R = 1.46 min, m/z = 361 $[M + 1]^+$. HPLC: t_R = 7.54 min, purity 99.7%.

HRMS calcd for $C_{19}H_{25}N_2O_5$ $[M + 1]^+$ 361.17368, found 361.17368.

^{1}H NMR (400 MHz DMSO-d_6): δ 7.45 (d, J = 2.6 Hz, 1H), 7.36 (m, 2H), 3.79 (m, 5H), 3.54 (s, 3H), 3.48 (s, 3H), 2.23 (m, 1H), 1.85 (m, 2H), 1.71−1.61 (m, 3H), 1.20 (m, 2H), 1.03 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 176.0, 161.7, 155.4, 150.8, 135.2, 124.0, 117.0, 116.0, 109.0, 56.2, 51.9, 47.2, 42.8, 36.2, 31.3, 29.9, 28.8.

Synthesis of 4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-ylmethyl)-cyclohexanecarboxylic Acid (17). 4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-ylmethyl)-cyclohexanecarboxylic acid methyl ester **16** (1.0 kg, 2.8 mol, 1 equiv) was suspended in THF (4.0 L) in a 10 L jacketed reactor, and a solution of LiOH (76 g, 3.2 mol, 1.1 equiv) in water (1.0 L) was added at 20 °C over 15 min. An endotherm was observed during the addition (ΔT = −2 °C; from 19 to 17 °C). The mixture was heated to 60 °C (T_{int}) for 30 min, then another portion of water (1.0 L) was added to enable more efficient stirring. After 2.5 h the conversion was complete by HPLC.

The organic solvent was distilled under reduced pressure (T_{int} = 45−40 °C, p = 700−500 mbar; 3.5 L recovered in 1.5 h); water (3.0 L) was added and the mixture acidified to pH = 1 with HCl 3 M (1.1 L). Then acetone (1.0 L) was added and the suspension cooled to +5 °C for 30 min. The mixture was filter on a Buchner funnel, washing with water (2 × 1.0 L). The wet solid was dried in oven (p = 20 mbar; 2 d at T_{oven} = 50 °C, 8 h at T_{oven} = 80 °C), giving 930 g of **17** as a white solid. Yield: 90%.

UPLC−MS: t_R = 1.19 min, m/z = 347 $[M + 1]^+$.

HRMS calcd for $C_{18}H_{23}N_2O_5$ $[M + 1]^+$ 347.16073, found 347.16074.

HPLC: t_R = 6.33 min, purity 99.3%.

^{1}H NMR (400 MHz DMSO-d_6): δ 7.48 (d, J = 2.6, 1H), 7.38 (m, 2H), 3.81 (m, 5H), 3.48 (s, 3H), 2.10 (m, 1H), 1.85 (m, 2H), 1.64 (m, 3H), 1.18 (m, J = 12.5 Hz, 3.4 Hz, 2H), 1.02 (m, J = 12.5 Hz, 3.4 Hz, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 177.3, 161.7, 155.4, 150.8, 135.2, 124.0, 116.9, 115.9, 109.9, 56.2, 47.3, 43.0, 36.3, 31.3, 30.1, 28.9.

KF: 6.8 wt % water.

Synthesis of 4-[4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-ylmethyl)-cyclohexanecarbonyl]-piperazine-1-carboxylic Acid *tert*-Butyl Ester (18). 1,1-Carbonyldiimidazole (723 g, 4.5 mol, 2.1 equiv) and acetonitrile (4.5 L) were respectively charged to a 10 L jacketed reactor under nitrogen flux, and the suspension was stirred at 20 °C for 15 min. 4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-ylmethyl)-cyclohexanecarboxylic acid **17** (750 g, 2.1 mol corrected for water content, 1 equiv) was added portionwise (6 × 120 g + 1 × 30

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g) over 40 min. Gas evolution (CO_2) and exotherm (ΔT= +10 °C; from 17 to 27 °C) were observed. The mixture was heated to +50 °C (T_{int}) for 1 h and checked by HPLC (activation complete). Then a solution of *N*-Boc-piperazine (524 g, 2.8 mol, 1.3 equiv) in acetonitrile (1 L) was added over 10 min. An endotherm was observed during the addition (ΔT= −1 °C; from 53 to 52 °C). The reaction was heated to reflux (T_{int}= 80 °C) for 2 h (99.5% a/a HPLC conversion) and at 45 °C (T_{int}) overnight. The obtained white suspension was cooled to 10 °C for 30 min and filtered on a Buchner funnel, followed by washing with acetonitrile (500 mL).

The product was dried on filter by suction until constant weight to give 960 g of **18** as a white solid. Yield: 91%.

UPLC−MS: t_R = 1.51 min, m/z = 515 $[M + 1]^+$.

HRMS calcd for $C_{27}H_{39}N_2O_4$ $[M + 1]^+$ 515.28701, found 515.28702.

HPLC: t_R = 7.54 min, purity 99.8%.

^{1}H NMR (400 MHz DMSO-d_6): δ 7.46 (d, J = 2.6 Hz, 1H), 7.41−7.35 (m, 2H), 3.83 (m, 5H), 3.48 (s, 3H), 3.41 (m, 4H), 3.26 (m, 4H), 2.51 (m, 1H), 1.71−1.61 (m, 5H),1.49 (s, 9H), 1.28−1.05 (m, 4H).

^{13}C NMR (100 MHz DMSO-d_6): δ 174.1, 161.8, 155.4, 154.4, 150.9, 135.5, 124.0, 117.0, 116.0, 109.9, 79.8, 56.2, 47.4, 45.2, 41.4, 40.6, 36.4, 31.4, 30.1, 29.2, 28.7.

Synthesis of 6-Methoxy-1-methyl-3-[4-(piperazine-1-carbonyl)-cyclohexylmethyl]-quinazoline-2,4-dione (19). 4-[4-(6-Methoxy-1-methyl-2,4-dioxo-quinazolin-3-yl-methyl)-cyclohexanecarbonyl]-piperazine-1-carboxylic acid tert-butyl ester **18** (700 g, 1.4 mol, 1 equiv) was dissolved in dichlomethane (3.5 L) at 20 °C in a 10 L jacketed reactor. A solution of TFA (911 mL, 11.8 mol, 8.4 equiv) in dichlomethane (0.7 L) was added over 10 min. An exotherm was observed during the addition (ΔT= +3 °C; from 20 to 23 °C). The solution was heated to 38 °C (T_{int}) for 2.5 h (99.8% HPLC conversion) and then cooled to 5 °C under nitrogen flux and stirred overnight at this temperature.

Dichloromethane (3.0 L) was distilled under reduced pressure (p = 400−500 mbar, T_{int} = 20−30 °C, T_{jacket} = 45−80 °C). The organic mixture was basified with aqueous NaOH 15 wt % until pH = 13, maintaining the temperature at 10−20 °C during the addition of the base. The organic layer was washed with water (3 × 3.0 L). The organic solvent was removed under reduced pressure (p = 400−500 mbar, T_{int} = 20−30 °C, T_{jacket} = 45−80 °C), and the residual was rinsed with MTBE (2.5 L). The suspension was cooled to 10 °C for 1 h and filtered on a Buchner funnel. The isolated product was dried in the vacuum oven (p = 20 mbar, T_{oven} = 25 °C) until constant weight in order to obtain 320 g of **19**. The mother liquors were concentrated under reduced pressure (p = 300−400 mbar, T_{int} = 20−45 °C, T_{jacket} = 45−70 °C; 1.0 L of distillate recovered), affording a suspension which was stirred at 10 °C for 1 h and then filtered on a Buchner funnel to give a second crop. The isolated product was dried in the vacuum oven (p = 20 mbar, T_{oven} = 25 °C) until constant weight in order to obtain 135 g of **19**. Yield (combined): 81%.

UPLC−MS: t_R = 0.92 min, m/z = 415 $[M + 1]^+$.

HRMS calcd for $C_{22}H_{31}N_4O_4$ $[M + 1]^+$ 415.23456, found 415.23455.

HPLC: t_R = 3.41 min, purity 98.6%.

^{1}H NMR (400 MHz DMSO-d_6): δ 7.47 (d, J = 2.6 Hz, 1H), 7.41−7.35 (m, 2H), 3.82 (m, 5H), 3.48 (s, 3H), 3.34 (m, 4H), 2.62−2.45 (m, 5H), 1.75−1.60 (m, 5H), 1.28−1.04 (m, 4H).

^{13}C NMR (100 MHz DMSO-d_6): δ 173.7, 161.8, 155.4, 150.8, 135.3, 124.0, 117.0, 116.0, 109.9, 56.2, 47.4, 46.8, 46.2, 42.7, 36.4, 31.4, 30.1, 29.3.

Synthesis of 6-Methoxy-3-{4-[4-(2-methoxy-acetyl)-piperazine-1-carbonyl]-cyclohexylmethyl}-1-methyl-quinazoline-2,4-dione (1). To a solution of 6-Methoxy-1-methyl-3-[4-(piperazine-1-carbonyl)-cyclohexyl-methyl]-quinazoline-2,4-dione **19** (430 g, 1.0 mol, 1 equiv) in dichloromethane (2.0 L) mechanically stirred in a 10 L jacketed reactor, triethylamine (172 mL, 1.2 mol, 1.2 equiv) was added all at once at 18 °C under nitrogen flux. A slight exotherm was observed after the addition (ΔT= +1 °C; from 18 to 19 °C). Then a solution of 2-methoxyacetyl chloride (120 g, 1.1 mol, 1.1 equiv) in dichloromethane (0.8 L) was added over 15 min, observing an exotherm with ΔT= +10 °C (from 18 to 28 °C). The mixture was stirred at 20 °C for 1 h when HPLC indicated complete conversion.

The reaction mixture was washed with water (1 × 1.5 L), aqueous $NaHCO_3$ 2.5 wt % (1 × 2.0 L) and water (2 × 1.5 L). Part of the organic solvent (2.0 L) was distilled under reduced pressure (T_{int}= 20 °C, T_{jacket} = 70 °C, p = 400 mbar), and EtOAc (2.0 L) was added, resulting in a precipitation of a white solid. The suspension was stirred at 0 °C for 18 h, filtered on a Buchner funnel, and then washed with EtOAc (1 × 1.0 L). The isolated product was dried in the vacuum oven (T_{oven}= 25 °C, p = 20 mbar) until constant weight: 460 g of SEN461 **1** as an off-white solid was obtained. Yield: 90%.

UPLC−MS: t_R= 1.10 min, m/z = 415 $[M + 1]^+$.

HRMS calcd for $C_{25}H_{35}N_4O_6$ $[M + 1]^+$ 487.25569, found 487.25570.

HPLC: t_R= 5.34 min, purity 99.7%.

^{1}H NMR (400 MHz $CDCl_3$): δ 7.66 (d, J = 3.0, 1H), 7.27 (dd, J = 9.1 Hz, 3.0 Hz, 1H), 7.15 (d, J = 9.1, 1H), 4.11 (s, 2H), 3.93 (d, J = 7.2 Hz, 2H), 3.89 (s, 3H), 3.60−3.42 (m, 11H), 3.41 (s, 3H), 2.43 (m, 1H), 1.92 (m, 1H), 1.82−1.76 (m, 4H), 1.53 (m, 2H), 1.17 (m, 2H).

^{13}C NMR (100 MHz DMSO-d_6): δ 174.2, 168.0, 161.8, 155.4, 150.8, 135.3, 124.0, 117.0, 116.0, 109.9, 71.2, 59.0, 56.3, 47.4, 45.3, 44.7, 42.2, 36.4, 31.4, 30.1, 29.2.

M.p. (DSC): 164 °C.

Water content (KF): 1.7 wt %.

Pd (ICP-MS): 5 ppm.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H NMR and ^{13}C NMR spectra. Experimental procedures for the preparation of most compounds in plant scale. Copies of analytical data of SEN461 process impurity identification. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the many operators and support personnel at Aptuit Verona. We especially recognize Arianna Ribecai, Angelo Manzo, Federico Scaravelli for their support in process development; Simone Battaggia, Simone Guelfi, Francesco Tinazzi for their work in the pilot-plant campaign; Yves Saint-Denis for managing multiple aspects of the research timeline with the business timelines; Stefano Provera for his NMR studies and interpretation. We also gratefully acknowledge Arianna Nencini and Maurizio Varrone for the early medicinal chemistry work in Siena Biotech.

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(9) Aldrich; 25 g at €154/g; 500 g at €2.90/g.
(10) Direct coupling of benzoic acid **14** with amino acid **4** would cut the current synthesis by two steps and was therefore investigated. While activation of **14** as acyl chloride was complete, subsequent addition of amino acid **4** gave only very poor conversion.
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(16) SEN461 was nominated for clinical evaluation, and the manufacturing of a GMP batch was outsourced. The process development studies and the manufacturing campaign were carried out within Aptuit API Development Manufacturing facilities based in Verona, Italy.
(17) The experiments were run in a RC1 Reaction Calorimeter, Mettler Toledo.
(18) The activation step was monitored by HPLC (at 220 nm) previously adding benzylamine to the reaction sample and having an indication of the residual oxalyl chloride by observing the UV−visible product obtained.
(19) The possibility of introducing a purification step at the beginning of stage 7 was considered with the aim of avoiding contamination by inorganics and potential risks in the pilot-plant campaign. Even if the rework procedure was put in place and tested, it was not used since a wash from inorganics was not required.
(20) The permeation rate during washing was improved using a DCM/MTBE mixture instead of pure MTBE. For further details see the Supporting Information.
(21) We explain the dimer formation as a consequence of a double nucleophilic attack of the starting amine **19** and the methylene bridge position of the 2-methylacetyl moiety of **1** to DCM in basic conditions. For further details see the Supporting Information.