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Article

New Scalable Synthetic Routes to ELQ-300, ELQ-316, and Other Antiparasitic Quinolones

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ABSTRACT: The endochin-like quinolone (ELQ) compound class may yield effective, safe treatments for a range of important human and animal afflictions. However, to access the public health potential of this compound series, a synthetic route needed to be devised, which would lower costs and be amenable to large-scale production. In the new synthetic route described here, a substituted β-keto ester, formed by an Ullmann reaction and subsequent acylation, is reacted with an aniline via a Conrad−Limpach reaction to produce 3-substituted 4(1*H*)-quinolones such as **ELQ-300** and **ELQ-316**. This synthetic route, the first described to be truly amenable to industrial-scale production, is relatively short (five reaction steps), does not require palladium, chromatographic separation, or protecting group chemistry, and may be performed without high vacuum distillation.

KEYWORDS: *antimalarial, antiparasitic, **ELQ-300**, **ELQ-316**, practical synthesis, process development, Conrad−Limpach reaction, Ullmann reaction*

INTRODUCTION

When endochin (Figure 1) was first synthesized in 1940 by Andersag and colleagues, its high activity in the avian malaria model led to the hope that a new and promising class of antimalarials had been discovered. Endochin and a series of derivatives were tested against malaria in humans, but the results were disappointing and the compounds were not pursued further as antimalarials.[1] This compound class was reexamined in the early 2000s, when a series of new endochin derivatives were discovered, which were curative of patent malaria in mice. The outstanding representatives of this new generation of endochin-like quinolones (ELQs) bore a methyl group at position 2, a diphenyl ether substituent at position 3, and additional substituents in the second ring of the quinolone system (Figure 1). These ELQs demonstrated high antimalarial potency in vitro and in vivo, parasite selectivity, chemical and metabolic stability, desirable pharmacokinetics, and low mammalian cell toxicity. In addition to their antimalarial activity, compounds in the series were later found to be highly active against other Apicomplexa, for which satisfactory treatments are urgently needed. These include various *Babesia* species (affecting humans, cattle, horses, and dogs),[2] *Theileria equi* (horses),[2] *Neospora caninum* (cattle, sheep, goats, deer, horses, and dogs),[3] *Besnoitia besnoiti* (cattle),[4,5] and *Toxoplasma gondii* (humans, sheep, goats, cats, and marine mammals).[6−9] Finally, an ELQ compound has been found to have a potent, low-dose inhibitory effect on the nematode *Echinococcus multilocularis*, a fox-transmitted tapeworm that may be fatal to its hosts, including humans.[10]

With favorable properties and broad-spectrum activity, the ELQ compound class may yield effective, safe treatments for a range of important human and animal afflictions. Of these, malaria is a particularly serious and prevalent human disease. In 2019 alone, it afflicted 229 million people worldwide and caused an estimated 409,000 deaths.[11] **ELQ-300**, in the form of a prodrug **ELQ-331**, has recently been accepted as a preclinical candidate by the Medicines for Malaria Venture for potential use in the prevention and treatment of malaria.[12] **ELQ-316** and its prodrugs, on the other hand, have the greatest potency against *T. gondii* and *Babesia microti*. Toxoplasmosis may have infected up to one-third of all humankind; this infection can be serious for immunocompromised individuals and can cause harm to the fetus when contracted during pregnancy.[13,14] Babesiosis affects both humans and livestock and is, together with neosporosis and besnoitiosis, a significant problem for livestock husbandry.[2−4]

If compounds in this series are to become practical drug candidates, a synthetic route is needed that is amenable to industrial-scale production. Historically, 3-alkyl-substituted 4(1*H*)-quinolones, such as endochin, have been synthesized by the reaction of a substituted β-keto ester with an aniline via the Conrad−Limpach reaction (Scheme 1).[15,16] Endochin itself is easily synthesized by the Conrad−Limpach reaction, a sequence of two reactions. In the first stage, 2-*n*-heptylacetoacetic ester is condensed with *meta*-anisidine to form β-anilinocrotonate; in the second stage, thermal cyclization

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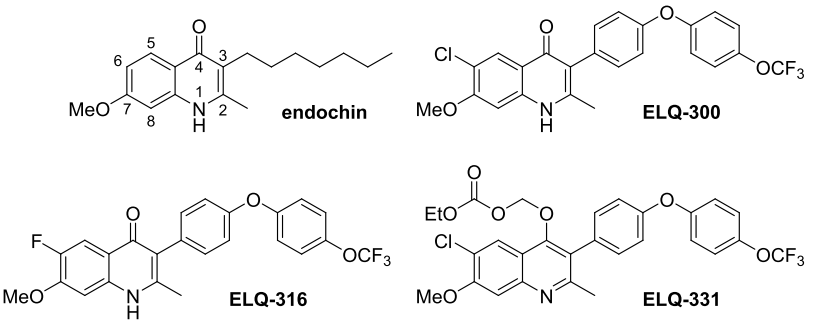
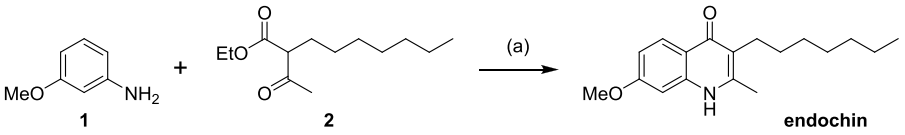


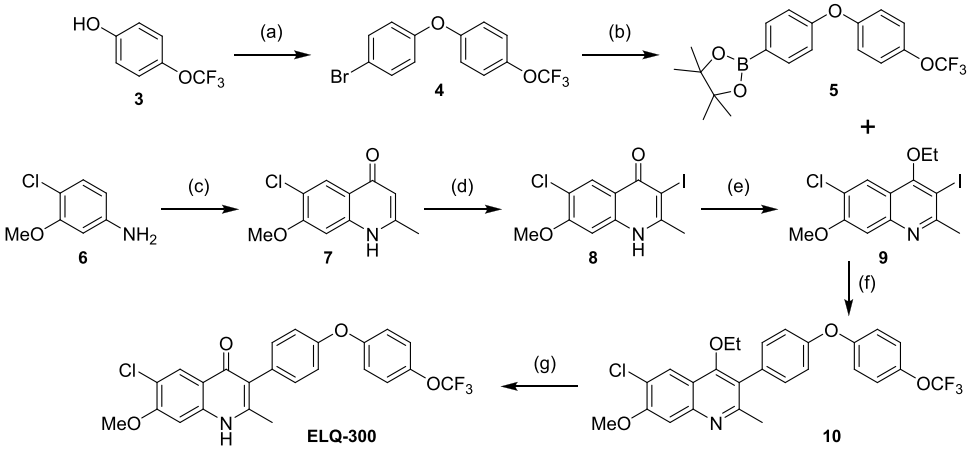
Figure 1. Structures of endochin, **ELQ-300**, **ELQ-316**, and **ELQ-331**.

Scheme 1. Original Synthesis of Endochin[a]



[a]Reaction (a), 1: catalytic H^+ and benzene, reflux; 2: Dowtherm A, 250 °C, 40−50% yield.

Scheme 2. Optimized Original Synthesis of ELQ-300[a]



[a]Reaction (a): 1,4-Dibromobenzene, CuCl, K_2CO_3, DMG, and DMF, 160 °C, 90 min, 60−80%; (b): $Pd(dppf)Cl_2$, bis(pinacolato)diboron, KOAc, and DMF, 90 °C, 98%; (c), 1: ethyl acetoacetate, cat. *p*-TsOH, and PhH, reflux, and 2: Dowtherm A, 250 °C, 68%; (d): I_2, $NaHCO_3$, and MeOH, 96%; (e): EtI, K_2CO_3, and DMF, 81%; (f): $Pd(dppf)Cl_2$, aqueous K_2CO_3, and DMF, 90 °C, 70%; (g): aqueous HBr and AcOH, 90 °C, 95%; for synthetic details, see Supporting Information.

produces endochin, usually in Dowtherm A boiling at 250 °C. Of the two isomers formed in the second step, endochin, the 7-methoxy isomer, crystallizes out of the reaction mixture upon cooling, while the 5-methoxy isomer remains dissolved. The yield of endochin is around 40−50% with this straightforward procedure.[1,17,18] Alternative cyclization methods for 4(1*H*)-quinolones have also been explored.[19−21]

3-Aryl-substituted 4(1*H*)-quinolones, such as **ELQ-300**, have been synthesized by the reaction of 4(1*H*)-quinolone with a reactive aryl moiety primarily via a Suzuki−Miyaura reaction.[22−24] However, 4(1*H*)-quinolones are known to be sparingly soluble in organic solvents and water and are difficult to isolate by chromatography. As a result, reactions that produce a mixture of 3-aryl-4(1*H*)-quinolones have proven difficult to separate and generally give poor yields of pure 4(1*H*)-quinolone products.

The original route to synthesize **ELQ-300**, involving in parallel the formation of 4(1*H*)-quinolone **7** via a Conrad−Limpach reaction (Scheme 2, reaction c) and the formation of a diaryl ether side chain **4** via an Ullmann reaction (reaction a), was designed to allow for late-stage structural variation at the 3-position using a Suzuki−Miyaura reaction (reaction f). The synthesis presented in Scheme 2 is a version of the originally published synthesis that was optimized for a larger scale, the details of which can be found in the Supporting Information. We discovered that conversion of 3-halo-4(1*H*)-quinolone **8** to the corresponding 4-ethoxy-3-halo-quinoline **9** provided a protected 4(1*H*)-quinolone intermediate that performed well in a Suzuki−Miyaura reaction and provided a product (**10**) that could be isolated by chromatography and then readily converted back to the desired 4(1*H*)-quinolone, such as **ELQ-300**.[6,25−27] However, though this route has been used to prepare hundreds of grams of various ELQ compounds, it is not ideal for industrial-scale production because it is relatively long (seven reaction steps), requires the use of a somewhat expensive palladium catalyst, and involves high vacuum distillation (compound **4**), at least two chromatographic

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

separations (compounds **5** and **10**), and multiple recrystallizations.

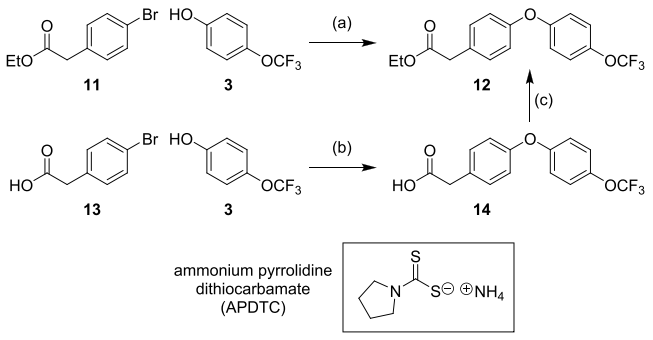
Additional complications arose when performing large-scale versions of the reactions shown in Scheme 2. The original iodination reaction (iodine, potassium iodide and *n*-butylamine in DMF) did not scale well, so it was replaced with reaction (d) that required at least 72 h and did not progress much beyond 96% completion. During the formation of the 4-*O*-ethyl ether (reaction e), *N*-ethylation occurred, resulting in a small amount of an *N*-ethylated side product that was difficult to separate. Unwanted reduction of the 3-iodo group during the Suzuki reaction (f) resulted in a small amount of reduced side product that was also difficult to separate by chromatography and was inseparable after the quinolone was re-formed in reaction (g). Finally, removal of the 4-*O*-ethyl ether protecting group (reaction g) required relatively harsh reaction conditions (HBr in acetic acid (AcOH) at 90 °C) and long reaction times (>48 h), which can result in demethylation of the 7-OMe ether.

As part of our effort to establish a synthetic route to sythesize **ELQ-300** which would be amenable to industrial-scale preparation, we returned to Andersag's original method by which 3-substituted 4(1*H*)-quinolones were prepared from substituted *β*-keto esters using the Conrad−Limpach reaction as the final step. Recently, the synthesis of 3-diaryl ether 4(1*H*)-quinolones via substituted *β*-keto esters has been revisited.[28,29] However, these routes were not shown to be amenable to industrial-scale production. Our optimized approach, presented here, is relatively short (five reaction steps), does not require palladium, involves no chromatographic separation, and avoids high vacuum distillation. Additionally, it requires no protecting group chemistry because the poorly soluble 4(1*H*)-quinolone is not formed until the final reaction step.

RESULTS AND DISCUSSION

The first step of the synthesis of diaryl ether **12** is an Ullmann reaction.[30] The copper-mediated coupling of 4-trifluoromethoxyphenol **3** and ethyl 2-(4-bromophenyl)acetate **11** with copper(I) chloride (CuCl), *N*,*N*-dimethylglycine (DMG) as a copper(I) chloride chelator, and potassium carbonate (K_2CO_3) in DMF at 160 °C for 90 min afforded the diaryl ether **12** (Scheme 3).[31,32] The reaction was monitored by GC−MS and was determined to be complete when **11** was consumed. The product **12** was isolated with a yield of 48−60% by high vacuum distillation, a method that facilitated the nearly complete removal of copper. The copper content of **12** using these conditions was <21 ppm, determined by inductively coupled plasma−atomic emission spectroscopy (ICP-AES). Other copper couplings were explored, but they required harsh conditions (e.g., copper metal) or were not scalable (e.g., copper(II) acetate).[33] The copper(II) acetate reaction (not shown) used in the original ELQ synthetic method[25,26] did not scale well because it was difficult to maintain a dry, well-oxygenated, homogenous copper(II) acetate mixture under bulk conditions.

Scheme 3. Two Alternate routes for the Ullmann Synthesis of Ethyl 2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)acetate 12[a]



[a]Reaction (a): CuCl, K_2CO_3, DMG, and DMF, 160 °C, 90 min, 48−60% yield; (b), 1: CuCl, K_2CO_3, DMG, and DMF, 160 °C, 3.5 h, and 2: APDTC, 78%; (c) catalytic HCl and EtOH, 18 h, 91%.

In order to avoid high vacuum distillation, which may be problematic on an industrial scale, we attempted to find an alternative method for the preparation of diaryl ether **12**. By substituting the corresponding carboxylic acid **13** for the ester **11** in the Ullmann reaction, we obtained a solid intermediate, diaryl ether **14**, which could be purified without distillation (Scheme 3, reaction b): 4-bromophenylacetic acid **13** was allowed to react with 4-trifluoromethoxyphenol **3** in the presence of a catalytic amount of CuCl, DMG, and K_2CO_3 in DMF at 160 °C. After 3.5 h, **13** was completely consumed as determined by GC−MS. During the workup, the copper catalyst was removed by the addition of ammonium pyrrolidinedithiocarbamate (APDTC) according to the procedure described by Gallagher and Vo.[34] The solid carboxylic acid **14** was easily purified by treatment with hot water to remove most of the primary impurity (starting material, phenol **3**) and water-soluble residues, and was obtained in 78% yield with >95% purity by GC−MS and ^{1}H-NMR. Esterification of **14** with ethanol in the presence of catalytic hydrochloric acid over 18 h afforded the desired diaryl ether **12** in 91% yield after removal of ethanol in vacuo and passage of the crude product through a silica gel plug, which was rinsed with 3:1 hexanes/ethyl acetate. The product **12** was at least 95% pure as observed by GC−MS and ^{1}H-NMR and was suitable for use in the next step without further purification. The overall yield of this two-step process was 71%. The successful preparation of the solid intermediate **14** and its conversion to the high-purity key intermediate **12** show that this ELQ compound synthesis can be performed without high vacuum distillation.

It was important to verify that the copper content of **12** obtained via the carboxylic acid route (from **13**) was as low as the copper content of **12** obtained via the ester route (from **11**). By an ICP-AES analysis, it was determined that the copper content of **14** after treatment with APDTC was 73 ppm. After esterification and passage through a silica gel plug, the copper content of **12** was <12 ppm. Thus, the copper content of **12** obtained from **13** was no higher than the copper content of **12** (<21 ppm) obtained from **11** after high vacuum distillation.

The synthesis of diaryl ether **12** using hypervalent iodine was also explored as a possible alternative approach to the synthesis of **ELQ-300**. This approach would avoid the use of copper in the synthetic sequence, as iodine(III) mediates the formation of the diaryl ether C−O bond. A similar strategy has been used in previous syntheses of **ELQ-300** with symmetrical diaryliodonium salts.[28,29] In our case, we considered the use of an unsymmetrical diaryliodonium salt in which a relatively inexpensive aryl auxiliary replaces half of the desired aryl group, which would otherwise go to the waste stream, in the

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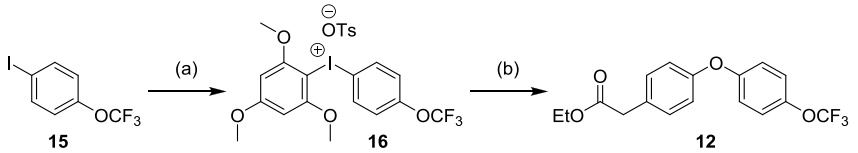
Scheme 4. Hypervalent Iodine Synthesis of Ethyl 2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)acetate 12[a]

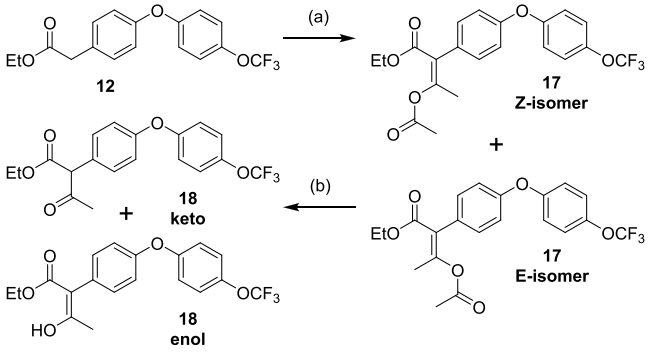


[a]Reaction (a), 1: *meta*-Chloroperoxybenzoic acid, *para*-toluenesulfonic acid, and acetonitrile, 65 °C, 40 min, and 2: 1,3,5-trimethoxybenzene, 5 min, 84% yield; (b): ethyl 2-(4-hydroxyphenyl)acetate, K_2CO_3, and toluene, 55 °C, 2.5 h, 54% (0.5 mmol scale), 43% (3.6 mmol scale), and 48% (10 mmol scale).

coupling with an appropriate phenol. Aryl(TMP)iodonium salts (TMP = 2,4,6-trimethoxyphenyl) were a logical choice given their established chemoselective aryl transfer,[35] scalable synthesis,[36] and use in C−O coupling,[37] and the low cost of the auxiliary precursor, 1,3,5-trimethoxybenzene. Moreover, process safety for the synthesis of aryl(TMP)iodonium salts has been evaluated, including thermal stability of intermediates, and the synthesis was deemed safe within the operating temperature window.[32] Based on the cost and availability of starting materials, aryl iodide **15** was selected as one of the coupling partners (Scheme 4). Large-scale oxidation of **15** proceeded smoothly under slightly modified literature conditions to deliver >200 g of unsymmetrical diaryliodonium salt **16** in 84% yield. The reaction of **16** with ethyl 2-(4-hydroxyphenyl)acetate in the presence of potassium carbonate as a base provided access to **12** via metal-free C−O coupling in moderate yield on various scales (43−54%, 48% average). The overall yield of **12** using the iodonium salt route is lower than the copper-catalyzed route (ca. 36−45% compared to 48−71%), and the impurity profile required a column chromatography step that was not required in the copper-catalyzed route. Because of these disadvantages the iodonium route was not pursued further. Additionally, other phenol coupling partners that would provide direct access to **18** or a methyl ketone analog of **12** were considered but led to complex mixtures of products in coupling with **16**.

We next explored the most challenging part of this route, the formation of the key intermediate, the substituted *β*-keto ester **18**. The acylation reaction (Scheme 5) did not proceed using strong bases such as NaH, *n*-butyllithium, lithium diisopropylamide, and commercially prepared lithium hexamethyldisilazide (LiHMDS) solution. We found that the acylation proceeded only when freshly prepared LiHMDS was used to deprotonate **12** at −20 °C. Preliminary attempts to acylate **12** suggested that C-acylation occurs initially followed by rapid O-acylation of the newly introduced acetyl group, producing enol acetate **17**. If the reaction is quenched at −20 °C immediately after addition of acetic anhydride, C-acylated **18**, bis-acylated **17**, and the starting material **12** can be detected by thin-layer chromatography (TLC). This finding suggested that it was necessary to use excess acetic anhydride with freshly prepared LiHMDS in tetrahydrofuran (THF) to force the reaction to consume all of the starting material **12**, thereby forming only the O-acylated *β*-keto ester **17**. Under these conditions, **17** was obtained in quantitative yield and in sufficient purity (>95%) to be used in the next step without further purification. Compound **17** exists as a mixture of equally reactive *E*- and *Z*-isomers, which can be isolated by chromatography. The identity of the *Z*-isomer was determined by 2D NOESY NMR, and the percent of the *Z*-isomer was estimated to be 90−95% using GC−MS and ^{1}H-NMR.

Scheme 5. Synthesis of Ethyl 3-oxo-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl) butanoate, *β*-keto ester 18[a]



[a](a) LiHMDS, Ac_2O, and THF, −20 °C to RT over 16 h, 99%; (b) 10% *p*-TsOH and AcOH, 100 °C, 60−90 min, 99%.

Other acylating reagents were also explored. Ethyl acetate and acetylimidazole have been reported to give 2-phenylacetoacetic esters in one step,[38] and indeed, our model reactions using ethyl 2-(4-bromophenyl)acetate provided direct access to the corresponding *β*-keto ester. Unfortunately, reactions of these reagents with diaryl ether **12** proved to be more complex. In the case of ethyl acetate, we observed no reaction. In the case of acetylimidazole, *β*-keto ester **18** was formed predominantly along with some of the bis-acylated product **17** and some other uncharacterized products as shown by ^{1}H-NMR. Under these conditions, in order to obtain *β*-keto ester **18** in sufficient purity for the next reaction, chromatography would be required. Thus, in our case, we found that acylation with acetylimidazole is less desirable and may not be suitable for large-scale synthesis, especially considering the relative ease of work-up and low cost associated with acetic anhydride.

We found that it is possible to quantitatively convert the bis-acylated **17** to the desired *β*-keto ester **18** using catalytic *para*-toluenesulfonic acid (*p*-TsOH) in AcOH. The *β*-keto ester **18** exists in both keto and enol forms with a keto/enol ratio of approximately 7:3 as determined by ^{1}H-NMR. Since the *β*-keto ester **18** could not be detected by GC−MS, TLC and ^{1}H-NMR were used to assess purity and characterize the product. The isolated *β*-keto ester **18** was sufficiently pure for use in the next reaction and already contained a 10% mole fraction of *p*-TsOH, which is a suitable catalyst for the subsequent acid-catalyzed condensation with anilines.

The target ELQ compounds were prepared from *β*-keto ester **18** using a Conrad−Limpach reaction, which comprises a Schiff base formation followed by high-temperature cyclization (Scheme 6).[15,16] Continuous removal of water using a Dean−Stark trap and a water-carrying solvent afforded the desired Schiff bases **20a−d** via condensation with anilines **19a−d**.

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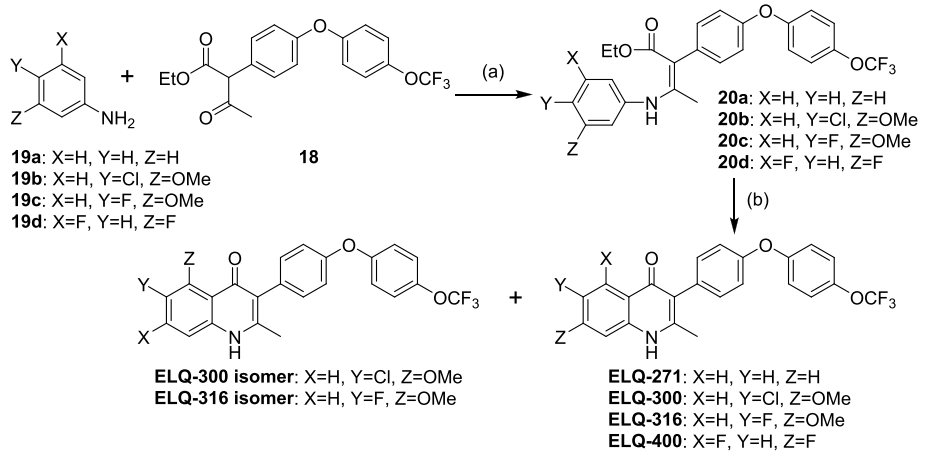
Scheme 6. Synthesis of a Series of ELQ Compounds from β-Keto Ester Intermediate 18[a]



[a](a) 10% *p*-TsOH, cyclohexane, and reflux, 24−72 h; (b) Dowtherm A, 230 or 250 °C, 0.5 h.

Traditionally, benzene has been used for such reactions.[1,17,18] However, since benzene is not suitable for pharmaceutical preparations, cyclohexane (which boils at nearly the same temperature and also forms an azeotrope with water) was used as an alternative. Anilines **19a−d** were allowed to react with β-keto ester **18** in the presence of catalytic *p*-TsOH in refluxing cyclohexane with a Dean−Stark trap to give the imines **20a−d**, which were then used without further purification in the final cyclization step.

The cyclization step of the Conrad−Limpach reaction is classically performed at high temperatures (230 or 250 °C).[39] We compared these two temperatures for a series of ELQ derivatives and investigated the effect of a lower temperature of 200 °C for **ELQ-300** (Table 1). All temperatures investigated led to acceptable yields of products with minimal impurity; however, the reaction at 200 °C led to a somewhat lower yield of **ELQ-300**. Although aniline **19b** was used as the limiting reagent, it was not completely consumed even after extending the reaction time to 72 h. Excess aniline **19b**, being poorly soluble in cyclohexane, was partially recoverable by filtration. To demonstrate the potential scalability of this synthetic route, we performed the reaction using 50.0 g (131 mmol) of β-keto ester **18** and 2.25 g of *p*-TsOH to form the Schiff base **20b**, followed by cyclization at 250 °C. Under these conditions, the reaction proceeded as expected and gave **ELQ-300** in a yield of 57.1%, higher than at the 1−10 g (2−20 mmol) scale. The crude product from this reaction was >99% pure by ^{1}H-NMR and HPLC. At all temperatures investigated, only a negligible amount of the 6-chloro-5-methoxy regioisomer of **ELQ-300** was formed, which was below the limit of detection by ^{1}H-NMR. To allow detection and quantification of the **ELQ-300** regioisomer, the product was converted to its corresponding 4-chloro derivative using phosphorus oxychloride ($POCl_3$) and analyzed by GC−MS. The results confirmed that a negligible amount (<0.5%) of the regioisomer was formed. In this study, when the reactions were performed at a 1−10 g (2−20 mmol) scale, 230 °C appeared to be the optimal cyclization temperature as it gave the highest yield. However, a higher yield was obtained when the reaction was scaled up to 50.0 g (131 mmol) of β-keto ester **18** at 250 °C. It is important to emphasize that since starting materials **11** and **13** may also be acetylated (Scheme 3, reaction b), diaryl ether **12** must be obtained in a pure form without contamination from **11** or **13** in order to afford high purity of the final ELQ products. Other solvents may work in this reaction.[40] Tsoung *et al.* have shown that 4(1*H*)-quinolones can be prepared by performing a Conrad−Limpach reaction in a flow reactor, which indicates that this approach should be amenable to implementation on an industrial scale.[41,42]

As shown in Table 1, the β-keto ester **18** was found to be equally useful as an intermediate for the synthesis of a series of ELQ compounds in addition to **ELQ-300**, including **ELQ-271**, **ELQ-316**, and **ELQ-400**.[25,26,43] In all cases, the condensation of β-keto ester **18** with the corresponding aniline **19a**, **19c**, and **19d**, followed by thermal cyclization, afforded the desired quinolones in relatively good yield. In the case of **ELQ-316**, the reaction proceeded well using 115.2 g of β-keto ester **18** to form the corresponding Schiff base **20c** followed by cyclization at 250 °C. Similar to the case of **ELQ-300**, in the cyclization of **20c** to form **ELQ-316**, only trace formation of the undesired regioisomer was detected by GC−MS of the corresponding 4-chloro derivative. The copper content of this sample was below the limit of detection (<17 ppm), as with **12** obtained in Scheme 3 (<21 ppm), suggesting that removal of copper is not a concern in this reaction sequence.

Table 1. Conrad−Limpach Reaction Conditions for the Synthesis of a Series of ELQ Compounds

ELQ #	amount of **14**	cyclization temperature	yield[a]	purity[b]
ELQ-300	131 mmol	250 °C	57%	>99%
ELQ-300	21.5 mmol[d]	250 °C	43%	>99%[c]
ELQ-300	8.3 mmol[d]	230 °C	48%	>99%
ELQ-300	1.8 mmol[d]	200 °C	30%	>99%
ELQ-316	302 mmol	250 °C	54%	>99%
ELQ-316	13.1 mmol[d]	250 °C	53%	97%
ELQ-316	7.6 mmol[d]	230 °C	50%	98%
ELQ-400	7.8 mmol[d]	250 °C	32%	>99%
ELQ-400	7.3 mmol[d]	230 °C	33%	>99%
ELQ-271	8.1 mmol[d]	250 °C	63%	>99%

[a]Yields are calculated from the originally used **14**, as the intermediate Schiff bases **20a−d** were used without purification or characterization in the subsequent cyclization step. [b]Purity determined by reversed-phase HPLC. [c]Yield and purity of recrystallized **ELQ-300**. [d]Back-calculated from the weight of Schiff bases **20a−d** used.

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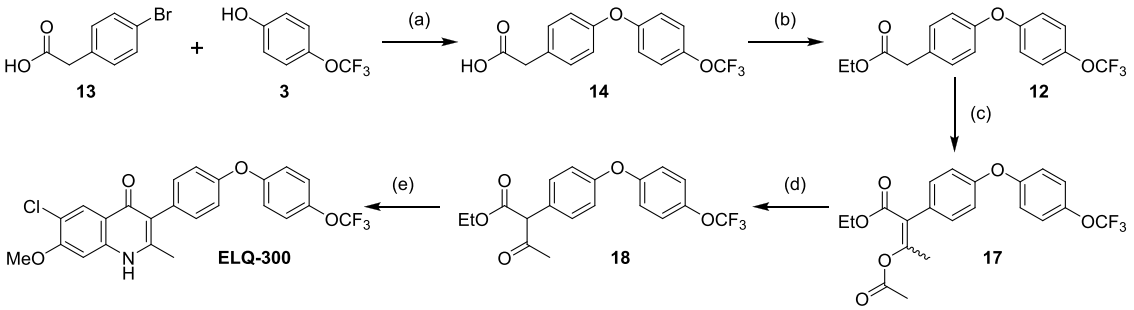
Scheme 7. New Efficient Synthesis of ELQ-300[a]



[a]Reaction (a) 1: CuCl, K_2CO_3, DMG, and DMF, 160 °C, 210 min, and 2: APDTC, 78%; (b) catalytic HCl and EtOH, 91%; (c) LiHMDS, Ac_2O, and THF, −20 °C to RT over 16 h, 100%; (d) 10%, *p*-TsOH and AcOH, 100 °C, 60−90 min, 100%; (e) 1: 10% *p*-TsOH and cyclohexane, reflux, 72 h, and 2: Dowtherm A, 230 or 250 °C, 30 min, 57%

At this early stage in development, it is difficult to obtain an accurate estimate of the cost of goods for the industrial production of **ELQ-300** and **ELQ-316**. However, using **ELQ-300** as an example, it is possible to compare the relative cost of the original synthesis (Scheme 2) to the new efficient synthesis (Scheme 7). On one hand, the original synthesis comprises seven reaction steps and has a 36% yield over its longest linear sequence (five reaction steps), which does not take into account the yield of the two non-linear steps. Additionally, it requires the use of a relatively expensive palladium catalyst, at least two chromatographic separations, and high vacuum distillation. On the other hand, the new efficient synthesis comprises five reaction steps and has a 41% overall yield. It requires no expensive reagents, such as palladium, and no chromatographic separations. Based on length, relative cost of reagents, and simplicity of purification, we estimate the cost of the new efficient synthesis of ELQ compounds to be 10−20% of the cost of the original synthesis.

CONCLUSIONS

We have successfully developed an efficient late-stage cyclization synthetic route to synthesize **ELQ-300** and other structurally related ELQ derivatives (Scheme 7), bypassing the more expensive palladium-catalyzed Suzuki−Miyaura reaction originally developed by our lab to explore the initial structure−activity relationship of quinolone and side chain modifications to the core scaffold (Scheme 2).[25,26] Additionally, we have found an efficient synthesis route to the key intermediate diaryl ether **12** that does not require high vacuum distillation. The overall yield in this two-step synthesis (71%, Scheme 3) is better than the yield of the single-step synthesis of **12** involving high vacuum distillation (48−60%, Scheme 3). The key to the success of this efficient late-stage cyclization scheme is our ability to synthesize the bis-acylated product **17** and then to selectively convert it to the desired intermediate *β*-keto ester **18** using *p*-TsOH as a catalyst. This synthetic route is inexpensive and contains five scalable steps, with purification via a single recrystallization (as needed) and no chromatography or distillation. **ELQ-300** and other ELQ compounds are obtained in a pure form with an overall yield of ca. 30−40%. Further, the steps have not been fully optimized at an industrial scale, and it is believed that improvements may be achieved with the adjustment of reaction conditions (e.g., time and temperature), solvents, and equipment. Compared to the synthetic route to **ELQ-300** and other ELQ compounds originally described by us,[25,26] this late-stage cyclization synthesis appears to be readily scalable and promises to considerably reduce the cost of goods. This is especially important in the case of **ELQ-300** production given that it is the active component of the preclinical candidate antimalarial prodrug, **ELQ-331**, and the fact that many malaria-endemic countries are among the world's most impoverished.

In addition to the advantages described above, this efficient synthetic route avoids the relatively harsh conditions (HBr in AcOH) required to deprotect the 4-*O*-ethyl ether quinoline intermediate in the previously described synthesis (Scheme 2).[25,26] These conditions regularly resulted in the partial demethylation of the 7-OMe ether of both **ELQ-300** and **ELQ-316**. Because the milder conditions of this new route do not result in demethylation of the 7-OMe ether, we are currently exploring using the new route to synthesize quinolone analogs with sensitive functional groups that were not accessible by the original route.

It is also noteworthy that **ELQ-316** can be made efficiently and with high purity and yield using this new late-stage cyclization synthetic pathway. While **ELQ-316** exhibits slightly less antimalarial activity than **ELQ-300** in vivo, it has superior antiparasitic activity against a broader range of Apicomplexan protozoan species including *T. gondii* and *B. microti*, which also cause severe and potentially fatal disease in humans. Recent studies show that both **ELQ-300** and **ELQ-316** are also active against a range of Apicomplexan parasites of importance to veterinary medicine.[2,4,5]

METHODS

Unless otherwise stated, all chemicals and reagents were from Sigma-Aldrich Chemical Company in St. Louis, MO (USA), Combi-Blocks in San Diego (CA), or TCI America, Portland (OR) and were used as received. Melting points were obtained using an Optimelt Automated Melting point system from Stanford Research Systems, Sunnyvale, CA (USA). Analytical TLC utilized Merck 60F-254,250 micrometer precoated silica gel plates, and spots were visualized under 254 nm UV light. GC−MS was performed using an Agilent Technologies 7890B gas chromatograph (30 m, DBS column set at either 100 or 200 °C for 2 min then at 30 °C/min to 300 °C with inlet temperature set at 250 °C) using an Agilent Technologies 5977A mass-selective detector operating at 70 eV. Flash chromatography on silica gel was performed using an Isolera One flash chromatography system from Biotage, Uppsala, Sweden. ^{1}H-NMR spectra were obtained using a Bruker AMX-400 NMR spectrometer operating at 400.14 MHz unless

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

specified in the text. The NMR raw data were analyzed using iNMR Spectrum Analyst software. ^{1}H chemical shifts are reported in parts per million (ppm) relative to an internal (TMS) or residual solvent peak. Coupling constant values (J) are reported in Hertz (Hz). Decoupled ^{19}F operating at 376 MHz was also obtained for compounds containing fluorine (data not shown). HPLC analyses were performed using an Agilent 1260 Infinity instrument with detection at 254 nm and a Phenomenex, Luna 5 μm C8(2) 100 Å reversed-phase LC column (150 × 4.6 mm) at 40 °C, eluting with a gradient of A/B at 25%/75% to A/B at 25% to 90% (A: 0.05% formic acid in water, B: 0.05% formic acid in methanol). High-resolution mass spectrometry (HRMS) was performed using a high-resolution (30,000) Thermo LTQ-Orbitrap Discovery hybrid mass spectrometry instrument (San Jose, CA) equipped with an electrospray ionization source operating in the positive or negative ion mode. The Orbitrap was externally calibrated prior to data acquisition allowing accurate mass measurements for $[M + H]^+$ ions to be obtained within 4 ppm. Copper content was determined by inductively coupled plasma−atomic emission spectroscopy (ICP-AES) by Galbraith Laboratories, Knoxville, TN.

Ethyl 2-(4-(4-(Trifluoromethoxy)phenoxy)phenyl)-acetate (12) from 11 and 3. A round-bottom flask, stir bar, and potassium carbonate were oven dried at 150 °C for at least 24 h prior to use. Copper chloride (CuCl) (12.2 g, 123 mmol, 0.15 equiv), DMG (8.5 g, 82.3 mmol, 0.1 equiv), and dimethylformamide (DMF, 200 mL) were placed into the hot round-bottom flask and degassed for 20 min at 50 °C under house vacuum, while stirring. To the intensely blue-colored catalyst mixture were added K_2CO_3 (227 g, 1.65 mol, 2.0 equiv), ethyl 2-(4-bromophenyl)acetate **11** (200 g, 0.823 mol, 1.0 equiv) in 200 mL of degassed DMF, and 4-(trifluoromethoxy)phenol **3** (161.7 g, 0.908 mol, 1.1 equiv). The reaction mixture was heated to 50 °C, degassed for 10 min under house vacuum, and purged with argon for 5 min. The temperature was then raised and maintained at 160 °C for 90 min under argon, whereupon the GC−MS analysis showed that **11** was consumed. Upon cooling, the solid residue was filtered, boiled with 500 mL of ethyl acetate to extract the products from the residue, cooled, and then filtered again. This process was repeated once more. All of the filtrates were combined and concentrated to give crude **12** as a black, oily product (355 g) that was purified by distillation under high vacuum (0.5−0.6 mTorr) at 140 °C to give **12** (134 g, 48% yield) as a yellow oil. GC−MS shows one major peak with M^+ = 340 (37%), 267 (100%). ^{1}H-NMR ($CDCl_3$): δ 7.32−7.28 (m, 2H), 7.22−7.18 (m, 2H), 7.04−6.98 (m, 4H), 4.20 (q, J = 7.1 Hz, 2H), 3.63 (s, 2H), 1.30 (t, J = 7.1 Hz, 3H). Copper <21 ppm. HRMS calculated for $C_{17}H_{16}F_3O_4$ $[M + H]^+$ = 341.0995 observed for $[M + H]^+$ = 341.0993.

2-(4-(4-(Trifluoromethoxy)phenoxy)phenyl)acetic Acid (14) from 13 and 3. A round-bottom flask, stir bar, and K_2CO_3 were oven dried at 150 °C for at least 24 h, and DMF was degassed under house vacuum for 1 h prior to use. Copper(I) chloride (CuCl) (1.38 g, 13.9 mmol, 0.15 equiv), DMG (0.96 g, 9.30 mmol, 0.10 equiv), and DMF (30 mL) were placed into the hot round-bottom flask and degassed for 20 min at 50 °C, while stirring. To the blue-colored catalyst mixture, DMF (100 mL) and K_2CO_3 (38.5 g, 279 mmol, 3.0 equiv) were added. Next, 2-(4-bromophenyl)acetic acid (**13**, 20 g, 93.0 mmol, 1.0 equiv) was slowly added to avoid excessive foaming followed by 4-(trifluoromethoxy)phenol (**3**, 19.9 g, 111.6 mmol, 1.2 equiv). The reaction mixture was heated to 50 °C, degassed for 10 min, and purged with argon for 5 min. The temperature was then raised and maintained at 160 °C for 3.5 h under argon, when 2-(4-bromophenyl)acetic acid was consumed as shown by GC−MS analysis. The crude mixture was cooled to room temperature, and 200 mL of water and ammonium pyrrolidinedithiocarbamate (APDTC) (5.0 g, 30.6 mmol, 2.2 equiv with respect to CuCl used) were added followed by stirring at 50 °C for 1 h. The resulting slurry was passed through a Celite pad (50 g) and washed thoroughly with water (250 mL). To the filtrate were added ice (150 g) and concentrated HCl (12.1 N, 55 mL) until the pH was around 2. The light yellow solid that precipitated out of the solution was filtered, washed with hot water (100 mL), boiled in 300 mL of water, cooled with 200 g of ice, filtered again, and air dried. The above treatment with water was used to remove water soluble residues and some of the remaining phenol **3**. The resulting 2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-acetic acid **14** (22.7 g, 78% yield) was used in the subsequent step without further purification. GC−MS showed one major peak with M^+ = 312 (53%), 267 (100%). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.29 (d, J = 8.4 Hz, 2H), 7.20 (dd, J = 9.1, 0.8 Hz, 2H), 7.04−6.99 (m, 4H), 3.67 (s, 2H). Copper <73 ppm. HRMS calculated for $C_{15}H_{11}F_3O_4$ $[M + H]^+$ = 313.0682, observed for $[M + H]^+$ = 313.0678, and calculated for $C_{15}H_{11}F_3O_4$ $[M + Na]^+$ = 335.0504, observed for $[M + Na]^+$ = 335.0497. The product was at least 95% pure as shown by GC−MS and ^{1}H-NMR. The only impurity observed was the phenol starting material **3**.

Ethyl 2-(4-(4-(Trifluoromethoxy)phenoxy)phenyl)-acetate (12) from 14. Into a round-bottom flask equipped with a stir bar were added **14** (22.6 g, 72.4 mmol, 1.0 equiv), absolute ethanol (167 g, 3.6 mol, 50 equiv), and 12.1 N HCl (0.75 mL, 9.1 mmol, 0.13 equiv). The solution was heated to reflux for 18 h, at the end of which time only ∼1% of the starting material **14** was present. The solvent was removed in vacuo, and the resulting thick slurry was passed through a silica gel plug (70 g), rinsing with a mixture of hexanes/ethyl acetate (3:1) until no more **12** came through as monitored by TLC. After solvent removal, ethyl 2-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)acetate **12** was recovered (22.5 g, 91% yield) as a light orange oil. GC−MS showed one major peak with M^+ = 340 (37%), 267 (100%). ^{1}H-NMR ($CDCl_3$): δ 7.32−7.28 (m, 2H), 7.22−7.18 (m, 2H), 7.04−6.98 (m, 4H), 4.20 (q, J = 7.1 Hz, 2H), 3.63 (s, 2H), 1.30 (t, J = 7.1 Hz, 3H). Copper <12 ppm. HRMS calculated for $C_{17}H_{16}F_3O_4$ $[M + H]^+$ = 341.0995, observed for $[M + H]^+$ = 341.0993. The product was at least 95% pure as shown by GC−MS and ^{1}H-NMR, sufficient for use in the subsequent step without further purification.

4-(Trifluoromethoxy)phenyl(2′,4′,6′-trimethoxyphenyl)iodonium Tosylate (16) from 15. To a 1 L three-necked round-bottom flask at room temperature was added toluenesulfonic acid monohydrate (73.04 g, 0.38 mol, 1 equiv), acetonitrile (384 mL), and **15** (62 mL, 0.39 mol, 1 equiv). An overhead stirrer was inserted into the middle neck and stirring was commenced. *meta*-Chloroperbenzoic acid (*m*-CPBA) (97.84 g, 0.43 mmol, 1.1 equiv) was then added slowly to the flask. The flask was then lowered into a preheated oil bath (65 °C) and a yellow slurry formed as the reaction progressed. After 40 min, 1,3,5-trimethoxybenzene (64.58 g, 0.38 mol, 1.0 equiv) was added to the flask. The slurry dissolved, and the reaction mixture became an orange liquid.

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

The reaction was removed from the heat after 5 min and cooled to room temperature. Trituration with diethyl ether followed by filtration and drying afforded **16** (205.3 g, 0.33 mol, 84% yield) as a yellow solid. ^{1}H-NMR (600 MHz; DMSO-d_6) δ 8.03 (d, J = 8.7 Hz, 2 H), 7.47 (m, 4 H), 7.10 (d, J = 7.8 Hz, 2H), 6.48 (s, 2H), 3.95 (s, 6H), 3.87 (S, 3H), 2.28 (s, 3H) ppm. ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.8, 159.8, 150.7, 146.1, 138.1, 137.2, 128.9, 125.9, 124.3, 120.3 (q, J = 171.2 Hz) 114.2, 92.6, 87.7, 57.8, 56.6, 21.2 ppm. FTIR (λ): 3054, 2984, 2945, 2845, 1581, 1263, 1228, 1161, 1121, 1031, 1008, 816, 732, 702 cm^{-1}. HRMS (ESI) m/z: $[M - OTs]^+$ Calculated: 454.9962; Observed: 454.9959. Melting point: 190−192 °C.

Ethyl-3-acetoxy-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)but-2-enoate (12) from 16. This reaction was performed at three different scales using the following general procedure. Compound **16** (1.0 equiv) was added to a round-bottom flask containing toluene (5 mL per mmol, **7**) and potassium carbonate (3.0 equiv). Ethyl 2-(4-hydroxyphenyl)-acetate (1.5 equiv) was added to the flask, and the reaction was placed in a preheated (55 °C) oil bath and stirred for 2.5 h. The reaction mixture was removed from the heat and concentrated in vacuo. The resulting brown oil was subjected to a short silica gel column using 5% ethyl ether in hexanes as the eluting solvent mixture to yield the product in an impure state. Product peaks corresponding to pure materials were used to determine the yield of **12** based on ^{1}H-NMR spectroscopy. The yields of **12** obtained at the three scales used were as follows: 54% (0.5 mmol scale), 43% (3.6 mmol scale), and 48% (10 mmol scale).

Ethyl-3-acetoxy-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)but-2-enoate (17) from 12. Temperatures given were recorded by an internal thermometer. A stirred solution of dry THF (350 mL) and hexamethyldisilazane (HMDS) (109.1 g, 676 mmol, 2.3 equiv) under Ar was cooled to −20 °C in an 80% ethylene glycol, 20% ethanol, and dry ice bath. While monitoring the temperature to ensure that it did not exceed −20 °C, n-butyl-lithium (2.5 M) in hexane (n-BuLi) (258.6 mL, 646 mmol, 2.2 equiv) followed by a solution of **12** (100 g, 293 mmol, 1.0 equiv) in THF (250 mL) were added dropwise. After stirring for 35 min at −20 to −30 °C, acetic anhydride (66.0 g, 74.2 mL, 646 mmol 2.2 equiv) was added dropwise while monitoring the temperature to ensure that it did not exceed −10 °C. The solution turned from intense yellow to orange-yellow. At this point TLC showed no more starting material and the presence of the products **17** and **18**. GC−MS showed the presence of the desired product **17**. The solution was then allowed to warm gradually to room temperature, and after 16 h, the solution took on the appearance of a yellow gel. The reaction mixture was then added to 700 mL of 10% HCl and 200 g of ice. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 250 mL). The organic layers were combined, washed with water (200 mL) and brine (200 mL), dried over Na_2SO_4 (100 g), filtered, and concentrated to give crude **17** (129.2 g, 104% yield) as a yellow oil. GC−MS showed one major peak at 7.18 min with M^+ = 424 (22%), 336 (100%) corresponding to the Z-isomer of **17** and one minor peak at 6.97 min with M^+ = 424 (30%), 336 (100%) corresponding to the E-isomer. The crude product did not contain the starting material **12** and was approximately 95% pure by GC−MS, sufficient for further reaction. For analysis, pure Z and E isomers were obtained by flash chromatography using a gradient of ethyl acetate/hexane (5:25) as the eluting solvent mixture. Isomers of **17** were assigned using 2D-NOESY NMR. ^{1}H-NMR (400 MHz; $CDCl_3$) of the Z-isomer of **17**: δ 7.29−7.27 (m, 2H), 7.21−7.19 (m, 2H), 7.05−6.99 (m, 4H), 4.18 (q, J = 7.1 Hz, 2H), 2.23 (s, 3H), 1.94 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H). ^{1}H-NMR (400 MHz; $CDCl_3$) of the E-isomer of **17**: δ 7.21−7.17 (m, 4H), 7.05−6.96 (m, 4H), 4.23 (q, J = 7.1 Hz, 2H), 2.40 (s, 3H), 1.90 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H). HRMS of the product **17** as an isomer mixture calculated for $C_{21}H_{20}F_3O_6$ $[M + H]^+$ = 425.1206, observed for $[M + H]^+$ = 425.1199. HRMS of the **17** Z-isomer calculated for $C_{21}H_{20}F_3O_6$ $[M + H]^+$ = 425.1206, observed for $[M + H]^+$ = 425.1200. HRMS of the **17** E-isomer calculated for $C_{21}H_{20}F_3O_6$ $[M + H]^+$ = 425.1206, observed for $[M + H]^+$ = 425.1201.

Ethyl 3-Oxo-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)butanoate (18) from 17. A stirred solution of the bis-acylated **17** (125.2 g, 295 mmol) in glacial AcOH (250 mL) and p-TsOH monohydrate (5.6 g, 29.5 mmol, 0.1 equiv) was heated at 100 °C. After 2 h, starting material **17** was not detected by TLC. The dark brown solution was cooled to room temperature and concentrated under reduced pressure. After most of the AcOH was eliminated, to remove the residual AcOH, cyclohexane (2 × 50 mL) was added to the brown oil and concentrated again to give 116.5 g of **18** as a dark brown oil. Because this material still contained 5.6 g of p-TsOH, the yield of **18** was 110.9 g (98% yield). The product can be used without purification in the following Conrad−Limpach reaction. If desired, pure **18** can be obtained by flash chromatography using 2:8 ethyl acetate/hexanes as the eluting solvent mixture. GC−MS cannot be used to characterize **18** as it decomposes in the injection port. Both the keto and enol forms can be detected by ^{1}H-NMR. Integration of the aromatic region does not provide an accurate count of the number of protons in **18** because of the existence of the keto/enol form. ^{1}H-NMR (400 MHz; $CDCl_3$) δ 13.15 (s, 0.4), 7.37−7.34 (m, 2H), 7.23−7.20 (m, 3H), 7.16−7.14 (m, 1H), 7.08−7.00 (m, 7H), 4.71 (s, 1H), 4.27−4.20 (m, 4H), 2.24 (s, 3H), 1.90 (s, 1H), 1.31 (t, J = 7.1 Hz, 3H), 1.22 (t, J = 7.1 Hz, 2H). HRMS calculated for $C_{19}H_{18}F_3O_3$ $[M + H]^+$ = 383.1101, observed for $[M + H]^+$ = 383.1097.

General Procedure for the Preparation of the Schiff Bases (20a−d, Scheme 6). A stirred mixture of substituted anilines **19a−d** and β-keto ester **18** containing 10% of p-TsOH in cyclohexane was heated at reflux for 24−72 h using a Dean−Stark trap to continuously remove the water formed during the condensation. After cooling to room temperature, the mixture was filtered and the solid thus removed (containing unreacted anilines **19a−d** and some unidentified materials) was washed with cyclohexane. The filtrate combined with the cyclohexane washes was concentrated in vacuo to give the products **20a−d** as yellow-brown, highly viscous oils, which were used without purification in the next phase of the reaction.

General Procedure for the Conrad−Limpach Reaction[15,16] (ELQ, Scheme 6). The Conrad−Limpach reactions were performed at different temperatures. The 250 °C cyclization was conducted at the boiling point of Dowtherm A. For other temperatures, the cyclization was conducted in Dowtherm A maintained at the desired temperature, determined by an internal thermometer. To facilitate addition of the highly viscous Schiff base obtained above, it was diluted with Dowtherm A with slight warming. This mixture was added to the heated Dowtherm A over approximately 10 min

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

with vigorous stirring, so that the desired temperature was always maintained. After stirring for a further period of time (specified for individual reactions below), the reaction mixture was cooled to room temperature and diluted with hexanes, resulting in the formation of a white precipitate. The precipitate was recovered by filtration, washed with hexanes, and air dried to give the crude ELQ compound. Yields were determined over two steps based on the β-keto ester **18** used to form the initial Schiff base.

2-Methyl-3-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)quinolin-4(1*H*)-one (ELQ-271). *1-Ethoxy-3-(phenylimino)-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)but-1-en-1-ol (**20a**, Scheme 6).* Following the general procedure for the preparation of the Schiff base, a mixture of aniline **19a** (1.85 g, 19.9 mmol, 1.0 equiv) and 8.07 g of β-keto ester **18** containing 10% of *p*-TsOH (7.69 g, 20.1 mmol, 1.0 equiv of **18** and 0.38 g, 2.6 mmol, 0.1 equiv of *p*-TsOH) in cyclohexane (200 mL) was heated at reflux for 24 h. Cyclohexane (30 mL) was used to wash the precipitate. The filtrate was concentrated in vacuo to give the crude Schiff base **20a** (9.60 g) as a yellow, highly viscous oil.

*Conrad−Limpach Cyclization of **20a**.* A portion of the Schiff base **20a** (3.90 g) was diluted with Dowtherm A (5 mL) with slight warming. This Schiff base solution was added over 5 min to boiling Dowtherm A (100 mL, 250 °C) with vigorous stirring so that boiling was always maintained. After stirring for another 20 min at 250 °C, the stirred reaction mixture was cooled to room temperature resulting in the formation of a white precipitate. Hexane (150 mL) was then added to the mixture. The resulting solid was filtered, washed with hexanes (30 mL) and acetone (100 mL), and air-dried to give crude **ELQ-271** (2.11 g, 63%) as a white solid. ^{1}H-NMR (400 MHz; DMSO-d_6): δ 11.66 (s, 1H), 8.10−8.08 (m, 1H), 7.64 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H), 7.54 (ddd, *J* = 8.3, 1.1, 0.6 Hz, 1H), 7.44−7.41 (m, 2H), 7.32−7.28 (m, 3H), 7.19−7.15 (m, 2H), 7.10−7.07 (m, 2H), 2.27 (s, 3H). HPLC analysis indicated that the obtained **ELQ-271** was >99% pure. GC−MS analysis of the 4-chloro derivative of **ELQ-271** obtained by chlorination with $POCl_3$ showed only a few very small uncharacterized impurities besides the major component with M^+ = 429.5.

6-Chloro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1*H*)-one (ELQ-300). *3-(4-Chloro-3-methoxyphenyl)imino)-1-ethoxy-2-(4-(4-(trifluoromethoxy)phenoxy)-phenyl)but-1-en-1-ol (**20b**).* Following the general procedure for the preparation of the Schiff base, a mixture of 4-chloro-3-methoxyaniline **19b** (20.63 g, 131 mmol, 1.0 equiv) and 52.25 g of keto ester **18** containing 10 mol % of *p*-TsOH (50.0 g, 131.0 mmol, 1.0 equiv of **18** and 2.25 g, 1.31 mmol, 0.1 equiv of *p*-TsOH) in cyclohexane (300 mL) was heated at reflux for 44 h. Cyclohexane (100 mL) was used to wash the precipitate. The condensation was practically complete after 20 h, as no substantial increase in the volume of water occurred after this time. The combined filtrates were concentrated in vacuo to give the crude Schiff base **20b** (67.5 g) as a yellow, highly viscous oil.

*Conrad−Limpach cyclization of **20b**.* The Schiff base **20b** (67.5 g) was diluted with Dowtherm A (30 mL) with slight warming. This Schiff base **20b** solution was added in small portions over 25 min to boiling Dowtherm A (550 mL, 250 °C) with vigorous stirring so that boiling was always maintained. After stirring for another 10 min at 250 °C, the stirred reaction mixture was cooled to room temperature resulting in the formation of a thick white precipitate. The mixture was then allowed to stand without stirring at room temperature overnight. Hexane (1000 mL) was added and the mixture was stirred for 15 min and filtered. The resulting white precipitate was washed with ethyl acetate (500 mL) and acetone (250 mL) and air dried to give **ELQ-300** (35.5 g, 57% yield). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 11.66 (s, 1H), 8.00 (s, 1H), 7.43−7.41 (m, 2H), 7.30−7.26 (m, 2H), 7.18−7.14 (m, 2H), 7.09−7.05 (m, 3H), 3.97 (s, 3H), 2.24 (s, 3H). HPLC analysis indicated that **ELQ-300** was >99% pure. No **ELQ-300** regioisomer was observed by ^{1}H-NMR, and GC−MS analysis of the 4-chloro derivative of **ELQ-300** obtained by chlorination with $POCl_3$ showed only a single compound to be present, M^+ = 493.1.

To assess the performance of the Conrad−Limpach reaction at temperatures below 250 °C using the Schiff base **20b**, 10.0 g of β-keto ester **18** containing 10 mol % of *p*-TsOH (9.5 g, 25 mmol, 1.0 equiv of **14** and 0.5 g, 2.9 mmol, 0.1 equiv of *p*-TsOH) and 1.0 equiv of aniline **19b** (4.0 g, 25 mmol) were condensed as described above, and the cold cyclohexane solution was filtered and diluted to a total volume of 300 mL. From this stock solution (**Solution A**), aliquots of specific volume were removed, stripped of the solvent, diluted with Dowtherm A, and cyclized at the specified temperatures.

The cyclization at 230 °C was performed similarly to that at 250 °C above. To 100 mL of rapidly stirred Dowtherm A held at 230 °C, the residue of a 100 mL aliquot of **Solution A** (corresponding to 8.3 mmols of **18** condensed with **19b**) was dissolved in Dowtherm A (10 mL) and added dropwise over the course of 10 min. The temperature was carefully monitored and the heat reservoir was sufficiently large to keep temperature changes within 1 °C. After stirring for another 20 min at 230 °C, the stirred reaction mixture was cooled to room temperature resulting in the formation of a white precipitate. The mixture was filtered, washed with hexanes and acetone, and air dried to give crude **ELQ-300** (1.88 g, 48% yield) as a white solid. As in the case of the cyclization at 250 °C, no **ELQ-300** regioisomer was observed by ^{1}H-NMR and GC−MS analysis of the 4-chloro derivative of **ELQ-300** obtained by chlorination with $POCl_3$. The ^{1}H-NMR spectrum of **ELQ-300** obtained from the reaction at 230 °C was identical to that of **ELQ-300** obtained at 250 °C. The HPLC analysis indicated that the obtained **ELQ-300** was >99% pure.

The cyclization at 200 °C was performed similarly to the above. To 100 mL of rapidly stirred Dowtherm A held at 200 °C, the residue of a 22 mL aliquot of **Solution A** (corresponding to 1.8 mmol **18** condensed with **19b**) in Dowtherm A (5 mL) was added dropwise over the course of 10 min. The temperature was carefully monitored and the heat reservoir was sufficiently large to keep temperature changes within 1 °C. After stirring for another 60 min at 200 °C, the stirred reaction mixture was allowed to cool to room temperature and let stand overnight, resulting in the formation of a white precipitate. Hexane (100 mL) was added and the mixture was filtered, washed with hexanes, and air-dried to give **ELQ-300** (0.26 g, 30% yield) as a white solid. As in the cases of the cyclizations at 250 and at 230 °C, no **ELQ-300** regioisomer was observed by ^{1}H-NMR and GC−MS analyses of the 4-chloro derivative of **ELQ-300** obtained by chlorination with $POCl_3$. The ^{1}H-NMR spectrum of **ELQ-300** obtained from the reaction at 200 °C was identical to that

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

of **ELQ-300** obtained at 250 °C. The HPLC analysis indicated that the obtained **ELQ-300** was >99% pure.

6-Fluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1*H*)-one (ELQ-316). *1-Ethoxy-3-((4-fluoro-3-methoxyphenyl)imino)-2-(4-(4-(trifluoromethoxy)-phenoxy)phenyl)but-1-en-1-ol (**20c**).* Following the general procedure for the preparation of the Schiff base, a mixture of 4-fluoro-3-methoxyaniline **19c** (42.5 g, 0.301 mol, 1 equiv) and 120.7 g of *β*-keto ester **18** containing 10% of *p*-TsOH (115.2 g, 0.302 mol, 1.0 equiv of **18** and 5.5 g, 0.1 equiv of *p*-TsOH) in cyclohexane (600 mL) was heated at reflux for 72 h. Cyclohexane (50 mL) was used to wash the precipitate. The combined filtrates were concentrated in vacuo to give the crude Schiff base **20c** as a yellow, highly viscous oil.

*Conrad–Limpach Cyclization of **20c**.* The Schiff base **20c** was diluted with Dowtherm A (50 mL) with slight warming. This Schiff base solution was added over 30 min to boiling Dowtherm A (700 mL, 250 °C) with vigorous stirring so that boiling was always maintained. After stirring for another 15 min at 250 °C, the reaction mixture was cooled to room temperature, resulting in the formation of a firm, white cake. Ethyl acetate (2 L) was added, and the cake was broken up with a glass rod and stirred until no large pieces remained. The mixture was filtered, washed with methanol (500 mL) and then with acetone (250 mL), and air-dried to give crude **ELQ-316** (73 g, 54%) as a white solid. ^{1}H-NMR (400 MHz; DMSO-d_6): δ 11.63 (s, 1H), 7.70 (d, J = 11.8 Hz, 1H), 7.43−7.40 (m, 2H), 7.30−7.26 (m, 2H), 7.18−7.14 (m, 2H), 7.11−7.05 (m, 3H), 3.96 (s, 3H), 2.24 (s, 3H). The HPLC analysis indicated that the obtained **ELQ-316** was >99% pure. Copper <17 ppm. No **ELQ-316** regioisomer was observed by ^{1}H-NMR, and GC−MS analysis of the 4-chloro derivative of **ELQ-316** obtained by chlorination with $POCl_3$ showed only a single compound to be present, M^+ = 477.5.

The cyclization at 230 °C was performed similarly to the above. To 100 mL of rapidly stirred Dowtherm A held at 230 °C, 3.84 g of the condensation product **20c** in Dowtherm A (2 mL) was added dropwise over the course of 10 min. The temperature was carefully monitored and the heat reservoir was sufficiently large to keep temperature changes within 1 °C. After stirring for another 30 min at 230 °C, the stirred reaction mixture was cooled to room temperature resulting in the formation of a white precipitate. Hexane (100 mL) was then added to the mixture. It was then filtered, washed with hexanes (30 mL) and ethyl acetate (2 × 10 mL), and air-dried to give **ELQ-316** (1.75 g, 50%) as a white solid. No **ELQ-316** regioisomer was observed by ^{1}H-NMR and GC−MS analyses of the 4-chloro derivative of **ELQ-316** obtained by chlorination with $POCl_3$. The ^{1}H-NMR spectrum of **ELQ-316** obtained from the reaction at 230 °C was identical to that of **ELQ-316** obtained at 250 °C. HPLC analysis indicated that the obtained **ELQ-316** was 98% pure.

5,7-Difluoro-7-methoxy-2-methyl-3-(4-(4-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1*H*)-one (ELQ-400). *1-Ethoxy-3-((3,5-difluorophenyl)imino)-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)but-1-en-1-ol (**20d**).* Following the general procedure for the preparation of the Schiff base, a mixture of 3,5-difluoro aniline **19d** (3.26 g, 25.3 mmol, 1.0 equiv) and 10.14 g of *β*-keto ester **18** containing 10% of *p*-TsOH (9.66 g, 25.3 mmol, 1.0 equiv of **18** and 0.48 g, 2.8 mmol, 0.1 equiv of *p*-TsOH) in cyclohexane (200 mL) was heated at reflux for 24 h. After cooling to room temperature, the clear solution was separated from the residue stuck to the walls of the reaction flask and was concentrated in vacuo to give the crude Schiff base **20d** (13.0 g) as a yellow, highly viscous oil.

*Conrad–Limpach cyclization of **20d**.* A portion of the Schiff base **20d** (4.00 g) was diluted with Dowtherm A (2 mL) with slight warming. The Schiff base solution was added over 5 min to boiling Dowtherm A (90 mL, 250 °C) with vigorous stirring so that boiling was always maintained. After stirring for another 15 min at 250 °C, the stirred reaction mixture was cooled to room temperature, resulting in the formation of a white precipitate. When cold, hexane (100 mL) was added to the mixture. After brief stirring, it was filtered, washed with hexanes (30 mL), and air-dried to give crude **ELQ-400** (1.90 g, 32%) as a white solid. ^{1}H-NMR (400 MHz; DMSO-d6): δ 11.74 (s, 1H), 7.43−7.40 (m, 2H), 7.28−7.25 (m, 2H), 7.18−7.14 (m, 2H), 7.10−7.00 (m, 4H), 2.21 (s, 3H). The HPLC analysis indicated that the obtained **ELQ-400** was >99% pure.

The cyclization at 230 °C was performed similarly to the above. To 100 mL of rapidly stirred Dowtherm A held at 230 °C, 3.80 g of the condensation product **20d** in Dowtherm A (5 mL) was added dropwise over the course of 10 min. The temperature was carefully monitored and the heat reservoir was sufficiently large to keep temperature changes within 1 °C. After stirring for another 30 min at 230 °C, the stirred reaction mixture was cooled to room temperature resulting in the formation of a white precipitate. Hexane (150 mL) was then added, and the precipitate was collected by filtration, washed with hexanes (50 mL) and acetone (10 mL), and air-dried to give crude **ELQ-400** (1.14 g, 33%) as a slightly yellow solid. The ^{1}H-NMR spectrum of **ELQ-400** obtained from the reaction at 230 °C was identical to that of **ELQ-400** obtained at 250 °C. HPLC analysis indicated that the obtained **ELQ-400** was >99% pure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.1c00099.

Synthetic details for the optimized original synthesis of **ELQ-300** (Scheme 2) (PDF)

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Org. Process Res. Dev. XXXX, XXX, XXX−XXX

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Author Contributions

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Notes

The authors declare the following competing financial interest(s): OHSU and S.P., R.D., L.F., K.L, H.J., A.N., J.D., M.R., and R.W. have a financial interest in a company that may have a commercial interest in the results of this research and technology. A patent application on the intellectual property described herein has been filed by OHSU.

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■ ABBREVIATIONS

ELQ, endochin-like quinolone; HBr, hydrobromic acid; DMG, *N*,*N*-dimethylglycine; K_2CO_3, potassium carbonate; DMF, *N*,*N*-dimethylformamide; TMP, 2,4,6-trimethoxyphenyl; TLC, thin-layer chromatography; LiHMDS, lithium hexamethyldisilazide; THF, tetrahydrofuran; NOESY NMR, nuclear Overhauser effect spectroscopy nuclear magnetic resonance; ^{1}H-NMR, proton nuclear magnetic resonance; GC−MS, gas chromatography−mass spectrometry; *p*-TsOH, *para*-toluenesulfonic acid; AcOH, acetic acid; OMe, *o*-methyl; HPLC, high-performance liquid chromatography; $POCl_3$, phosphorus oxychloride; TMS, tetramethylsilane; HRMS, high-resolution mass spectrometry

■ REFERENCES

(1) Salzer, W. T.; Timmler, H.; Andersag, H. Über einen neuen, gegen Vogelmalaria wirksamen Verbindungstypus. *Chem. Ber.* **1948**, *81*, 12−19.
(2) Silva, M. G.; Bastos, R. G.; Stone Doggett, J.; Riscoe, M. K.; Pou, S.; Winter, R.; Dodean, R. A.; Nilsen, A.; Suarez, C. E. Endochin-like quinolone-300 and ELQ-316 inhibit *Babesia bovis, B. bigemina, B. caballi* and *Theileria equi*. *Parasites Vectors* **2020**, *13*, 606.
(3) Müller, J.; Aguado, A.; Laleu, B.; Balmer, V.; Ritler, D.; Hemphill, A. In vitro screening of the open source Pathogen Box identifies novel compounds with profound activities against *Neospora caninum*. *Int. J. Parasitol.* **2017**, *47*, 801−809.
(4) Eberhard, N.; Balmer, V.; Müller, J.; Müller, N.; Winter, R.; Pou, S.; Nilsen, A.; Riscoe, M.; Francisco, S.; Leitao, A.; Doggett, J. S.; Hemphill, A. Activities of Endochin-Like Quinolones Against in vitro Cultured *Besnoitia besnoiti Tachyzoites*. *Front. Vet. Sci.* **2020**, *7*, 96.
(5) Anghel, N.; Balmer, V.; Müller, J.; Winzer, P.; Aguado-Martinez, A.; Roozbehani, M.; Pou, S.; Nilsen, A.; Riscoe, M.; Doggett, J. S.; Hemphill, A. Endochin-Like Quinolones Exhibit Promising Efficacy Against *Neospora Caninum in vitro* and in Experimentally Infected Pregnant Mice. *Front. Vet. Sci.* **2018**, *5*, 285.
(6) Doggett, J. S.; Nilsen, A.; Forquer, I.; Wegmann, K. W.; Jones-Brando, L.; Yolken, R. H.; Bordon, C.; Charman, S. A.; Katneni, K.; Schultz, T.; Burrows, J. N.; Hinrichs, D. J.; Meunier, B.; Carruthers, V. B.; Riscoe, M. K. Endochin-like quinolones are highly efficacious against acute and latent experimental toxoplasmosis. *Proc. Natl. Acad. Sci.* **2012**, *109*, 15936−15941.
(7) McConnell, E. V.; Bruzual, I.; Pou, S.; Winter, R.; Dodean, R. A.; Smilkstein, M. J.; Krollenbrock, A.; Nilsen, A.; Zakharov, L. N.; Riscoe, M. K.; Doggett, J. S. Targeted Structure-Activity Analysis of Endochin-like Quinolones Reveals Potent Qi and Qo Site Inhibitors of *Toxoplasma gondii* and *Plasmodium falciparum* Cytochrome bc_1 and Identifies ELQ-400 as a Remarkably Effective Compound against Acute Experimental Toxoplasmosis. *ACS Infect. Dis.* **2018**, *4*, 1574−1584.
(8) Dubey, J. P.; Jones, J. L. *Toxoplasma gondii* infection in humans and animals in the United States. *Int. J. Parasitol.* **2008**, *38*, 1257−1278.
(9) Shapiro, K.; VanWormer, E.; Packham, A.; Dodd, E.; Conrad, P. A.; Miller, M. Type X strains of *Toxoplasma gondii* are virulent for southern sea otters (*Enhydra lutris nereis*) and present in felids from nearby watersheds. *Proc. R. Soc. B* **2019**, *286*, 20191334.
(10) Rufener, R.; Dick, L.; D'Ascoli, L.; Ritler, D.; Hizem, A.; Wells, T. N. C.; Hemphill, A.; Lundström-Stadelmann, B. Repurposing of an old drug: *In vitro* and *in vivo* efficacies of buparvaquone against *Echinococcus multilocularis*. *Int. J. Parasitol. Drugs Drug Resist.* **2018**, *8*, 440−450.
(11) World malaria report *2020: 20 years of global progress and challenges*; World Health Organization: Geneva, 2020.
(12) Frueh, L.; Li, Y.; Mather, M. W.; Li, Q.; Pou, S.; Nilsen, A.; Winter, R. W.; Forquer, I. P.; Pershing, A. M.; Xie, L. H.; Smilkstein, M. J.; Caridha, D.; Koop, D. R.; Campbell, R. F.; Sciotti, R. J.; Kreishman-Deitrick, M.; Kelly, J. X.; Vesely, B.; Vaidya, A. B.; Riscoe, M. K. Alkoxycarbonate Ester Prodrugs of Preclinical Drug Candidate ELQ-300 for Prophylaxis and Treatment of Malaria. *ACS Infect. Dis.* **2017**, *3*, 728−735.
(13) Alday, P. H.; Doggett, J. S. Drugs in development for toxoplasmosis: advances, challenges, and current status. *Drug Des. Dev. Ther.* **2017**, *Volume11*, 273−293.

(14) Bigna, J. J.; Tochie, J. N.; Tounouga, D. N.; Bekolo, A. O.; Ymele, N. S.; Youda, E. L.; Sime, P. S.; Nansseu, J. R. Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. *Sci. Rep.* **2020**, *10*, 12102.
(15) Conrad, M.; Limpach, L. synthesen von Chinolinderivaten mittelst Acetessigester. *Ber. Dtsch. Chem. Ges.* **1887**, *20*, 944−948.
(16) Conrad, M.; Limpach, L. Synthese von Chinolinderivaten mittelst Acetessigester. *Ber. Dtsch. Chem. Ges.* **1891**, *24*, 2990−2992.
(17) Winter, R.; Kelly, J. X.; Smilkstein, M. J.; Hinrichs, D.; Koop, D. R.; Riscoe, M. K. Optimization of endochin-like quinolones for antimalarial activity. *Exp. Parasitol.* **2011**, *127*, 545−551.
(18) Winter, R. W.; Kelly, J. X.; Smilkstein, M. J.; Dodean, R.; Hinrichs, D.; Riscoe, M. K. Antimalarial quinolones: synthesis, potency, and mechanistic studies. *Exp. Parasitol.* **2008**, *118*, 487−497.
(19) Torii, S.; Okumoto, H.; Long, H. X. A direct approach to 2-substituted 1,4-dihydr-4-oxo-qinoline-3-carboxylates by palladium-catalyzed carbonylative cyclization. *Tetrahedron Lett.* **1990**, *31*, 7175−7178.
(20) Pidathala, C.; Amewu, R.; Pacorel, B.; Nixon, G. L.; Gibbons, P.; Hong, W. D.; Leung, S. C.; Berry, N. G.; Sharma, R.; Stocks, P. A.; Srivastava, A.; Shone, A. E.; Charoensutthivarakul, S.; Taylor, L.; Berger, O.; Mbekeani, A.; Hill, A.; Fisher, N. E.; Warman, A. J.; Biagini, G. A.; Ward, S. A.; O'Neill, P. M. Identification, design and biological evaluation of bisaryl quinolones targeting *Plasmodium falciparum* type II NADH:quinone oxidoreductase (PfNDH2). *J. Med. Chem.* **2012**, *55*, 1831−1843.
(21) Ho, H. E.; Stephens, T. C.; Payne, T. J.; O'Brien, P.; Taylor, R. J. K.; Unsworth, W. P. Merging π-Acid and Pd Catalysis: Dearomatizing Spirocyclization/Cross-Coupling Cascade Reactions of Alkyne-Tethered Aromatics. *ACS Catal.* **2019**, *9*, 504−510.
(22) Ward, T. R.; Turunen, B. J.; Haack, T.; Neuenswander, B.; Shadrick, W.; Georg, G. I. Synthesis of a quinolone library from ynones. *Tetrahedron Lett.* **2009**, *50*, 6494−6497.
(23) Cross, R. M.; Manetsch, R. Divergent route to access structurally diverse 4-quinolones via mono or sequential cross-couplings. *J. Org. Chem.* **2010**, *75*, 8654−8657.
(24) Ravi, M.; Chauhan, P.; Kant, R.; Shukla, S. K.; Yadav, P. P. Transition-Metal-Free C-3 Arylation of Quinoline-4-ones with Arylhydrazines. *J. Org. Chem.* **2015**, *80*, 5369−5376.
(25) Nilsen, A.; LaCrue, A. N.; White, K. L.; Forquer, I. P.; Cross, R. M.; Marfurt, J.; Mather, M. W.; Delves, M. J.; Shackleford, D. M.; Saenz, F. E.; Morrisey, J. M.; Steuten, J.; Mutka, T.; Li, Y.; Wirjanata, G.; Ryan, E.; Duffy, S.; Kelly, J. X.; Sebayang, B. F.; Zeeman, A.-M.; Noviyanti, R.; Sinden, R. E.; Kocken, C. H. M.; Price, R. N.; Avery, V. M.; Angulo-Barturen, I.; Jiménez-Díaz, M. B.; Ferrer, S.; Herreros, E.; Sanz, L. M.; Gamo, F.-J.; Bathurst, I.; Burrows, J. N.; Siegl, P.; Guy, R. K.; Winter, R. W.; Vaidya, A. B.; Charman, S. A.; Kyle, D. E.; Manetsch, R.; Riscoe, M. K. Quinolone-3-diarylethers: a new class of antimalarial drug. *Sci. Transl. Med.* **2013**, *5*, 177ra37.
(26) Nilsen, A.; Miley, G. P.; Forquer, I. P.; Mather, M. W.; Katneni, K.; Li, Y.; Pou, S.; Pershing, A. M.; Stickles, A. M.; Ryan, E.; Kelly, J. X.; Doggett, J. S.; White, K. L.; Hinrichs, D. J.; Winter, R. W.; Charman, S. A.; Zakharov, L. N.; Bathurst, I.; Burrows, J. N.; Vaidya, A. B.; Riscoe, M. K. Discovery, synthesis, and optimization of antimalarial 4(1*H*)-quinolone-3-diarylethers. *J. Med. Chem.* **2014**, *57*, 3818−3834.
(27) Cross, R. M.; Flanigan, D. L.; Monastyrskyi, A.; LaCrue, A. N.; Sáenz, F. E.; Maignan, J. R.; Mutka, T. S.; White, K. L.; Shackleford, D. M.; Bathurst, I.; Fronczek, F. R.; Wojtas, L.; Guida, W. C.; Charman, S. A.; Burrows, J. N.; Kyle, D. E.; Manetsch, R. Orally bioavailable 6-chloro-7-methoxy-4(1*H*)-quinolones efficacious against multiple stages of *Plasmodium*. *J. Med. Chem.* **2014**, *57*, 8860−8879.
(28) Monastyrskyi, A.; Namelikonda, N. K.; Manetsch, R. Metal-free arylation of ethyl acetoacetate with hypervalent diaryliodonium salts: an immediate access to diverse 3-aryl-4(1*H*)-quinolones. *J. Org. Chem.* **2015**, *80*, 2513−2520.
(29) Namelikonda, N. K.; Monastyrskyi, A.; Manetsch, R. Scalable Multigram Syntheses of Antimalarial 4(1*H*)-Quinolones ELQ-300 and P4Q-391. *Eur. J. Org. Chem.* **2017**, *2017*, 3328−3334.
(30) Fui, C. J.; Sarjadi, M. S.; Sarkar, S. M.; Rahman, M. L. Recent Advancement of Ullmann Condensation Coupling Reaction in the Formation of Aryl-Oxygen (C-O) Bonding by Copper-Mediated Catalyst. *Catalysts* **2020**, *10*, 1103.
(31) Ma, D.; Cai, Q. *N*,*N*-dimethyl glycine-promoted Ullmann coupling reaction of phenols and aryl halides. *Org. Lett.* **2003**, *5*, 3799−3802.
(32) Kancharla, P.; Dodean, R. A.; Li, Y.; Pou, S.; Pybus, B.; Melendez, V.; Read, L.; Bane, C. E.; Vesely, B.; Kreishman-Deitrick, M.; Black, C.; Li, Q.; Sciotti, R. J.; Olmeda, R.; Luong, T.-L.; Gaona, H.; Potter, B.; Sousa, J.; Marcsisin, S.; Caridha, D.; Xie, L.; Vuong, C.; Zeng, Q.; Zhang, J.; Zhang, P.; Lin, H.; Butler, K.; Roncal, N.; Gaynor-Ohnstad, L.; Leed, S. E.; Nolan, C.; Ceja, F. G.; Rasmussen, S. A.; Tumwebaze, P. K.; Rosenthal, P. J.; Mu, J.; Bayles, B. R.; Cooper, R. A.; Reynolds, K. A.; Smilkstein, M. J.; Riscoe, M. K.; Kelly, J. X. Lead Optimization of Second-Generation Acridones as Broad-Spectrum Antimalarials. *J. Med. Chem.* **2020**, *63*, 6179−6202.
(33) Sunil, U. T.; Vivekanand, B. J.; Vijay, P. P.; Sushma, S. K.; Digambar, D. G.; Rajendra, P. P. Application Progress of Recent Advances in Some Copper Catalyzed Coupling Reactions. *Mini-Rev. Org. Chem.* **2013**, *10*, 281−301.
(34) Gallagher, W. P.; Vo, A. Dithiocarbamates: Reagents for the Removal of Transition Metals from Organic Reaction Media. *Org. Process Res. Dev.* **2015**, *19*, 1369−1373.
(35) Malmgren, J.; Santoro, S.; Jalalian, N.; Himo, F.; Olofsson, B. Arylation with unsymmetrical diaryliodonium salts: a chemoselectivity study. *Chemistry* **2013**, *19*, 10334−10342.
(36) Seidl, T. L.; Moment, A.; Orella, C.; Vickery, T.; Stuart, D. R. Synthesis of 4-Methylbenzoate(2′,4′,6′-trimethoxy-phenyl)iodonium Tosylate. *Org. Synth.* **2019**, *96*, 137−149.
(37) Gallagher, R. T.; Basu, S.; Stuart, D. R. Trimethoxyphenyl (TMP) as a Useful Auxiliary for *in situ* Formation and Reaction of Aryl(TMP)iodonium Salts: Synthesis of Diaryl Ethers. *Adv. Synth. Catal.* **2020**, *362*, 320−325.
(38) Vaswani, R. G.; Albrecht, B. K.; Audia, J. E.; Côté, A.; Dakin, L. A.; Duplessis, M.; Gehling, V. S.; Harmange, J.-C.; Hewitt, M. C.; Leblanc, Y.; Nasveschuk, C. G.; Taylor, A. M. A practical synthesis of indoles via a Pd-catalyzed C-N ring formation. *Org. Lett.* **2014**, *16*, 4114−4117.
(39) Elderfield, R. C., *Heterocyclic Compounds*; John Wiley & Sons, Inc.: New York, NY, 1961.
(40) Brouet, J.-C.; Gu, S.; Peet, N. P.; Williams, J. D. A Survey of Solvents for the Conrad-Limpach Synthesis of 4-Hydroxyquinolones. *Synth. Commun.* **2009**, *39*, 5193−1569.
(41) Tsoung, J.; Bogdan, A. R.; Kantor, S.; Wang, Y.; Charaschanya, M.; Djuric, S. W. Synthesis of Fused Pyrimidinone and Quinolone Derivatives in an Automated High-Temperature and High-Pressure Flow Reactor. *J. Org. Chem.* **2017**, *82*, 1073−1084.
(42) Bogdan, A. R.; Dombrowski, A. W. Emerging Trends in Flow Chemistry and Applications to the Pharmaceutical Industry. *J. Med. Chem.* **2019**, *62*, 6422−6468.
(43) Stickles, A. M.; Ting, L.-M.; Morrisey, J. M.; Li, Y.; Mather, M. W.; Meermeier, E.; Pershing, A. M.; Forquer, I. P.; Miley, G. P.; Pou, S.; Winter, R. W.; Hinrichs, D. J.; Kelly, J. X.; Kim, K.; Vaidya, A. B.; Riscoe, M. K.; Nilsen, A. Inhibition of cytochrome bc_1 as a strategy for single-dose, multi-stage antimalarial therapy. *Am. J. Trop. Med. Hyg.* **2015**, *92*, 1195−1201.

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