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Development of a Scalable Synthetic Route to GSK369796 (*N-tert*-Butyl Isoquine), a Novel 4-Aminoquinoline Antimalarial Drug

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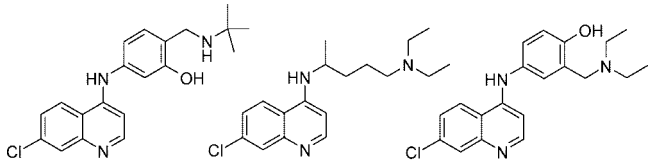
Abstract:

An improved process to the novel 4-aminoquinoline antimalarial GSK369796 is described. Although the initial synthetic route consisted of only two steps from readily available starting materials, the product isolated via the key Mannich reaction was hampered by both low yields and low purity. In addition to instability under the reaction conditions used for the Mannich reaction, the drug substance was found to decompose during attempts to purify by recrystallisation. Reaction conditions were developed that resolved these issues, culminating in the successful production of multi-kg quantities of GSK369796 in >98% a/a purity and in 57% overall yield.

Introduction

*N-t*ert-Butyl isoquine **1** is a member of the 4-aminoquinoline class of compounds, which include chloroquine **2** and amodiaquine **3**.[1,2] These compounds have been successfully used for the treatment of malaria for many years but have associated problems. Parasite resistance has developed to chloroquine,[3,4] and there is idiosyncratic toxicity associated with amodiaquine.[5]



GSK369796
1

2

3

GSK369796 has been selected from the isoquine series of compounds developed at University of Liverpool to remove the potential for metabolism-dependent drug toxicity seen with amodiaquine. GSK369796 (**1**) retains low nanomolar activity against chloroquine-resistant *Plasmodium falciparum* strains and appears to be resistant to metabolism to chemically reactive metabolites responsible for amodiaquine toxicity. In addition to excellent in vivo efficacy, extensive characterisation of GSK369796 has identified this molecule as having the best DMPK and developability characteristics of the isoquine series analysed.[6]

GSK369796 is being developed in a public private partnership between GSK, Medicines for Malaria Venture (MMV), and the University of Liverpool. In line with current best practice for the treatment of uncomplicated *P. falciparum* malaria, GSK369796 will be developed as a fixed dose combination with another antimalarial agent.[7] This combination strategy is advocated by disease experts to reduce or prevent the development of resistance to new antimalarial agents.[8] In this paper we describe the optimisation of the chemistry for the synthesis of *N-tert*-butyl isoquine.

Results and Discussion

The original route is depicted in Scheme 1 and involves a two-step synthesis from commercially available 3-hydroxy acetanilide **4**. Step 1 involved Mannich reaction of **4** with *tert*-butylamine/aqueous formaldehyde in refluxing ethanol and produced the desired product **5a** in low to moderate yields.[6] The major drawback with this procedure was the formation of bis-Mannich addition products that proved difficult to remove by chromatography on silica gel and the requirement for repeated chromatography to produce gram quantities of analytical material. Step 2 involved hydrolysis of the amide **5a** and coupling of the intermediate 3-aminophenol with 4,7-dichloroquinoline. This step also required modification over the corresponding route to amodiaquine since we found that the 3-aminophenol intermediate was unstable at neutral to basic pH. However, the 3-aminophenol intermediate is sufficiently nucleophilic to couple with 4,7-dichloroquinoline at lower pH, that is, after hydrolysis of the amide, the sequential reaction can be carried out in ethanol by addition of 4,7-dichloroquinoline. For purification, analogues were chromatographed on silica as their hydrochloride salts using methanol/dichloromethane (10–20% MeOH/dichloromethane) as eluent. The free bases could be conveniently obtained by dissolving pure columned solid hydrochloride product in distilled water and adding saturated sodium bicarbonate solution with filtration of the precipitated free base. Although this method provided the parent drug

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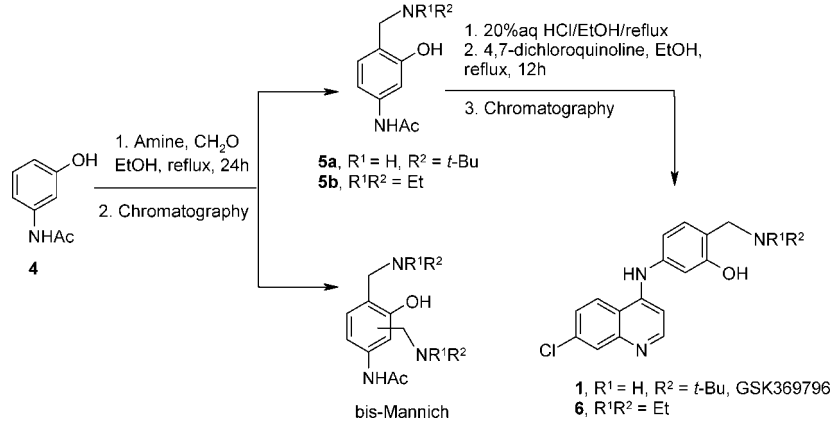
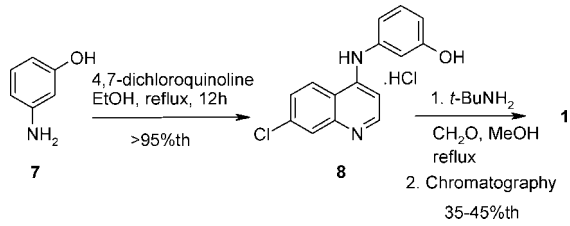
Scheme 1. **Original route to GSK369796**



Scheme 2. **Improved route to GSK369796**



isoquine **6** from amide **5b** in analytically pure form, the *N-tert* butyl derivative **1** required further purification by chromatography.

To improve the yield and reduce the number of chromatography steps, we decided to reverse the order of reactions; thus 4-aminophenol was coupled first with 4,7-dichloroquinoline to provide intermediate **8**, which was then allowed to react with *tert*-butylamine in the second stage of the process (Scheme 2).

Burckhalter, as an alternative method for the synthesis of amodiaquine, employed a similar reverse strategy that provided amodiaquine in 80% yield.[9] In terms of yield, this was to be a major improvement as the product **8** proved to be insoluble in cold ethanol and could be filtered and washed to give analytically pure material (monohydrochloride) without the need for chromatography.

Step 2 involved the Mannich reaction, and on a laboratory scale this procedure provided **1** in moderate yield and purity following chromatography. However, laboratory scale-up of step 2 prior to the manufacture of multi-kg quantities of **1** resulted in low isolated yields (25–35% th) and purity (75–85% a/a) of **1**. These reactions were hampered by a laborious pH adjusted aqueous work-up that included several filtrations to remove unreacted starting material **8**. The formation of the bis-Mannich addition products was also observed. Attempted purification of the crude product by recrystallisation at elevated temperature in methanol or ethanol also resulted in further decomposition. From these results, it was clear that significant improvements to the Mannich reaction at step 2 and subsequent isolation of **1** would be required in order to enhance the efficiency of the process and this is the subject of this publication.

Prior to the optimization phase, we carried out a stability screen of **1**, as the free base, in a variety of solvents and at elevated temperature. The results of this screen are depicted in Figure 1.

It was clear from this stability data that an alternative solvent to methanol was required for the Mannich step and ideally, the reaction should be carried out at a lower temperature. We decided to test 2-propanol, 2-butanol, and mixtures of 2-propanol/NMP 1:1 and 2-propanol/25% DMF. The solvent mixtures gave only very slow conversion after 24 h at 40 °C with only 20% a/a product by HPLC. Running the reaction in either 2-propanol or 2-butanol at the same temperature gave more promising results. A DoE factor screen also confirmed that high dilution conditions (35 vol of solvent), low reaction temperature (≤40 °C), and high equivalents of Schiff base (5 equiv) were beneficial to maximising the solution yield of product (Figure 2).

A verification reaction carried out in 2-propanol on a 10-g scale showed that after 4 h at T_i = 40 °C HPLC indicated a conversion of 75% a/a along with 22% a/a bis-Mannich products **9** and **10** combined and 1% a/a starting material **8**. Leaving the reaction mixture overnight resulted in precipitation of the benzoxazine **11** (Figure 3). The benzoxazine **11** could also be isolated directly from the reaction mixture by addition of water followed by filtration of the precipitated product in 58–63% th yield and in high purity. The lability of this molecule under acidic conditions was underlined by the fact that attempted purity determination by HPLC (eluent contained TFA) resulted in a peak coinciding with the desired product **1** and with the same mass by HPLC-MS (electrospray). A direct injection of a sample of **11** into the LC-MS system (bypassing the HPLC) revealed the mass 367, which corresponded to the benzoxazine. Additional ^{13}C NMR measurement confirmed this picture. The formation of the benzoxazine is presumably due to the presence of excess imine in the Mannich reaction.[10,11]

Interestingly, when the same reaction was repeated using 2-butanol as solvent, a similar reaction profile was observed but without the precipitation of the benzoxazine. Quenching the reaction mixture directly with 5 M HCl solution gave a yellow suspension, which was filtered, affording **1** as the dihydrochloride salt and as a yellow, free-flowing powder (overall yield of 46% with a purity of 99.9% a/a by HPLC) (Scheme 3). This result was a significant improvement over the original methanol process, which for comparison purposes

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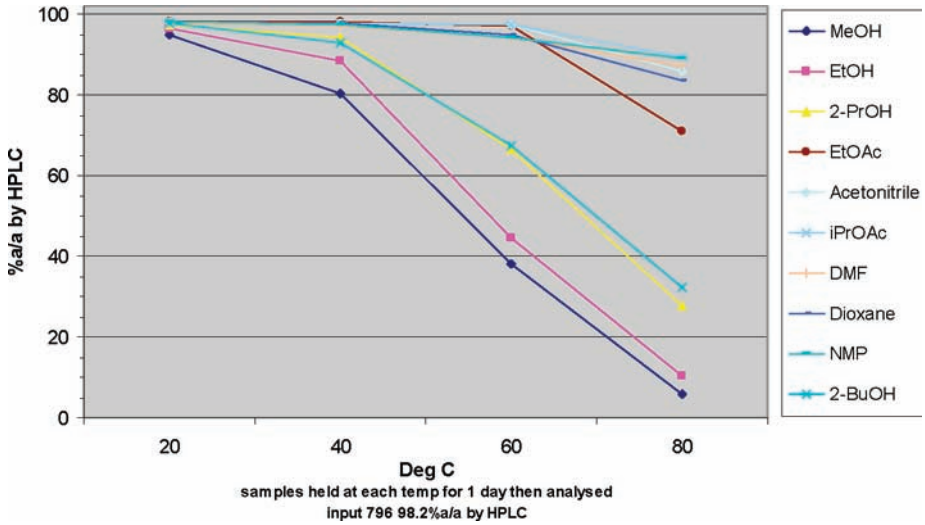


Figure 1. **Purity versus temperature plot for GSK369796 free base in solvents.**

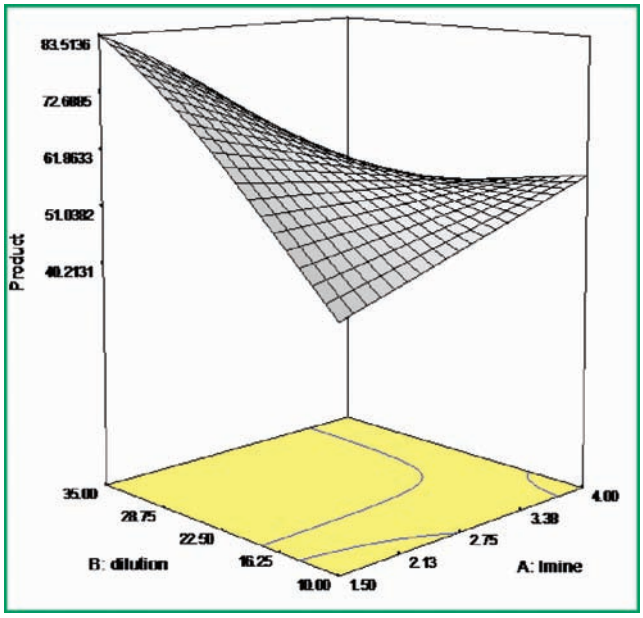


Figure 2. **Impact of dilution and imine equivalents on solution yield of 1 at 40 °C in 2-propanol.**

gave 9% a/a bis-Mannich, 49% a/a product, and 42% a/a starting material **8** during in-process monitoring.

During our optimisation of the Mannich step, we found it was critical to push the reaction to completion, to ensure only trace amounts of starting material **8** remained even if levels of **9** and **10** increased relative to product. The bis-Mannich impurities were highly soluble in alcohols, and the starting material was not, such that coprecipitation of unreacted **8** was the principle reason for the elaborate work-up procedures employed during the early phase of process development. The bis-Mannich impurities were simply retained in the mother liquors after the filtration of the product.

To further streamline the process, we investigated the preparation of the "neat" Schiff base. In a first run to establish a protocol for the neat Schiff base preparation, 1.0 equiv of *tert*-butyl amine was added over 0.5 h at 20–30 °C to 1.05 equiv of paraformaldehyde to give a white suspension. After 3 h of stirring, a colourless biphasic system was obtained. The layer containing the Schiff base was filtered over $MgSO_4$ and stored as crude material in 87% yield. The proton NMR showed a monomer to trimer ratio of 1:3, which was also confirmed by GC analysis. The neat Schiff base was subsequently produced in the pilot plant on a 35-kg scale (Scheme 4).

Following on from further solid-state studies carried out at GSK, the decision was taken to isolate **1** as the dihydrochloride salt (and not the free base). In addition, it was known that the di-HCl salt existed in two polymorphic forms (form I, the kinetically formed polymorph, and form II, the thermodynamic product). Form II was selected for development, so ideally we wanted to be able to isolate this form directly from step 2. This was achieved by seeding the reaction mixture with authentic form II directly after the quench with 5 M HCl solution and stirring overnight at 10 °C.

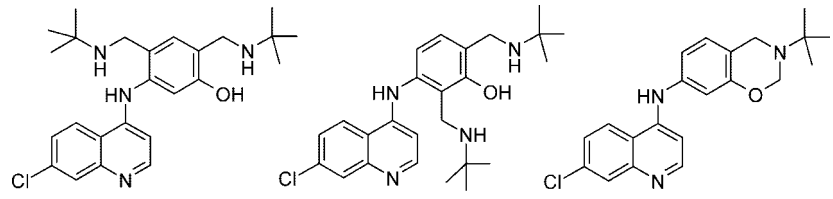
The development activities described above resulted in the successful implementation of step 2 on scale-up in the pilot plant. Two batches at step 2 were performed on 12.5-kg and 13.5-kg inputs of **8**, with 59% yield obtained for both batches; purity was 98.2−98.3% a/a, 0.4−0.5% a/a of residual **8**, and 1.1% a/a bis-Mannich products. A portion of one batch was recrystallised further from 2-propanol/water to provide 6.7 kg of **1** for future clinical studies.

Conclusions

GSK369796 dihydrochloride salt **1** was prepared in 57% th overall yield from 4,7-dichloroquinoline. Significant development activities on the step 2 Mannich reaction allowed the successful scale-up to prepare multi-kg quantities of **1** to support further preclinical and clinical studies.

Experimental Section

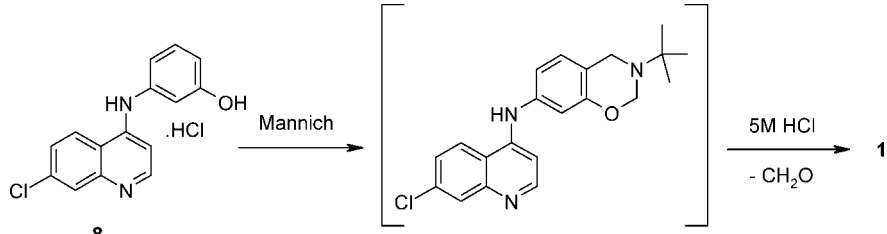
3-[(7-Chloro-4-quinolinyl)amino]phenol Hydrochloride Salt (8). 3-Aminophenol (**7**, 2.45 kg, 0.1 equiv) and 4,7-dichloroquinoline (49.5 kg, 1.1 equiv) were dissolved in EtOH (340 L) at 25 °C. The reaction mixture was heated to 60 °C over a period of 1 h, giving a smooth yellow suspension. The suspension was further heated to 75 °C over 15 min. To the suspension was added via a feeding tank a solution of the remainder of the 3-aminophenol (**7**, 22.19 kg, 0.9 equiv) in EtOH (140 L, the solution was prepared in a stirring vessel)



9 **10** **11**

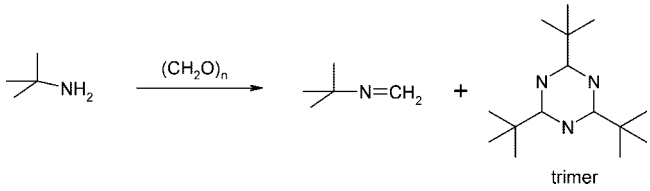
Figure 3. **bis-Mannich and benzoxazine impurities.**

Scheme 3. **Preparation of 1 via benzoxazine 11**



8 **11**

Scheme 4. **Structure of imine "trimer"**



over a period of 75 min at 75 °C, affording a thicker suspension. The yellow suspension was stirred at 75 °C for a further 2.5 h, and a sample taken for HPLC analysis indicated a conversion of 100% a/a. The suspension was cooled to 25 °C over 2.5 h and kept for 18 h at 25 °C. The suspension was filtered and washed successively with EtOH (50 L) and DCM (50 L). The yellow solid was then dried in two portions in the conical stirred dryer at 45 °C for 15 h at 20 mbar to give the title compound **8** as a yellow solid (67.12 kg, 97% th, 99.2% a/a purity).

Representative Method for the Preparation of Schiff Base. *tert*-Butylamine (35.1 kg, 1.0 eqiv) was loaded into the 630-L reactor at 20 °C. Paraformaldehyde (15.14 kg, 1.05 equiv) was added to the liquid in four portions over a period of 2.5 h at 20–35 °C (reaction is exothermic). The resultant white suspension was stirred for a further 3 h at 25 °C, affording a slightly turbid biphasic mixture. This was cooled to 20 °C, and the Schiff base (top layer) was separated in the feeding tank and then filtered over $MgSO_4$ (8.7 kg) in a glass nutsch (filtration time 3.5 h).

The neat Schiff base was stored at room temperature prior to use in the Mannich reaction (36.8 kg, 90% th, 91% a/a purity by GC).

***N*-(7-Chloro-4-quinolinyl)-3-(1,1-dimethylethyl)-3,4-dihydro-2*H*-1,3-benzoxazin-7-amine (11).** 3-[(7-Chloro-4-quinolinyl)amino]phenol hydrochloride salt (**8**, 13.1 kg, 1 equiv) was suspended in 2-propanol (460 L) at 25 °C. The yellow suspension was heated to 40 °C. To the suspension was added via the feeding tank the Schiff base (5 equiv) over a period of 10 min, affording a clear yellow solution. The solution was stirred over a period of 3 h at 40 °C. The reaction was cooled over 1 h to 10 °C, and the resultant suspension was kept at 10 °C for 0.5 h, filtered, and washed successively with water (2 × 26 L) and 2-propanol (2 × 26 L). The off-white solid was then dried under reduced pressure at a bath temperature of 45 °C on the rotary evaporator to give the title compound **11** as an off-white solid (4.58 kg, 30% th, purity 98.9% a/a).

5-[(7-Chloro-4-quinolinyl)amino]-2-{[(1,1-dimethylethyl)-amino]methyl}phenol Dihydrochloride Salt (GSK369796) (1). 3-[(7-Chloro-4-quinolinyl)amino]phenol hydrochloride salt (**8**, 12.5 kg, 1 equiv) was suspended in 2-butanol (437 L) at 20 °C. The yellow suspension was heated to 40 °C. Neat Schiff base (17.5 kg, 5.0 equiv) was added via the feeding tank to the suspension over a period of 10 min, affording a clear yellow solution. The solution was stirred for an additional 3 h at 40 °C, and a sample was withdrawn and analysed by HPLC (70% a/a GSK369796, 27% a/a bis-Mannich products, and 3% a/a residual starting material **8**). The reaction was quenched with 5 M HCl solution (5.8 equiv) added over 15 min to give a clear yellow solution (pH determined 0.80 via pH electrode). Seeds of authentic **1** (5.6 g, form II) suspended in 2-butanol (1.2 L) were added at 40 °C, affording a fine suspension. The suspension was cooled over a period of 110 min to 10 °C and then aged at 10 °C for an additional 16.5 h. The product was filtered and washed with 2-butanol/water 9:1 (3 × 25 L). The yellow solid was dried under reduced pressure at a bath temperature of 45 °C on the rotary evaporator to give GSK369796 dihydrochloride salt **1** as a yellow solid (10.38 kg, 59% th, purity 98.22% a/a).

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