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Discovery and Structural Optimization of Acridones as Broad-Spectrum Antimalarials

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Abstract

Malaria remains one of the deadliest diseases in the world today. Novel chemoprophylactic and chemotherapeutic antimalarials are needed to support the renewed eradication agenda. We have discovered a novel antimalarial acridone chemotype with dual stage activity against both liver stage and blood stage malaria. Several lead compounds generated from structural optimization of a large library of novel acridones exhibit efficacy in the following systems: 1) Picomolar inhibition of in vitro *Plasmodium falciparum* blood stage growth against multi-drug resistant parasites; 2) Curative efficacy after oral administration in erythrocytic *P. yoelii* murine malaria model; 3)

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SUPPORTING INFORMATION

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Summary of single nucleotide polymorphisms (SNPs) in 73-resistant clones of Dd2-V259L relative to Dd2 parental line and reference line 3D7 (PDF)

Spectra (NMR and HRMS) of all final compounds (PDF)

HPLC chromatograms of key target compounds (PDF)

Molecular formula strings and in vitro data (CSV)

Prevention of in vitro *P. berghei* sporozoite-induced development in human hepatocytes; and 4) Protection of in vivo *P. berghei* sporozoite-induced infection in mice. This study offers the first account of liver stage antimalarial activity in acridone chemotype. Details of the design, chemistry, structure-activity relationships, safety, metabolic/pharmacokinetic studies, and mechanistic investigation are presented herein.

Graphical Abstract

Keywords

Malaria; antimalarials; acridones; *Plasmodium falciparum*; multi-drug resistance

INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* parasites, among which *P. falciparum* is the most dangerous, with the highest rate of complications and mortality. It remains one of the world's deadliest diseases and the global effort to eradicate malaria is compromised by lack of effective vaccine, emergence of artemisinin resistance^{1–5}, as well as the escalated burden of *Plasmodium vivax*^{6–10}. Each year, malaria causes about 200 million clinical cases and claims nearly half a million lives, mostly children under the age of five and pregnant women¹. Although the burden of malaria is primarily borne by those living in developing countries, dramatic increases in international travel now place approximately 125 million Western travelers at risk of infection annually. More than 10000 of these are reported to acquire malaria each year^{11–14}. In the absence of a clinically useful vaccine^{15, 16}, there will continue to be the need for new, low cost, effective and safe malaria preventions and treatments, particularly for the most vulnerable populations: pregnant women and young children in endemic countries, as well as for non-immune travelers into the areas of risk.

Liver stage malaria is the first step of any natural infection, a symptomatically silent but obligatory phase before the onset of the clinical symptoms of disease, which makes it an attractive target for prophylactic antimalarial intervention strategies and the development of an antimalarial vaccine^{17–22}. Drugs targeting liver stage malaria (often referred to as causal prophylactics) are highly sought after as they offer many advantages over drugs that merely target the blood stage infection, but remain a formidable challenge¹⁹. Previously, we reported the discovery of dual-function acridones with both intrinsic antimalarial activity and resistance-counteracting ability^{23,24}. We made modifications with acridones lacking the (dialkylamino)alkyl moiety at *N*-10 position of the middle ring (Figure 1) and intriguingly they demonstrated broad-spectrum efficacy against both liver stage and blood stage infection of malaria. Lead optimization effort followed, focusing on structure–activity relationship

(SAR) studies with various substituents on ring-A and ring-B. The in vitro and in vivo blood stage and liver stage antimalarial activity/efficacy of broad-spectrum acridones are described in this report, as well as safety profiles and metabolic/pharmacokinetic parameters. Notably, this study produced highly-potent drug candidates with picomolar IC₅₀ values against a panel of multidrug-resistant (MDR) *Plasmodium* parasites, as well as full protection of liver stage infection at low oral doses in animal model.

RESULTS AND DISCUSSION

Chemistry.

Initially, a series of acridones **7–15**, **23** and **24** (Scheme 1 and Table 1) with a substitution at the 3 position of ring-A and various (alkylamino)alkyl ethers at the 6 position of ring-B was synthesized starting with commercially available 2-chlorobenzoic acid precursors **1a–d** or methyl 4-methoxysalicylate (**16**). The copper-catalyzed amination^{23, 25} of **1a–d** with *m*-anisidine (**2**), and Buchwald-Hartwig cross coupling²⁶ of triflate **17** with 3-(trifluoromethyl)-aniline (**18**) yielded the corresponding anthranilic acid/ester intermediates **3a–d** and **19**, respectively. The Eaton's acid-mediated cyclization of **3a–d** and **19** provided a mixture of corresponding methoxy-acridones **4a–d**, **5a–d**, and **20**, **21**, which were separated by recrystallization using a mixture of DMF and MeOH in 4:1 ratio for **4a–d** and **5a–d**, and EtOAc and MeOH in 6:3 ratio for **20** and **21**. The compounds **5a–d** and **21** were then demethylated either with HI/phenol or boron tribromide (BBr₃) to obtain the hydroxy-acridones **6a–d** and **22**, respectively, which when further treated with various 2-(dialkylamino)alkyl chlorides and K₂CO₃ in dry acetone provided the desired acridones **7–15**, **23** and **24** (Scheme 1)²³. Acridones **29** and **30**, in which ring-A is substituted with Cl at the 2 position and ring-B is substituted with (dialkylamino)ethoxy moiety at the 6 position, were synthesized from triflate **17** and *p*-chloroaniline (**25**), via the similar chemistry. The acridone **32** with (*tert*-butylamino)propyloxy moiety at the 6 position of ring-B was obtained from hydroxyacridone **28** via a two-step reaction sequence, in which **28** was first converted to chloropropyloxy acridone **31** prior to amination with *tert*-butylamine in the presence of NaI (Scheme 1, bottom panel).

To investigate the effect of disubstituent pattern at 1 and 3 positions of ring-A on potency, a large number of acridones **38–55**, **62** and **63** (Schemes 2, and Table 2) were prepared. These have the ring-A is substituted either with Cl/F atoms or combination of Cl and OMe groups, and ring-B substituted with a variety of (dialkylamino)alkyl/chloroalkyl ethers at the 6 position. Acridones **38–55** were synthesized from the triflate **17** and 3,5-dichloroaniline (**33**)/3,5-difluoroaniline (**34**), via the same chemistry as described in the Scheme 1. Conversely, a different reaction strategy was designed and executed for the synthesis of acridones **62** and **63** (Scheme 2, bottom panel), in which the (dipropylamino)ethoxy group was installed at the 6 position of ring-B before the Buchwald-Hartwig cross coupling. Synthesis of **62** and **63** began with the coupling of methyl 2,4-dihydroxybenzoate (**56**) with 1,2-dibromoethane in the presence of KI and K₂CO₃ to provide *O*-alkyl bromide **57**, which was further converted into the dipropylamino-ethoxy intermediate **58**, by coupling with dipropylamine in the presence of KI and K₂CO₃. The intermediate **58** was treated with triflic anhydride (Tf₂O) in the presence of pyridine to give the triflate **59**, which was further

subjected to Buchwald-Hartwig cross coupling with 3-chloro-5-methoxyaniline (**60**) to give the anthranilate intermediate **61**. Eaton's acid-mediated cyclization of **61** provided a mixture of two acridones **62** and **63**, which were separated by a column chromatography using a mixture of dichloromethane and MeOH as mobile phase. It is noteworthy that our new strategy (Scheme 2, bottom panel) is feasible to generate acridones that contain OMe group on ring-A and the (dialkylamino)alkyl ethers on ring-B.

To investigate the effect of 1, 2- or 2, 3-disubstitution pattern of ring-A on potency, a large number of acridones **70–86**, **92**, **93**, **98** and **99** (Scheme 3, Table 3 and Table 4) were generated. These acridones have the ring-A substituted either with Cl/F atoms or combination of halogen and alkoxy groups at the 1 and 2 or 2 and 3 positions, and ring-B substituted with a variety of (dialkylamino)alkoxy/chloroalkoxy moieties at the 6 position. Acridones **70–86**, **92** and **93**, were synthesized from the triflate **17** and 3,4-dichloroaniline (**64**)/3,4-difluoroaniline (**87**)/4-fluoro-3-methoxyaniline (**88**), via the same chemistry as described in Scheme 2. The key acridone intermediates **66** and **67**, obtained in 3:1 ratio, respectively, from **65**, were separated by recrystallization using a mixture of DMF and MeOH (3:7). The acridones **98** and **99**, which contain a F atom at the 2 position and OMe group at the 3 position of ring-A, and (diethylamino)ethoxy and (diethylamino)ethyl-amino groups at the 6 position of the ring-B, were synthesized from the methyl 2,4-dihydroxybenzoate (**56**) and methyl 4-iodosalicylate (**94**), respectively. Coupling of **56** with 2-bromo-*N,N*-diethylethylamine in the presence of tetrabutylammonium chloride (TBAC)²⁷ and K₂CO₃ provided compound **95a**. Coupling of **94** with 2-diethylaminoethylamine in the presence of CuI/Cs₂CO₃²⁸ and 2-acetylcyclohexanone provided **95b**. By treating with triflic anhydride, **95a–b** were then transformed into the corresponding triflates **96a–b**, which when further subjected to Buchwald-Hartwig cross coupling²⁹ with **88**, followed by Eaton's acid-mediated cyclization gave acridones **98** and **99** (Scheme 3, bottom panel).

To investigate the isomeric effect on potency, three dichloro-acridones **100–102** (Figure 2), which are isomeric to **45**, **73** and **83** (Table 2–4), were prepared from triflate **17** and the corresponding dichloroanilines, via the similar chemistry as described in Scheme 3.

Next our attention was turned toward the synthesis of acridones that contain (dialkylamino)alkyl ethers at the 7 position of ring-B. A series of acridones **109–113**, **119**, **120**, **123** and **128** (Scheme 4), in which ring-A is substituted either with halogens or a combination of halogen and alkoxy groups and ring-B is substituted with the (alkylamino)alkyl ethers at the 7 position, was synthesized starting with commercially available precursors **103**, **114** and **1d**, via the same synthetic methods that were employed in the previous Schemes.

Biological Activity.

In this work, the structure–activity relationship (SAR) studies were conducted with the focus on various substitutions at different positions on ring-A and ring-B of the acridones. We have synthesized a large library of the acridones and evaluated these for antimalarial blood stage and liver stage efficacy, safety, mode of action, and metabolic/pharmacokinetic properties. The in vitro antimalarial blood stage activity of acridone derivatives was tested

against a panel of *P. falciparum* with different geographic and genetic backgrounds using a SYBR Green based assay³⁰. Chloroquine (CQ) and atovaquone (ATV) were used as reference drugs in all experiments. These acridones exhibit potent activity against CQ sensitive and multi-drug resistant (MDR) parasites and cross-resistance patterns against ATV-resistant parasites vary significantly between positional isomers. The in vivo antimalarial blood stage efficacy was determined using a well-established modified Thompson 4-day suppression rodent model against *P. yoelii*^{23, 31, 32}. The acridone derivatives (via oral administrations) exhibited excellent blood stage efficacy. The in vitro liver stage activity of acridone derivatives was assessed utilizing luciferase-expressing *P. berghei* sporozoite infected human hepatocyte HepG2 cells^{33, 34}. A number of the acridone analogues exhibit potent activity (some superior to ATV) against the liver stage parasite, without toxic effect to the host liver cells. The in vivo antimalarial liver stage efficacy in rodent malaria model was conducted with real-time in vivo imaging system (IVIS), utilizing transgenic bioluminescent parasites^{33, 35, 36}. Several lead compounds exhibited true causal prophylactic efficacy providing full protection and cure in this animal model. Cytotoxicity test against human hepatic HepG2 cells indicates favorable therapeutic index (>1000 for most compounds). In vitro cardiotoxicity and mutagenicity were assessed as well, with lead acridone compounds demonstrating feasible safety profiles. No overt clinical toxicity or behavior change was observed in mice treated with acridones. The in vitro metabolic stability using mouse and human liver microsomes, and in vivo pharmacokinetic (PK) studies in mice were also conducted for lead compounds. Observations from preliminary drug resistance selection studies suggest a complex mode of action for broad spectrum acridones, including but not limited to inhibition of the parasite electron transport chain.

In Vitro Blood Stage Activity and SAR of Acridones with Mono-Substitution on Ring-A and Ring-B.

In our previous work²³, acridone **7**, which is an intermediate of dual-function novel antimalarial acridone T3.5, demonstrated excellent potency against a panel of CQ sensitive and multi-drug resistant (MDR) *P. falciparum* parasites (Table 1). Our initial lead optimizations focused on exploring substituents with the halogen and/or trifluoromethyl moieties on the 2 and 3 positions of ring-A, and hydrophilic moieties such as ((dialkylamino)alkyl ethers) on the 6 position of ring-B. To probe the chlorine atom effect on potency, we initially synthesized three acridones **8–10**, in which the Cl atom at the 3 position of acridone **7** is replaced with bromine (Br), fluorine (F) and hydrogen (H) atoms, respectively (Table 1), and examined for in vitro antimalarial activity.

It appears that acridone **8** with the Br atom at the 3 position of ring-A retained the potency against all tested *P. falciparum* strains with great selectivity and was slightly more potent than acridone **7**. Replacing Cl by F atom as in **9** and H atom as in **10** led to substantial decrease in the antimalarial activity (**9** IC₅₀ = 377 nM and **10** IC₅₀ = 1347 nM versus **7** IC₅₀ = 32 nM against D6) against all *P. falciparum* strains (Table 1), suggesting that halogen at the 3 position of ring-A is required for potent antimalarial activity and the size of the halogen also has impact on potency (potency order of substituents: Br > Cl > F >> H). We then investigated the effect of various substituents at the 6 position of ring-B on antimalarial activities. A series of acridones **11–15**, where the (diethylamino)ethoxy group at the 6

position of acridone **7** is replaced with various (dialkylamino)alkoxy moieties, was generated and tested for their in vitro antimalarial activity. Acridone **11** with the (dimethylamino)ethoxy moiety at the 6 position on ring-B showed roughly 2-fold decrease in activity as compared to **7** (**11** IC₅₀ = 56 nM versus **7** IC₅₀ = 32 nM against D6). In contrast, acridone **12** with the (dipropylamino)ethoxy moiety at the 6 position on ring-B showed increased potency over **7** (**12** IC₅₀ = 2.5 nM versus **7** IC₅₀ = 32 nM against D6). Significant loss of potency was observed for acridones **13**, **14** and **15**, containing ethoxypyrrolidin, (diethylamino)propoxy and (diethylamino)pentylloxy moieties, respectively. These results demonstrate that the length and nature of the alkyl chain of (dialkylamino)alkoxy moieties at the 6 position play a crucial role for antimalarial potency. Replacing the chlorine atom of acridones **7** and **12** with trifluoromethyl (CF₃) moiety as in **23** and **24** dramatically decreased the antimalarial activity (**23** IC₅₀ = 816 nM versus **7** IC₅₀ = 32 nM and **24** IC₅₀ = 26 nM versus **12** IC₅₀ = 2.5 nM against D6). This work suggested that the bulky moiety (CF₃) at the 3 position on ring-A had adverse effect on antimalarial activity.

Investigations on the 2 position of ring-A produced acridones **29–32**, where the chlorine atom is shifted from the 3 position to the 2 position on ring-A. Interestingly, the effect of varying the substituent on ring A depended to a degree on the ring B substituent. Thus shifting the Cl atom from the 3 position to the 2 position as in **29**, a positional isomer of **7**, led to reduced potency (**29** IC₅₀ = 212 nM versus **7** IC₅₀ = 32 nM against D6), whilst acridone **30**, which is a positional isomer of **12**, retained potency (**30** IC₅₀ = 3.6 nM versus **12** IC₅₀ = 2.5 nM against D6). It is noteworthy that the activity was retained even after replacing the (dialkylamino)alkoxy moieties at the 6 position of ring-B with chloropropoxy moiety as in **31** (IC₅₀ = 24 nM against D6). Significant loss of antimalarial potency was observed when the Cl atom at the terminal carbon of acridone **31** was replaced with *tert*-butylamine as in **32** (IC₅₀ = 674 nM against D6). SAR analyses of these acridones demonstrate that the nature of substituents on ring-A and the (dialkylamino)alkoxy moieties on ring-B play a pivotal role for antimalarial potency.

In Vitro Blood Stage Activity and SAR of Acridones with 1,3-Disubstitutions on Ring-A and a 6-Substitution on Ring-B.

Having determined the impact of substituents on the antimalarial activity of acridones that contain a halogen either at the 2 or 3 position of ring-A and an (alkylamino)alkyl ether at the 6 position of ring-B, we subsequently tested a hypothesis that two halogens on ring-A might represent an opportunity to make more potent and selective antimalarials. Therefore, we have generated a large number of acridones **38–55**, **62** and **63** with ring-A substituted either with two chlorine/fluorine atoms or a combination of halogen and methoxy moieties at the 1 and 3 positions and ring-B substituted with a variety of hydrophilic and/or hydrophobic moieties at the 6 position and subsequently tested their antimalarial potentials against a panel of *P. falciparum* strains (Table 2). Di-halogenated acridones **38–42** with various chloroalkyl ethers at the 6 position of ring-B exhibited excellent potency against all *P. falciparum* strains (IC₅₀ = 0.12–14 nM against D6) with high selectivity index (Table 2). 1,3-Difluoro acridones **43** and **44**, where ring-B is substituted with trifluorobutoxy and hexyloxy moieties at the 6 position, respectively, showed substantially higher potency (>10-fold) than

that of the chloroalkyl ether containing acridones **38–42** (Table 2). These results demonstrated that elongation of the chloroalkyl/alkyl ethers chain at the 6 position of ring-B (from chloroethyl ether; **38** IC₅₀ = 14 nM to hexyloxy ether; **44** IC₅₀ = 0.026 nM against D6) led to increase in antimalarial activity, regardless the di-chloro and/or di-fluoro substitutions on ring-A.

After establishing the optimal di halogen substituents pattern at the 1 and 3 positions on ring-A and chloroalkyl/alkyl ethers at the 6 position of ring-B, we focused further on exploring hydrophilic moieties at the 6 position of ring-B. A set of di-halogenated acridones **45–49**, where ring-B is substituted with various (dialkylamino)ethoxy moieties at the 6 position, were generated and screened for their antimalarial activity. Notably, di-halogenated acridones **45–49** were highly potent (IC₅₀ values as low as 0.00030 nM against D6, Table 2) compared to mono-halogenated acridones **7, 8** and **12** (Table 1) and di-halogenated acridones **38–42** (Table 2). Specifically, acridones **47–49** with (dipropylamino)ethoxy and (di-trifluoropropylamino)ethoxy moieties showed the highest potencies with picomolar IC₅₀ values. These results demonstrate that dipropylamine as part of hydrophilic moiety at the 6 position of ring-B plays a vital role in enhancing antimalarial potency. We also sought to explore the elongation of the alkyl chain between oxygen and dialkylamine as a part of hydrophilic moiety at the 6 position preparing three di-halogenated acridones **50–52** (Table 2). Although acridones **50–52** maintained relatively good activity against all *P. falciparum* strains (IC₅₀ < 32 nM against D6), analogues **50–52** showed a significant decline in activity (**50** IC₅₀ = 19 nM versus **45** IC₅₀ = 8.2 nM; **51** IC₅₀ = 32 nM versus **46** IC₅₀ = 7.8 nM; **52** IC₅₀ = 0.35 nM versus **48** IC₅₀ = 0.00030 nM against D6) comparing with their homologous acridones **45, 46** and **48**. Together these results again demonstrate that the length of alkyl chain between oxygen and dialkylamine as in a part of hydrophilic moiety at the 6 position plays an important role and it appears that two carbon chain length is optimal for antimalarial potency. We next investigated the effect of (monoalkylamino)alkoxy moieties at the 6 position. Replacement of (dialkylamino)alkoxy moieties with the (monoalkylamino)alkoxy moieties as in **53–55** led to reduced potency in general (Table 2). The biggest potency loss occurred when we introduced methoxy (OMe) group either at the 1 or 3 position on ring-A as in **62** and **63**. It appears that the OMe group on ring-A has an adverse effect, specifically at the 1 position (**62** IC₅₀ = 0.65 nM versus **47** IC₅₀ = 0.0050 nM; and **63** IC₅₀ = 251 nM versus **47** IC₅₀ = 0.0050 nM against D6). In general, SAR analyses demonstrate that di-halogen substitutions at the 1 and 3 positions of ring-A and (dialkylamino)ethoxy moieties at the 6 position of ring-B are well tolerated (Table 1 and 2).

In Vitro Blood Stage Activity and SAR of Acridones with 1,2-Dichloro-Substitutions on Ring-A and 6-Substitution on Ring-B.

Exploration of the SARs on ring-A and ring-B of acridones indicated that halogens at the 1 and 3 positions on ring-A and (dialkylamino)ethoxy moieties at the 6 position of ring-B were greatly favored. We next investigated the effect of halogens at the 1 and 2 positions on ring-A. We synthesized a series of 1,2-dichloro acridones **70–81** (Table 3) with various chloroalkyl/(dialkylamino)alkoxy moieties at the 6 position of ring-B. It is noteworthy that most of the 1,2-dichloro acridones **70–81** (Table 3) have shown great potency with low IC₅₀ values against all *P. falciparum* strains. However, they were significantly less potent than the

corresponding 1,3-dichloro/fluoro acridones **38–55** (Table 2 versus Table 3). More interestingly, these acridones **70–81**, followed the similar SAR trend as we observed for 1,3-dichloro/fluoro acridones **38–55** and it was consistent across all the acridone analogues (see Table 2 versus Table 3). These findings demonstrate that 1,2-dichloro substitutions on ring-A and chloroalkoxy/(dialkylamino)ethoxy moieties at the 6 position of ring-B are well tolerated.

In Vitro Blood Stage Activity and SAR of Acridones with 2,3-Disubstitutions on Ring-A and 6-Substitution on Ring-B.

Exploration of the SARs around ring-A of acridones indicated that di-substitutions at the 1,2 and 1,3 positions (Table 2 and Table 3) were greatly favored compared to the mono substitutions either at the 2 or 3 position of ring-A (Table 1). We next investigated the effect of di substituents at both the 2 and 3 positions. We generated a series of 2,3-disubstituted acridones **82–86**, **92**, **93**, **98** and **99** (Table 4). Of these acridones, **82–84** with 2,3-dichloro substitutions on ring-A and chloroalkoxy/(dialkylamino)alkoxy moieties at the 6 position on ring-B showed diminished activity compared with those of the corresponding 1,3-dichloro acridones (**82** IC₅₀ = 179 nM versus **40** IC₅₀ = 2.2 nM; **83** IC₅₀ = 44 nM versus **45** IC₅₀ = 8.2 nM against D6) and 1,2-dichloro acridones (**82** IC₅₀ = 179 nM versus **71** IC₅₀ = 3.2 nM; **83** IC₅₀ = 44 nM versus **73** IC₅₀ = 10 nM; **84** IC₅₀ = 3.4 nM versus **75** IC₅₀ = 0.022 nM against D6). 2,3-Dichloro acridones **85** and **86** with (*tert*-butylamino)propyloxy, and (*n*-butylamino)propyloxy moieties, respectively, showed slightly increased potencies (Table 4) as compared with those of the corresponding 1,3-dichloro/difluoro acridones (**85** IC₅₀ = 123 nM versus **53** IC₅₀ = 101 nM; **86** IC₅₀ = 36 nM versus **54** IC₅₀ = 115 nM against D6) and 1,2-dichloro acridones (**85** IC₅₀ = 123 nM versus **79** IC₅₀ = 196 nM; and **86** IC₅₀ = 36 nM versus **80** IC₅₀ = 46 nM against D6). The greatest loss of potency was observed in 2,3-difluoro acridone **92** when compared to the corresponding 1,3-difluoro/dichloro acridones (**92** IC₅₀ = 146 nM versus **46** IC₅₀ = 7.8 nM and **45** IC₅₀ = 8.2 nM against D6), 1,2-dichloro acridone (**92** IC₅₀ = 146 nM versus **73** IC₅₀ = 10 nM against D6) and 2,3-dichloro acridone (**92** IC₅₀ = 146 nM versus **83** IC₅₀ = 44 nM against D6). The adverse effect of substitutions at both the 2 and 3 positions on ring-A was further confirmed by the introduction of (diethylamino)ethoxy and OMe moieties at the 3 position as with the analogues **93** and **98**, respectively, which have an IC₅₀ of >116 nM against all *P. falciparum* strains (Table 4), suggesting that di-substitutions at the 2 and 3 positions are not preferred. Replacement of (diethylamino)ethoxy moiety at the 6 position of ring-B of **98** with (diethylamino)ethylamino group as with acridone **99**, led to further reduced potency (**99** IC₅₀ = 526 nM versus **98** IC₅₀ = 156 nM against D6). These findings demonstrate that the alkoxy moieties are well tolerated at the 6 position of ring-B.

To probe all possible di-substitution pattern on potency, we have prepared three dichloro representative analogues **100–102**, where chlorine atoms were substituted at 1,4/2,4/3,4 positions, respectively. It is noteworthy that the antimalarial potency significantly declined (> 10 fold) after shifting chlorine substitutions on ring-A from the 1,2/1,3/2,3 to 1,4/2,4/3,4 positions as in the acridones **100–102**, respectively (Table 4). These findings suggested that substitution at the 4 position on ring-A had an adverse effect on potency. Together, SAR analyses of the acridones (Tables 1–4) demonstrated that di-halogen substitutions at the 1,2

and 1,3 positions on ring-A and (dialkylamino)ethoxy moieties at the 6 position on ring-B are best tolerated.

In Vitro Blood Stage Activity and SAR of Acridones with the Substitutions at the 7 position on Ring-B.

Having identified the key pharmacophores at the 6 position of ring-B for enhanced antimalarial potency, we explored the effect of these substituents on the 7 position of ring-B. A series of acridones **109–113**, **119**, **120**, **123** and **128**, where ring-A is substituted either with halogens or a combination of halogen and alkoxy groups and ring-B is substituted with (dipropylamino)ethoxy and (diethylamino)ethoxy moieties at the 7 position, was generated and screened for their antimalarial activity (Table 5). Of these 7-substituted acridones, analogues **109** and **110** with the disubstitutions at the 1 and 3 positions on ring-A, exhibited greatest potency ($IC_{50} < 1.0$ nM against D6) (Table 5). However, the potency of these compounds **109** and **110**, significantly declined when compared to the corresponding isomer **48** (**109** $IC_{50} = 0.40$ nM, **110** $IC_{50} = 0.70$ nM versus **48** $IC_{50} = 0.00030$ nM against D6). Interestingly, interchange of fluorine and methoxy groups between the 1 and 3 positions on ring-A as in **111** resulted in a 50-fold decrease in potency (**111** $IC_{50} = 35$ nM versus **110** $IC_{50} = 0.70$ nM against D6). Again this result demonstrates that the methoxy group has an adverse effect at the 1 position on ring-A. Replacement of fluorine at the 1 position of **110** with chlorine as in **112**, showed a roughly 100-fold drop in activity (**112** $IC_{50} = 79$ nM versus **110** $IC_{50} = 0.70$ nM against D6). Compound **112** also exhibited diminished potency (>100-fold) when compared to the corresponding isomer **62** (**112** $IC_{50} = 79$ nM versus **62** $IC_{50} = 0.65$ nM against D6). Conversely, acridone **113**, showed better activity when compared to the corresponding positional isomers **63** and **112** (**113** $IC_{50} = 30$ nM versus **63** $IC_{50} = 251$ nM and **112** $IC_{50} = 79$ nM against D6). The greatest loss of potency (> 100-fold) was observed in two 7-substituted acridones **119** and **120** as compared to the corresponding positional isomer **75** (**119** $IC_{50} = 99$ nM, **120** $IC_{50} = 26$ nM versus **75** $IC_{50} = 0.022$ nM against D6). 7-(Diethylamino)ethoxy acridone **123**, where ring-A is substituted with one chlorine atom at the 2 position, showed a better activity than the corresponding isomer **29** (**123** $IC_{50} = 159$ nM versus **29** $IC_{50} = 212$ nM against D6), while acridone **128**, showed ~4-fold lesser potency than the corresponding isomer **7** (**128** $IC_{50} = 110$ nM versus **7** $IC_{50} = 32$ nM against D6). Cross-resistance patterns against ATV-resistant Tm90-C2B vary significantly between positional isomers, with acridones having substitutions at the 7 position on ring-B demonstrating similar or equal potency across the *P. falciparum* test panel (Table 1 to Table 5).

In Vitro Liver Stage Antimalarial Activity.

The in vitro liver stage activity of acridone derivatives was assessed utilizing luciferase-expressing *P. berghei* sporozoite infected human hepatocyte HepG2 cells^{33, 34} and the results are summarized in Table 6. A number of acridone analogues exhibited potent activity (some superior to ATV) against the liver stage parasite, without toxic effect to the host liver cells. Of these screened compounds, acridones **47–49**, **52**, **62**, **73–75**, **84**, **109** and **110**, showed the highest liver stage antimalarial potencies (IC_{50} range 0.090–7.8 nM). Interestingly, acridone

derivatives exhibited superb activities in this liver stage in vitro assay regardless of the different cross-resistance pattern against blood stage parasites Tm90-C2B.

In Vivo Blood Stage Efficacy in Rodent Malaria Models.

Given the remarkable antimalarial potencies of several acridones against various *P. falciparum* strains along with favorable toxicological properties against HepG2 cells, in vivo studies in a murine *P. yoelii* model^{23, 31, 32} were undertaken with the most potent and selective (Selective Index > 1000) acridones **7**, **44–50**, **52** and **73–75**, with CQ as a reference drug (Table 7). In these in vivo experiments, animals were randomly placed in groups of four and administered test drugs by oral gavage on 4 sequential days following the day of inoculation. The in vivo data are expressed as ED₅₀ and ED₉₀ values and reflect the dose (estimated from dose–response curves) for suppression of parasitemia by 50% and 90%, respectively, relative to vehicle-only controls as assessed on day 5 of each study. A number of acridones showed very promising in vivo oral efficacy (ED₅₀ and ED₉₀ values are shown in Table 7). Significantly, the acridones **48** (T36), **74** (T35) and **75** (T31), were curative in this model after 80 mg/kg × 4 days dosing. No overt clinical toxicity and/or behavior change was observed in mice treated with these acridones (highest dose tested was 80 mg/kg/d × 4).

In Vivo Liver Stage Efficacy in Rodent Malaria Model.

The assessment in vivo liver stage efficacy was conducted with real-time in vivo imaging system (IVIS), utilizing transgenic bioluminescent parasite^{33, 35, 36}. Mice were inoculated with 50000 luciferase-expressing *P. berghei* sporozoite intravenously (*i.v.*) through the tail vein on day 0, oral doses of acridones were administered on day –1, day 0, and day 1. The animals underwent imaging of luciferase activity at 24 and 48 h after the inoculation for liver stage development and at 72 h for blood stage infection. As illustrated in Figure 3, strong bioluminescence signals were detected in untreated mice at both 24 and 48 h in the area overlying the liver, followed by intense signal in the whole body of the mice, resulting from infection in the peripheral blood circulation. Acridones **7** (T2) and **73** (T13) provided full protection and cure at 160 mg/kg and 40 mg/kg, respectively. Significantly, acridones **48** (T36), **52** (T43), **74** (T35) and **75** (T31), provided full protection and cure at 10 mg/kg with no or negligible parasite visible in the liver of any of the tested mice at 24 h and 48 h, indicating true causal prophylactic efficacy (Figure 3). Notably, all treated mice were cured with no blood stage parasites to day 30. Indeed, the acridone **75** (T31) was able to provide full protection and blood stage cure even at 4 mg/kg (Figure 3). These results are comparable or superior to primaquine (full protection/cure at 25 mg/kg).

In Vitro Cardiotoxicity and Mutagenicity.

The in vitro effect of lead acridones on the hERG (*human-ether-a-go-go-related gene*) potassium channel current expressed in mammalian cells was evaluated using an automatic parallel patch clamp system (Eurofins Inc.) and the results are listed in Table 8. Some of the lead compounds demonstrated hERG inhibition level lower than CQ with IC₅₀ values higher than 10 μM, which is highly encouraging. Mutagenicity assessment of several lead acridones were conducted using the Ames assay (EPBI Inc.) at concentrations up to 10 μM, with and without S9 activation, against *Salmonella typhimurium* TA100 and TA98. Results were

negative; there was no increase over the background reversion rate with any of the tested acridones.

Metabolic Stability and Pharmacokinetic (PK) Studies.

The metabolic stability of the lead acridones was assessed using mouse and human liver microsomes^{37–39}. Most of the lead acridones appear to be stable for up to 60 minutes in both human and mouse microsomes (Table 9). In vivo pharmacokinetic (PK) study of lead acridones **7** (T2), **48** (T36), **52** (T43), **74** (T35) and **75** (T31) was also conducted following a single intragastric (*p.o.*) administration in ICR male mice at 80 mg/kg, with blood and liver samples taken at the following time points: 0, 0.5, 1, 2, 4, 7, 24, 48 and 72 h^{33, 39}. The key PK parameters of the lead acridones are summarized in Table 10. The PK data is supportive of the observed liver stage efficacy, as it is evident that acridones demonstrating strong prophylactic efficacy concentrate in the liver, with high liver/plasma ratio.

In Vitro 73-Resistance Selection and Acridone Target Validation.

To identify a potential target(s) of acridone inhibition in *P. falciparum*, we used in vitro resistance selection with acridone **73** followed by whole genome sequencing, an approach that has been used successfully on multiple occasions for putative target identification in malaria parasites⁴⁰. Several independent clonal lines of Dd2 and 3D7 were pressured for ~180 days with incrementally increasing concentrations of **73** up to 900 nM. Selections resulted in three resistant lines derived from Dd2 and one from 3D7. Whole genome sequencing of parental Dd2 line and two independent clones from the first **73** selection experiment on Dd2 showed a G→T nucleotide change in *pfcytb* (mal_mito_3; position 4266) corresponding to a V259L polymorphism (Dd2^{V259L}), which has been reported elsewhere in parasites selected for resistance to quinolones^{41, 42}. Relative to the Dd2 parental line and the 3D7 reference line, only three other SNPs were detected from genes encoding proteins not involved in antigenic variation (Supplementary Table S1). These included a conserved *Plasmodium* membrane protein of unknown function (A→G, position 629354, PF3D7_0615300), a putative RNA helicase (A→G position PF3D7_0615300) and an ApiAP2 transcription factor (C→T, position 330499, PF3D7_1107800). Given hypotheses that first-generation liver stage active acridones may target cytochrome B and selection of V259L with quinolones, these latter genes were not explored further. We therefore Sanger sequenced *pfcytb* from all other **73**-resistant lines. Alignment of sequences with parental lines revealed *pfcytb* non-synonymous SNPs in each case, resulting in three additional mutant alleles—two derived from Dd2 (Dd2^{M133I} and Dd2^{M133I-A138T}; Table 11) and one from 3D7 (3D7^{A82T+V259L}; Table 11). The M133I polymorphism have been previously selected in both *P. falciparum* and *P. berghei cytb* genes using ATV or other quinolones^{41, 43, 44}. Continued **73** selection on the Dd2^{M133I} line resulted in the acquisition of the second *pfcytb* mutation, yielding the Dd2^{M133I-A138T} line, while the 3D7^{A82T+V259L} line was discovered on our initial sampling of the **73** pressured 3D7 culture. To our knowledge, the A138T and A82T polymorphisms have not been previously reported. The Dd2^{V259L} clone had an IC₅₀ value of 1439 nM, or 36-fold greater than that of the parental Dd2 line (Table 11). The Dd2^{M133I} line showed modest **73** resistance at a 242 nM IC₅₀ or 6-fold increase. The acquisition of an additional A138T mutation increased resistance to an IC₅₀ of 1678 nM a 42-fold increase. Given that both M133I and A138T is predicted to occur in the

Q_o binding site of *PfCytB*⁴¹, the high IC₅₀ value for the double mutant is not surprising. Our results showed strikingly similar decreased sensitivity changes to ATV in response to single mutations of V259L or M133I with those reported by Lane *et al.*⁴¹, further supporting the role of PfCYTB in our reported drug response changes. The 3D7 double mutant, 3D7^{A82T-V259L} had a **73** IC₅₀ of 1518 nM, or 54-fold higher than the parental line. A82T is located in the second transmembrane domain but not in the Q_o site. Thus, the dominant effects on acridone decreased susceptibility are likely imparted by V259L, further supported by the comparable IC₅₀ results from the single mutant, Dd2^{V259L} (Table 11). The **73**-resistant parasites grown for up to three months without drug pressure had stable phenotypes when retested for susceptibility against a panel of acridones (data not shown). Additionally, no changes in susceptibilities were observed among the resistant clones to control antimalarial drugs chloroquine, mefloquine, lumefantrine and piperazine, relative to parental control lines (Table 11). None of these drugs are known to act against the *P. falciparum* cytochrome B.

Although these observations from preliminary mechanistic studies do not identify the site(s) of action, they suggest a complex mode of action for broad-spectrum acridones, including but not limited to inhibition of the parasite electron transport chain. The *cyt bc1* of the mitochondrial electron transport chain is a validated target and considered a “double-edge sword” for multi-stage antimalarial therapy, particularly given the recent discovery of the inability of malaria parasites to spread atovaquone resistance in the mosquito phase⁴⁵.

CONCLUSIONS

We report here the synthesis and antimalarial activities of various series of acridones against both blood stage and liver stage malaria. Acridone derivatives were synthesized via simple and inexpensive chemical procedures using easily available building blocks to respond to the demand for low-cost novel antimalarials. Several key findings emerged from these studies, specifically, a number of the acridones were (i) effective at low nanomolar concentrations against blood stage and liver stage parasites, (ii) curative in erythrocytic murine malaria model after oral administration and (iii) able to provide full protection and cure in sporozoite-induced infection in mice. In addition, lead acridones exhibited a favorable toxicological, metabolic and pharmacokinetic profiles. Our detailed SAR profiling of different substituents on ring-A and ring-B of acridones demonstrated that di-halogens either at the 1, 2 or 1, 3-positions on ring-A and (dialkylamino)alkoxy moieties at the 6 position on ring-B are required for the optimal antimalarial potency. Acridones **48** (T36), **74** (T35) and **75** (T31) have shown very promising in vivo efficacy against both blood stage and liver stage malaria at low oral doses. The preliminary drug resistance selection studies demonstrated a complex mode of action for broad-spectrum acridones. Our future optimization studies will focus on producing the second-generation of the novel acridone antimalarial chemotype that maintains efficacy against both blood stage and liver stage *Plasmodia*, with the ability to circumvent clinical atovaquone resistance and improved safety profiles.

The novel acridone chemotype described herein emphasizes a multi-stage targeting approach, highlighting compounds with efficacy against liver stage malaria to kill *Plasmodium* parasites before it reaches the blood stream where it can weaken and kill the

host^{18, 20–22}. It is noteworthy that a recent open-source screening of over 500000 compounds identified several acridone scaffolds as chemoprotective “hits”⁴⁶. The novel acridones described here are structurally distinct but it will be interesting to see whether there is a convergent path toward an optimized antimalarial acridone entity in the future. The ability to combat multiple stages (both exoerythrocytic and erythrocytic) of the infection represents a powerful tool, and one ideally suited to achieve the broadest possible benefit as a renewed malaria eradication effort proceeds.

EXPERIMENTAL SECTION

General.

NMR spectra were recorded on Bruker AMX-400 and AMX-600, spectrometers at 400 and 600 MHz, respectively. Experiments were recorded in CDCl₃ and DMSO-*d*₆ at 25 °C. Chemical shifts are given in parts per million (ppm) downfield from internal standard Me₄Si (TMS). HRMS (ESI) were recorded on a high-resolution (30000) thermo LTQ-Orbitrap Discovery hybrid mass spectrometer (San Jose, CA). Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions which required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of argon/nitrogen. Chromatography was executed on Biotage-Isolera and/or CombiFlash instruments, using silica gel (230–400 mesh) and/or neutral alumina as the stationary phase and mixtures of ethyl acetate (EtOAc) and hexane or dichloromethane (DCM) and methanol as eluents. Analytical HPLC analyses were performed on a Supelco Discovery HS C18 column (4.6 mm × 250 mm) with a linear elution gradient of water/methanol (containing 10 mM ammonium acetate) ranging from 80:20 to 0:100 for 50 min at a flow rate of 1 mL/min, at 254 nm. A purity of >95% has been established for all tested compounds.

Representative procedure for the synthesis of 4-chloro-2-((3-methoxyphenyl)amino)-benzoic acid (**3a**).

To a stirred suspension of *m*-anisidine (**2**) (18.5 g, 150.0 mmol), K₂CO₃ (27.6 g, 200.0 mmol) and Cu powder (0.12 g, 1.8 mmol) in pentanol (150 mL) at room temperature was added 2,4-dichlorobenzoic acid (**1a**) (19.1 g, 100.0 mmol), and the reaction mixture was refluxed for 5 h. Water was then added and the mixture was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with brine and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was recrystallized in methanol to yield the title compound **3a** (24.4 g, 88%) as a pale green solid. ¹H NMR (DMSO-*d*₆, 600 MHz) δ 13.31 (br s, 1H), 9.70 (s, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.32 (t, *J* = 8.1 Hz, 1H), 7.13 (s, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.85–6.69 (m, 2H), 6.74 (dd, *J* = 8.3, 2.3 Hz, 1H), 3.77 (s, 3H); HRMS (ESI) calcd for C₁₄H₁₃ClN₁O₃ (M + H)⁺ 278.0578, found 278.0564. Compounds **3b–c** were synthesized (65–85%) by the same procedure as described for **3a** and their identity was confirmed by NMR and GC-MS analyses.

Representative procedure for the synthesis of 3-chloro-6-methoxyacridin-9(10*H*)-one (5a).

120 mL of Eaton's acid was added to a flask containing **3a** (15.0 g, 54.2 mmol). The reaction mixture was then stirred at 90 °C for 5 h. After cooling to room temperature, water was added slowly to the flask and allowed to stir for 15 min and the solid material was filtered by Sintered funnel, and washed with water (500 mL). The pure compound **5a** (7.8 g, 55%) was obtained by recrystallization in DMF/CH₃OH (4:1). It is noteworthy that the minor isomer **4a**, was also observed in the crude material, however, our focus was on the major isomer **5a**. ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.68 (br s, 1H), 8.18 (d, *J* = 8.6 Hz, 1H), 8.14–8.08 (m, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.90–6.86 (m, 2H), 3.91 (s, 3H). Methoxy-acridones **5b–c** were synthesized in good yields (42–50%) by the same procedure as described for **5a**.

Representative procedure for the synthesis of 3-chloro-6-hydroxyacridin-9(10*H*)-one (6a).

To a stirred solution of **5a** (5.0 g, 17.98 mmol) in 100 mL of 57% hydriodic acid (HI) at room temperature was added phenol (27.2 g, 289.3 mmol), and the reaction mixture was stirred at reflux for 3 h. After cooling to room temperature, water was added slowly to the flask and the mixture was extracted with ethyl acetate (3 × 200 mL). The combined organic phases were washed with saturated NaHSO₃ solution (200 mL), and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure to yield the title compound **6a** (4.38 g, 93%) as a pale yellow solid. ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.51 (br s, 1H), 10.56 (s, 1H), 8.15 (d, *J* = 8.6 Hz, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 2.0 Hz, 1H), 7.22 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.90–6.86 (m, 2H); HRMS (ESI) calcd for C₁₃H₈ClN₁O₂ (M + H)⁺ 246.0316, found 246.0323. Hydroxy-acridones **6b–c** were synthesized in excellent yields (85–90%), by the same procedure as described for **6a**.

Representative procedure for the synthesis of 3-chloro-6-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (7).

To a stirred solution of **6a** (1.0 g, 4.08 mmol) in anhydrous acetone (50 mL) at room temperature were added K₂CO₃ (5.63 g, 40.08 mmol) and 2-(diethylamino)ethyl chloride hydrochloride (772 mg, 4.48 mmol), and the reaction mixture was stirred at reflux for 5 h. The mixture was then filtered and the solvent was removed by rotary evaporation. The crude product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired acridone **7** as a pale yellow solid (1.07 g, 76%). ¹H NMR (DMSO-*d*₆, 600 MHz) δ 11.64 (s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.11 (d, *J* = 9.1 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 7.24 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.89–6.86 (m, 2H), 4.15 (t, *J* = 6.1 Hz, 2H), 2.84 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁ClN₂O₂ (M + H)⁺ 345.1364, found 345.1366. **Note.** Most of the (dialkylamino)alkyl chlorides were obtained from commercial sources except the following (dialkylamino)alkyl chlorides that were prepared by adopting literature procedures^{47, 48}.

Synthesis of *N*-(2-chloroethyl)-*N*-propylpropan-1-amine hydrochloride.

A solution of 2-chloroethanol (5.00 g, 62.1 mmol) and dipropylamine (6.27 g, 62.1 mmol) in 25 mL of anhydrous toluene was refluxed for 3.5 h. Then the reaction mixture was brought to 0 °C and SOCl₂ (11.08 g, 93.2 mmol) was added dropwise, and the temperature of the

mixture was then gradually raised to 90 °C over 1 h period. Consumption of the 2-(dipropylamino)ethan-1-ol intermediate was confirmed by GC-MS analysis. Concentration of all solvents in vacuo gave the desired *N*-(2-chloroethyl)-*N*-propylpropan-1-amine hydrochloride in good yield (10.2 g, 82%). The product was used in subsequent reactions without further purification. The 3-chloro-*N,N*-dipropylpropan-1-amine hydrochloride was also prepared using the same procedure. These compounds were stored at –20 °C until further use.

Synthesis of 3-chloro-*N,N*-diethylpropan-1-amine.

To a stirred solution of SOCl₂ (30.8 mL, 422 mmol) in anhydrous chloroform (250 mL) at 0 °C was added gradually a solution of 3-(diethylamino)propan-1-ol (22.1 g, 168 mmol) in 35 mL chloroform. Then the reaction mixture was heated to reflux for 2 h. The solvent and excess SOCl₂ were removed in vacuo and the residue was cautiously treated with 20% NaOH solution (50 mL), extracted with diethyl ether (3 × 50 mL), dried over Na₂SO₄, and concentrated in vacuo. The product 3-chloro-*N,N*-diethylpropan-1-amine was obtained as a light yellow oil (22.9 g, 72%) and stored at –20 °C until further use.

Synthesis of acridones 8–15.

The final compounds **8–15** were synthesized in good yields (50–65%) by the same procedure as described for **7**.

3-Bromo-6-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (**8**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 10.32 (br s, 1H), 8.16 (d, *J* = 9.0 Hz, 1H), 8.10 (d, *J* = 8.6 Hz, 1H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.38 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.95 (dd, *J* = 9.0, 2.3 Hz, 1H), 4.51 (t, *J* = 4.6 Hz, 2H), 3.60 (t, *J* = 4.5 Hz, 2H), 3.28–3.20 (m, 4H), 1.27 (t, *J* = 7.2 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₂BrN₂O₂ (M + H)⁺ 389.0859, found 389.0856.

3-Fluoro-6-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (**9**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.65 (s, 1H), 8.24 (dd, *J* = 8.9, 6.5 Hz, 1H), 8.11 (d, *J* = 9.5 Hz, 1H), 7.20 (dd, *J* = 10.4, 2.3 Hz, 1H), 7.08 (m, 1H), 6.88–6.85 (m, 2H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₂FN₂O₂ (M + H)⁺ 329.1660, found 329.1661.

3-(2-(Diethylamino)ethoxy)acridin-9(10*H*)-one (**10**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.57 (s, 1H), 8.19 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.12 (d, *J* = 8.9 Hz, 1H), 7.69 (m, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.23 (m, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.84 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.14 (t, *J* = 6.2 Hz, 2H), 2.84 (t, *J* = 6.1 Hz, 2H), 2.58 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₃N₂O₂ (M + H)⁺ 311.1754, found 311.1771.

3-Chloro-6-(2-(dimethylamino)ethoxy)acridin-9(10*H*)-one (**11**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.68 (d, *J* = 0.3 Hz, 1H), 8.18 (d, *J* = 8.6 Hz, 1H), 8.12 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 1.9 Hz, 1H), 7.25 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.90–6.86 (m,

2H), 4.20 (t, $J = 5.8$ Hz, 2H), 2.69 (t, $J = 5.8$ Hz, 2H), 2.25 (s, 6H); HRMS (ESI) calcd for $C_{17}H_{18}Cl_1N_2O_2$ ($M + H$)⁺ 317.1051, found 317.1062.

3-Chloro-6-(2-(dipropylamino)ethoxy)acridin-9(10*H*)-one (12).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.65 (s, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.11 (d, $J = 9.6$ Hz, 1H), 7.49 (d, $J = 1.9$ Hz, 1H), 7.24 (dd, $J = 8.6, 2.0$ Hz, 1H), 6.87–6.85 (m, 2H), 4.13 (t, $J = 6.0$ Hz, 2H), 2.83 (t, $J = 6.0$ Hz, 2H), 2.45 (t, $J = 7.2$ Hz, 4H), 1.42 (m, 4H), 0.85 (t, $J = 7.3$ Hz, 6H); HRMS (ESI) calcd for $C_{21}H_{25}Cl_1N_2O_2$ ($M + H$)⁺ 373.1677, found 373.1687.

3-Chloro-6-(2-(pyrrolidin-1-yl)ethoxy)acridin-9(10*H*)-one (13).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.67 (br s, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.11 (d, $J = 8.8$ Hz, 1H), 7.50 (d, $J = 1.8$ Hz, 1H), 7.24 (dd, $J = 8.6, 2.0$ Hz, 1H), 6.90–6.86 (m, 2H), 4.21 (t, $J = 5.8$ Hz, 2H), 2.85 (t, $J = 5.8$ Hz, 2H), 2.54 (m, 4H), 1.69 (m, 4H); HRMS (ESI) calcd for $C_{19}H_{20}Cl_1N_2O_2$ ($M + H$)⁺ 343.1208, found 343.1212.

3-Chloro-6-(3-(diethylamino)propoxy)acridin-9(10*H*)-one (14).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.69 (br s, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.10 (d, $J = 9.2$ Hz, 1H), 7.49 (s, 1H), 7.23 (d, $J = 8.2$ Hz, 1H), 6.89–6.85 (m, 2H), 4.13 (t, $J = 5.9$ Hz, 2H), 2.55 (t, $J = 6.5$ Hz, 2H), 2.46 (q, $J = 7.0$ Hz, 4H), 1.91–1.85 (m, 2H), 0.95 (t, $J = 6.9$ Hz, 6H); HRMS (ESI) calcd for $C_{20}H_{24}Cl_1N_2O_2$ ($M + H$)⁺ 359.1521, found 359.1529.

3-Chloro-6-((5-(diethylamino)pentyl)oxy)acridin-9(10*H*)-one (15).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.67 (br s, 1H), 8.17 (d, $J = 8.6$ Hz, 1H), 8.11 (d, $J = 8.8$ Hz, 1H), 7.50 (s, 1H), 7.24 (d, $J = 8.6$ Hz, 1H), 6.88–6.86 (m, 2H), 4.11 (t, $J = 5.7$ Hz, 2H), 2.62 (q, $J = 6.8$ Hz, 4H), 1.91–1.79 (m, 4H), 1.51–1.45 (m, 4H), 1.00 (t, $J = 6.6$ Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{28}Cl_1N_2O_2$ ($M + H$)⁺ 387.1834, found 387.1835.

Representative procedure for the synthesis of methyl 4-methoxy-2-(((trifluoromethyl)sulfonyl)oxy)benzoate (17).

To a stirred solution of methyl 4-methoxysalicylate (**16**) (85 g, 467 mmol) and pyridine (50 mL) in anhydrous dichloromethane (100 mL) at 0 °C was added dropwise triflic anhydride (Tf₂O) (158 g, 560 mmol). Then the reaction mixture was stirred for 5 h while it was allowed to warm to room temperature. The mixture was poured into ice-cold water (500 mL), and extracted with diethyl ether (3 × 200 mL). The combined organic layers were washed with 2N HCl (3 × 50 mL), brine and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure to afford the desired product **17** (143.7 g, 98%) as a thick syrup. The product **17** was carried forward into the next reaction without further purification. ¹H NMR (CDCl₃, 400 MHz) δ 7.98 (d, $J = 8.9$ Hz, 1H), 6.88 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.70 (d, $J = 2.3$ Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H); HRMS (ESI) calcd for $C_{10}H_{10}F_3O_6S_1$ ($M + H$)⁺ 315.01447, found 315.01569.

Representative procedure for the synthesis of methyl 4-methoxy-2-((3-(trifluoromethyl)phenyl)amino)benzoate (19).

To a degassed solution of **17** (1.00 g, 3.18 mmol) and 3-(trifluoromethyl)aniline (**18**) (615 mg, 3.82 mmol) in anhydrous toluene (20 mL) at room temperature were added Pd(dba)₂ (183 mg, 0.31 mmol), DPPF (353 mg, 0.63 mmol) and KO^tBu (392 mg, 3.50 mmol), and the reaction mixture was heated at 100 °C for 8 h. After cooling to room temperature, the mixture was filtered through a pad of Celite and washed with dichloromethane (100 mL). The combined filtrates were concentrated under reduced pressure and the crude product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the title compound **19** in good yield (765 mg, 74%). ¹H NMR (DMSO-d₆, 400 MHz) δ 9.66 (br s, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.42 (s, 1H), 7.32–7.31 (m, 1H), 7.20 (d, *J* = 6.1 Hz, 1H), 6.63 (d, *J* = 2.2 Hz, 1H), 6.26 (dd, *J* = 9.0, 2.0 Hz, 1H), 3.76 (s, 3H), 3.64 (s, 3H).

Synthesis of 3-methoxy-6-(trifluoromethyl)acridin-9(10*H*)-one (21).

Compound **21** was synthesized along with compound **20** by the same procedure as described for **5a**. The pure compound **21** (69%) was obtained by recrystallization from a mixture of methanol and ethyl acetate (3:6). ¹H NMR (DMSO-d₆, 400 MHz) δ 11.88 (br s, 1H), 8.37 (d, *J* = 8.3 Hz, 1H), 8.15 (d, *J* = 8.9 Hz, 1H), 7.79 (s, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.91 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.86 (d, *J* = 2.1 Hz, 1H), 3.92 (s, 3H).

Synthesis of 3-hydroxy-6-(trifluoromethyl)acridin-9(10*H*)-one (22).

To a stirred solution of **21** (2.26 g, 7.71 mmol) in anhydrous dichloromethane (25 mL) maintained under an Argon atmosphere was added dropwise BBr₃ (9.63 g, 38.5 mmol) at –78 °C. Then the reaction mixture was stirred for 12 h while it was allowed to warm to room temperature. The reaction mixture was quenched with water (50 mL) and the organic layer was evaporated under reduced pressure. The resultant solid material was filtered by Sintered funnel and washed with water (100 mL) to afford the title compound **22** (2.01 g, 93%). ¹H NMR (DMSO-d₆, 400 MHz) δ 11.78 (br s, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.80 (s, 1H), 7.47 (dd, *J* = 8.4, 1.4 Hz, 1H), 6.82 (d, *J* = 2.1 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.2 Hz, 1H).

Synthesis of acridones 23 and 24.

Compounds **23** and **24** were synthesized (70–75%), by the same procedure as described for **7**.

3-Trifluoromethyl-6-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (23).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.87 (br s, 1H), 8.38 (d, *J* = 8.3 Hz, 1H), 8.15 (d, *J* = 8.9 Hz, 1H), 7.81 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 6.93–6.88 (m, 2H), 4.17 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 6.0 Hz, 2H), 2.58 (q, *J* = 7.1 Hz, 4H), 1.00 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₂₀H₂₂F₃N₂O₂ (M + H)⁺ 379.1628, found 379.1632.

3-Trifluoromethyl-6-(2-(dipropylamino)ethoxy)acridin-9(10*H*)-one (24).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.87 (s, 1H), 8.37 (d, *J* = 8.6 Hz, 1H), 8.15 (d, *J* = 8.9 Hz, 1H), 7.80 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 6.92–6.88 (m, 2H), 4.16 (t, *J* = 5.9 Hz, 2H),

2.85 (t, $J = 5.9$ Hz, 2H), 2.46 (t, $J = 7.2$ Hz, 4H), 1.46–1.40 (m, 4H), 0.86 (t, $J = 7.3$ Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{26}F_3N_2O_2$ ($M + H$)⁺ 407.1941, found 407.1943.

Synthesis of 2-chloro-6-hydroxyacridin-9(10*H*)-one (28).

By use of our standardized procedures, as described above for **19** (Buchwald-Hartwig cross coupling (Pd(dba)₂, DPPF, KO^{*t*}Bu)), **5a** (Eaton's acid-mediated cyclization) and **6a** (demethylation (HI/phenol)), the title compound **28** was synthesized in a good yield via the appropriate intermediates **26** and **27** from the triflate **17** and *p*-chloroaniline (**25**). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.61 (s, 1H), 10.56 (s, 1H), 8.04–8.12 (m, 2H), 7.69 (dd, $J = 8.8$, 2.7 Hz, 1H), 7.49 (d, $J = 8.9$ Hz, 1H), 6.80 (d, $J = 2.3$ Hz, 1H), 6.75 (dd, $J = 8.8$, 2.3 Hz, 1H); HRMS (ESI) calcd for $C_{13}H_9ClN_1O_2$ ($M + H$)⁺ 246.0316, found 246.0317.

Synthesis of acridones 29 and 30.

Acridones **29** (36%) and **30** (49%) were generated by the same procedure as described for **7** from **28**.

2-Chloro-6-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (29).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.11–8.09 (m, 2H), 7.69 (dd, $J = 8.9$, 2.6 Hz, 1H), 7.52 (d, $J = 8.9$ Hz, 1H), 6.89 (d, $J = 2.34$ Hz, 1H), 6.86 (dd, $J = 8.9$, 2.3 Hz, 1H), 4.15 (t, $J = 6.1$ Hz, 2H), 2.84 (t, $J = 6.1$ Hz, 2H), 2.57 (q, $J = 7.1$ Hz, 4H), 0.99 (t, $J = 7.1$ Hz, 6H); HRMS (ESI) calcd for $C_{19}H_{22}ClN_2O_2$ ($M + H$)⁺ 345.1364, found 345.1370.

2-Chloro-6-(2-(dipropylamino)ethoxy)acridin-9(10*H*)-one (30).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.74 (br s, 1H), 8.11 (m, 2H), 7.71 (dd, $J = 8.9$, 2.5 Hz, 1H), 7.52 (d, $J = 9.0$ Hz, 1H), 6.88–6.85 (m, 2H), 4.13 (t, $J = 6.0$ Hz, 2H), 2.84 (t, $J = 6.0$ Hz, 2H), 2.46 (t, $J = 7.2$ Hz, 4H), 1.45–1.40 (m, 4H), 0.85 (t, $J = 7.3$ Hz, 6H); HRMS (ESI) calcd for $C_{21}H_{26}ClN_2O_2$ ($M + H$)⁺ 373.1677, found 373.1680.

Representative procedure for the synthesis of 2-chloro-6-(3-chloropropoxy)acridin-9(10*H*)-one (31).

To a stirred solution of **28** (500 mg, 2.04 mmol) in anhydrous acetone (50 mL) at room temperature were added K₂CO₃ (1.40 g, 10.20 mmol) and 1-bromo-3-chloropropane (640 mg, 4.08 mmol), and the reaction mixture was stirred at reflux for 24 h. The mixture was then filtered and the solvent was removed by rotary evaporation. The obtained product was purified by recrystallization from a mixture of acetone and methanol (1:1), to afford the title compound **31** (498 mg, 76%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.78 (br s, 1H), 8.16–8.10 (m, 2H), 7.72 (dd, $J = 8.9$, 2.6 Hz, 1H), 7.53 (d, $J = 8.9$ Hz, 1H), 6.90–6.88 (m, 2H), 4.23 (t, $J = 6.1$ Hz, 2H), 3.84 (t, $J = 6.4$ Hz, 2H), 2.27–2.24 (m, 2H); HRMS (ESI) calcd for $C_{16}H_{14}Cl_2N_1O_2$ ($M + H$)⁺ 322.0396, found 322.0394.

Representative procedure for the synthesis of 6-(3-(*tert*-butylamino)propoxy)-2-chloroacridin-9(10*H*)-one (32).

To a stirred solution of **31** (400 mg, 1.24 mmol) in DMSO (25 mL) at room temperature were added NaI (747 mg, 4.98 mmol) and *tert*-butylamine (910 mg, 12.46 mmol), and the

reaction mixture was stirred at 145 °C for 2 h. The excess *tert*-butylamine was removed under reduced pressure and the mixture was poured into 50 mL of water. The precipitate that fell out was filtered and recrystallized from a mixture of methanol, ethyl acetate and triethylamine (1:1:1), to afford the titled compound **32** (240 mg, 54%). ¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.76 (br s, 1H), 8.10 (m, 2H), 7.72 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.53 (d, *J* = 8.9 Hz, 1H), 6.88 (m, 2H), 4.18 (t, *J* = 6.5 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H), 1.89–1.83 (m, 2H), 1.03 (s, 9H); HRMS (ESI) calcd for C₂₀H₂₄Cl₁N₂O₂ (M + H)⁺ 359.1521, found 359.1523.

Synthesis of acridones 38–55.

By use of our standardized procedures, as described above for **19** (Buchwald-Hartwig cross coupling (Pd(dba)₂, DPPF, KO^{*t*}Bu)), **5a** (Eaton's acid-mediated cyclization), **6a** (demethylation (HI/phenol)), **7** (*O*-alkylation by (alkylamino)-alkyl chlorides), **31** (*O*-alkylation by bromo-chloro alkanes) and **32** (amination by alkyl amines), the acridones **38–55** were synthesized in good yields (40–65%) via the appropriate intermediates **35–37** from the triflate **17** and 3,5-dichloroaniline (**33**)/3,5-difluoroaniline (**34**). The compounds **38–44** were purified by recrystallization from a mixture of acetone and methanol (1:1) and **45–55** were purified by recrystallization from a mixture of methanol, ethyl acetate and triethylamine (1:1:1).

1,3-Dichloro-6-(2-chloroethoxy)acridin-9(10*H*)-one (**38**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.71 (s, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.26 (d, *J* = 2.0 Hz, 1H), 6.91 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.79 (d, *J* = 2.4 Hz, 1H), 4.39 (t, *J* = 5.1 Hz, 2H), 4.03 (t, *J* = 5.1 Hz, 2H); HRMS (ESI) calcd for C₁₅H₁₀Cl₃Na₁N₁O₂ (M + H)⁺ 363.9669, found 363.9666.

6-(2-Chloroethoxy)-1,3-difluoroacridin-9(10*H*)-one (**39**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.78 (s, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.03–6.90 (m, 3H), 6.83 (d, *J* = 2.2 Hz, 1H), 4.39 (t, *J* = 5.0 Hz, 2H), 4.02 (t, *J* = 5.0 Hz, 2H); HRMS (ESI) calcd for C₁₅H₁₀Cl₁F₂Na₁N₁O₂ (M + H)⁺ 332.0260, found 332.0259.

1,3-Dichloro-6-(3-chloropropoxy)acridin-9(10*H*)-one (**40**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.72 (s, 1H), 8.07 (d, *J* = 9.1 Hz, 1H), 7.44 (d, *J* = 1.7 Hz, 1H), 7.28 (d, *J* = 1.3 Hz, 1H), 6.90 (dd, *J* = 8.9, 1.7 Hz, 1H), 6.82 (d, *J* = 1.8 Hz, 1H), 4.23 (t, *J* = 5.9 Hz, 2H), 3.84 (t, *J* = 6.3 Hz, 2H), 2.25 (m, 2H); HRMS (ESI) calcd for C₁₆H₁₂Cl₃Na₁N₁O₂ (M + H)⁺ 377.9826, found 377.9821.

6-(3-Chloropropoxy)-1,3-difluoroacridin-9(10*H*)-one (**41**).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.77 (s, 1H), 8.06 (d, *J* = 8.9 Hz, 1H), 7.02–7.00 (m, 1H), 6.95 (m, 1H), 6.88 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.84 (d, *J* = 2.3 Hz, 1H), 4.22 (t, *J* = 6.1 Hz, 2H), 3.83 (t, *J* = 6.4 Hz, 2H), 2.24 (m, 2H); HRMS (ESI) calcd for C₁₆H₁₂Cl₁F₂Na₁N₁O₂ (M + H)⁺ 346.0417, found 346.0416.

6-(4-Chlorobutoxy)-1,3-difluoroacridin-9(10H)-one (42).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.75 (br s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.02–6.92 (m, 2H), 6.87 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 4.14 (t, *J* = 5.7 Hz, 2H), 3.74 (t, *J* = 6.1 Hz, 2H), 1.94–1.88 (m, 4H); HRMS (ESI) calcd for C₁₇H₁₄ClF₂Na₁N₁O₂ (M + H)⁺ 360.0573, found 360.0572.

1,3-Difluoro-6-(4,4,4-trifluorobutoxy)acridin-9(10H)-one (43).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.77 (br s, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 7.02–6.93 (m, 2H), 6.89 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.82 (d, *J* = 2.3 Hz, 1H), 4.17 (t, *J* = 6.2 Hz, 2H), 2.47–2.44 (m, 2H), 2.05–1.98 (m, 2H); HRMS (ESI) calcd for C₁₇H₁₂F₅Na₁N₁O₂ (M + H)⁺ 380.0680, found 380.0679.

1,3-Difluoro-6-(hexyloxy)acridin-9(10H)-one (44).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.72 (br s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.02–6.95 (m, 2H), 6.86 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 4.09 (t, *J* = 6.5 Hz, 2H), 1.81–1.74 (m, 2H), 1.46–1.43 (m, 2H), 1.35–1.31 (m, 4H), 0.89 (t, *J* = 7.0 Hz, 3H); HRMS (ESI) calcd for C₁₉H₁₉F₂Na₁N₁O₂ (M + H)⁺ 354.1276, found 354.1275.

1,3-Dichloro-6-(2-(diethylamino)ethoxy)acridin-9(10H)-one (45).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.73 (br s, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.28 (d, *J* = 1.8 Hz, 1H), 6.88 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.81 (d, *J* = 1.9 Hz, 1H), 4.15 (t, *J* = 5.8 Hz, 2H), 2.85 (t, *J* = 5.9 Hz, 2H), 2.59 (q, *J* = 6.9 Hz, 4H), 1.00 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0974.

6-(2-(Diethylamino)ethoxy)-1,3-difluoroacridin-9(10H)-one (46).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.05 (d, *J* = 8.9 Hz, 1H), 7.02–6.92 (m, 2H), 6.86 (dd, *J* = 8.9, 2.0 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 4.14 (t, *J* = 6.1 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁F₂N₂O₂ (M + H)⁺ 347.1566, found 347.1565.

1,3-Dichloro-6-(2-(dipropylamino)ethoxy)acridin-9(10H)-one (47).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.68 (br s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 1.9 Hz, 1H), 7.26 (d, *J* = 1.9 Hz, 1H), 6.86 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.78 (d, *J* = 2.1 Hz, 1H), 4.12 (t, *J* = 5.9 Hz, 2H), 2.83 (t, *J* = 5.6 Hz, 2H), 2.46 (t, *J* = 7.1 Hz, 4H), 1.46–1.37 (m, 4H), 0.865 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₁H₂₅Cl₂N₂O₂ (M + H)⁺ 407.1288, found 407.1289.

6-(2-(Dipropylamino)ethoxy)-1,3-difluoroacridin-9(10H)-one (48).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.75 (br s, 1H), 8.05 (d, *J* = 8.9 Hz, 1H), 7.02–6.92 (m, 2H), 6.85 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.82 (d, *J* = 2.0 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H), 2.44 (t, *J* = 7.2 Hz, 4H), 1.46–1.37 (m, 4H), 0.85 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₁H₂₅F₂N₂O₂ (M + H)⁺ 375.1879, found 375.1878.

6-(2-(Bis(3,3,3-trifluoropropyl)amino)ethoxy)-1,3-difluoroacridin-9(10H)-one (49).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.75 (br s, 1H), 8.06 (d, *J* = 8.9 Hz, 1H), 7.01–6.92 (m, 2H), 6.84 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 4.15 (t, *J* = 5.5 Hz, 2H), 2.95 (t, *J* = 5.6 Hz, 2H), 2.83–2.79 (m, 4H), 2.48–2.42 (m, 4H); HRMS (ESI) calcd for C₂₁H₁₉F₈N₂O₂ (M + H)⁺ 483.1313, found 483.1306.

1,3-Dichloro-6-(3-(diethylamino)propoxy)acridin-9(10H)-one (50).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.68 (br s, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 1.5 Hz, 1H), 7.26 (d, *J* = 1.5 Hz, 1H), 6.86 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.78 (d, *J* = 1.8 Hz, 1H), 4.12 (t, *J* = 6.0 Hz, 2H), 2.55 (t, *J* = 6.4 Hz, 2H), 2.46 (q, *J* = 6.9 Hz, 4H), 1.92–1.84 (m, 2H), 0.95 (t, *J* = 7.0 Hz, 6H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1132.

6-(3-(Diethylamino)propoxy)-1,3-difluoroacridin-9(10H)-one (51).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.04 (d, *J* = 9.0 Hz, 1H), 7.00–6.87 (m, 2H), 6.76 (dd, *J* = 8.7, 9.6 Hz, 2H), 4.12 (t, *J* = 5.5 Hz, 2H), 2.53 (t, *J* = 7.3 Hz, 2H), 2.45 (q, *J* = 7.1 Hz, 4H), 1.87 (m, 2H), 0.95 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₂₀H₂₃F₂N₂O₂ (M + H)⁺ 361.1722, found 361.1727.

6-(3-(Dipropylamino)propoxy)-1,3-difluoroacridin-9(10H)-one (52).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.74 (br s, 1H), 8.05 (d, *J* = 8.9 Hz, 1H), 7.02–6.94 (m, 2H), 6.86 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.81 (d, *J* = 2.1 Hz, 1H), 4.14 (t, *J* = 6.3 Hz, 2H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.33 (t, *J* = 7.2 Hz, 4H), 1.92–1.85 (m, 2H), 1.44–1.34 (m, 4H), 0.83 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₂H₂₇F₂N₂O₂ (M + H)⁺ 389.2035, found 389.2032.

6-(3-(Butylamino)propoxy)-1,3-dichloroacridin-9(10H)-one (53).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.06 (d, *J* = 8.9 Hz, 1H), 7.44 (d, *J* = 1.5 Hz, 1H), 7.27 (d, *J* = 1.4 Hz, 1H), 6.87 (dd, *J* = 9.2, 1.6 Hz, 1H), 6.80 (d, *J* = 1.4 Hz, 1H), 4.17 (t, *J* = 6.0 Hz, 2H), 2.70 (t, *J* = 6.2 Hz, 2H), 2.55–2.50 (m, 2H), 1.94–1.91 (m, 2H), 1.42–1.28 (m, 4H), 0.87 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1134.

6-(3-(*tert*-Butylamino)propoxy)-1,3-difluoroacridin-9(10H)-one (54).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.07–8.05 (m, 1H), 7.06 (d, *J* = 9.8 Hz, 1H), 6.98–6.92 (m, 1H), 6.85–6.79 (m, 2H), 4.20 (t, *J* = 6.1 Hz, 2H), 2.88 (t, *J* = 7.2 Hz, 2H), 2.07–2.00 (m, 2H), 1.19 (s, 9H); HRMS (ESI) calcd for C₂₀H₂₃F₂N₂O₂ (M + H)⁺ 361.1722, found 361.1729.

6-(4-(*tert*-Butylamino)butoxy)-1,3-difluoroacridin-9(10H)-one (55).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.05 (d, *J* = 8.9 Hz, 1H), 7.04–6.93 (m, 2H), 6.87 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.83 (d, *J* = 1.5 Hz, 1H), 4.11 (t, *J* = 6.3 Hz, 2H), 2.62 (t, *J* = 6.4 Hz, 2H), 1.87–1.80 (m, 2H), 1.62–1.55 (m, 2H), 1.08 (s, 9H); HRMS (ESI) calcd for C₂₁H₂₅F₂N₂O₂ (M + H)⁺ 375.1879, found 375.1881.

Synthesis of methyl 4-(2-bromoethoxy)-2-hydroxybenzoate (57).

A suspension of methyl 2,4-dihydroxybenzoate (**56**) (5.0 g, 29.76 mmol) and K_2CO_3 (20.53 g, 148.8 mmol) in anhydrous acetone (100 mL) was stirred at reflux for 2 h. Then the reaction mixture was brought to room temperature and potassium iodide (KI) (1.48 g, 8.92 mmol) and 1,2-dibromoethane (11.13 g, 59.52 mmol) were added dropwise, and the reaction mixture was heated at reflux for overnight. The mixture was diluted with acetone (100 mL) and filtered through a pad of Celite. The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the title compound **57** as a white solid (3.85 g, 47%). 1H NMR ($CDCl_3$, 400 MHz) δ 10.98 (s, 1H), 7.76 (d, J = 8.7 Hz, 1H), 6.48 (d, J = 2.5 Hz, 1H), 6.46 (m, 1H), 4.32 (t, J = 6.2 Hz, 2H), 3.93 (s, 3H), 3.65 (t, J = 6.2 Hz, 2H).

Synthesis of methyl 4-(2-(dipropylamino)ethoxy)-2-hydroxybenzoate (58).

A suspension of dipropylamine (2.76 g, 27.37 mmol) and K_2CO_3 (6.3 g, 45.62 mmol) in anhydrous acetone (50 mL) was stirred at reflux for 2 h. Then the reaction mixture was brought to room temperature and KI (454 mg, 2.73 mmol) and a solution of **57** (2.5 g, 9.15 mmol) in anhydrous acetone (20 mL) were added dropwise, and the mixture was heated at reflux for 16 h. The mixture was diluted with acetone (100 mL), filtered through a pad of Celite and the solvent was removed by rotary evaporation to furnish the desired product **58** (2.21 g, 82%) as an orange syrup. The product **58** was carried forward into the next reaction without further purification. 1H NMR ($CDCl_3$, 400 MHz) δ 10.87 (s, 1H), 7.64 (d, J = 8.6 Hz, 1H), 6.36 (d, J = 2.6 Hz, 1H), 6.34 (m, 1H), 4.18 (t, J = 6.3 Hz, 2H), 3.94 (s, 3H), 2.78 (t, J = 6.3 Hz, 2H), 2.40 (t, J = 7.3 Hz, 4H), 1.41 (m, 4H), 0.89 (t, J = 7.1 Hz, 6H).

Synthesis of methyl 4-(2-(dipropylamino)ethoxy)-2-(((trifluoromethyl)sulfonyl)oxy)benzoate (59).

Compound **59** was synthesized by the same procedure as described for **17** and the product was chromatographed on silica gel with ethyl acetate/hexanes as eluent, to afford the pure product **59** (2.5 g, 86%) as a thick syrup. 1H NMR ($CDCl_3$, 400 MHz) δ 8.06 (d, J = 8.8 Hz, 1H), 6.97 (dd, J = 8.8, 2.4 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 4.09 (t, J = 6.2 Hz, 2H), 3.94 (s, 3H), 2.89 (t, J = 6.2 Hz, 2H), 2.49 (t, J = 7.5 Hz, 4H), 1.49 (m, 4H), 0.90 (t, J = 7.3 Hz, 6H).

Synthesis of methyl 2-((3-chloro-5-methoxyphenyl)amino)-4-(2-(dipropylamino)ethoxy)benzoate (61).

Compound **61** (1.0 g, 76%) was synthesized by the same procedure as described for **19**. 1H NMR ($CDCl_3$, 600 MHz) δ 9.62 (s, 1H), 7.92 (d, J = 8.9 Hz, 1H), 6.90 (d, J = 3.8 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.69 (d, J = 4.0 Hz, 1H), 6.63 (d, J = 4.0 Hz, 1H), 6.37 (dd, J = 8.9, 2.4 Hz, 1H), 4.01 (t, J = 6.4 Hz, 2H), 3.09 (s, 3H), 3.89 (s, 3H), 2.85 (t, J = 6.4 Hz, 2H), 2.48 (t, J = 7.6 Hz, 4H), 1.49 (m, 4H), 0.89 (t, J = 7.3 Hz, 6H).

Synthesis of acridones 62 and 63.

Compounds **62** and **63** were synthesized by the same procedure as described for **5a**.

1-Chloro-6-(2-(dipropylamino)ethoxy)-3-methoxyacridin-9(10*H*)-one (62).

¹H NMR (CDCl₃, 400 MHz) δ 9.68 (s, 1H), 8.29 (d, *J* = 8.9 Hz, 1H), 6.81–6.70 (m, 4H), 4.01 (t, *J* = 6.1 Hz, 2H), 3.79 (s, 3H), 2.89 (t, *J* = 6.1 Hz, 2H), 2.50 (t, *J* = 7.5 Hz, 4H), 1.49 (m, 4H), 0.88 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₂H₂₈Cl₁N₂O₃ (M + H)⁺ 403.1783, found 403.1781.

3-Chloro-6-(2-(dipropylamino)ethoxy)-1-methoxyacridin-9(10*H*)-one (63).

¹H NMR (CDCl₃, 400 MHz) δ 8.76 (br s, 1H), 8.30 (d, *J* = 9.0 Hz, 1H), 7.24 (d, *J* = 1.9 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 6.88 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.63 (d, *J* = 1.6 Hz, 1H), 4.10 (t, *J* = 6.2 Hz, 2H), 3.25 (s, 3H), 2.92 (t, *J* = 6.2 Hz, 2H), 2.52 (t, *J* = 7.6 Hz, 4H), 1.52 (m, 4H), 0.91 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₂H₂₈Cl₁N₂O₃ (M + H)⁺ 403.1783, found 403.1782.

Synthesis of acridones 70–86, 92 and 93.

By use of our standardized procedures, as described above for **19** (Buchwald-Hartwig cross coupling (Pd(dba)₂, DPPF, KO^{*t*}Bu)), **5a** (Eaton's acid-mediated cyclization), **6a** (demethylation (HI/phenol)), **7** (*O*-alkylation by (alkylamino)alkyl chlorides), **31** (*O*-alkylation by bromo-chloro alkanes) and **32** (amination by alkyl amines), the acridones **70–86**, **92** and **93** were prepared in good yields (40–75%) via the appropriate intermediates **65–69** and **89–91** from the triflate **17** and commercially available 3,4-dichloroaniline (**64**)/3,4-difluoroaniline (**87**)/4-fluoro-3-methoxyaniline (**88**). The compounds **70**, **71** and **83** were purified by recrystallization from a mixture of acetone and methanol (1:1) and **72–82**, **84–86**, **92** and **93** were purified by either recrystallized from a mixture of methanol, ethyl acetate and triethylamine (1:1:1) or trituration from aqueous acetone.

1,2-Dichloro-6-(2-chloroethoxy)acridin-9(10*H*)-one (70).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.09 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 9.2 Hz, 1H), 6.90 (dd, *J* = 9.1, 1.7 Hz, 1H), 6.82 (d, *J* = 1.8 Hz, 1H), 4.39 (t, *J* = 4.4 Hz, 2H), 4.03 (t, *J* = 4.7 Hz, 2H); HRMS (ESI) calcd for C₁₅H₁₀Cl₃Na₁N₁O₂ (M + H)⁺ 363.9669, found 363.9666.

1,2-Dichloro-6-(3-chloropropoxy)acridin-9(10*H*)-one (71).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.77 (br s, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 9.1 Hz, 1H), 6.88 (d, *J* = 9.1 Hz, 1H), 6.83 (s, 1H), 4.22 (t, *J* = 6.3 Hz, 2H), 3.84 (t, *J* = 6.2 Hz, 2H), 2.25 (m, 2H); HRMS (ESI) calcd for C₁₆H₁₂Cl₃Na₁N₁O₂ (M + H)⁺ 377.9826, found 377.9822.

1,2-Dichloro-6-(2-(dimethylamino)ethoxy)acridin-9(10*H*)-one (72).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.07 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 6.90–6.83 (m, 2H), 4.17 (t, *J* = 5.5 Hz, 2H), 2.68 (t, *J* = 5.5 Hz, 2H), 2.23 (s, 6H); HRMS (ESI) calcd for C₁₇H₁₇Cl₂N₂O₂ (M + H)⁺ 351.0662, found 351.0659.

1,2-Dichloro-6-(2-(diethylamino)ethoxy)acridin-9(10H)-one (73).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.08 (d, *J* = 8.8 Hz, 1H), 7.80 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 9.1 Hz, 1H), 6.87–6.83 (m, 2H), 4.14 (t, *J* = 6.0 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0974.

1,2-Dichloro-6-(2-(dipropylamino)ethoxy)acridin-9(10H)-one (74).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.08 (d, *J* = 8.9 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 6.87–6.83 (m, 2H), 4.13 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 6.0 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 4H), 1.45–1.40 (m, 4H), 0.86 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₁H₂₅Cl₂N₂O₂ (M + H)⁺ 407.1283, found 407.1287.

1,2-Dichloro-6-(3-(dibutylamino)propoxy)acridin-9(10H)-one (75).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.77 (br s, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.86 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.81 (d, *J* = 2.0 Hz, 1H), 4.13 (t, *J* = 6.1 Hz, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.36 (t, *J* = 6.3 Hz, 4H), 1.89–1.85 (m, 2H), 1.37–1.23 (m, 8H), 0.82 (t, *J* = 7.2 Hz, 6H); HRMS (ESI) calcd for C₂₄H₃₁Cl₂N₂O₂ (M + H)⁺ 449.1757, found 449.1759.

1,2-Dichloro-6-(2-(1-methylpyrrolidin-2-yl)ethoxy)acridin-9(10H)-one (76).

¹H NMR (DMSO-d₆, 600 MHz) δ 11.78 (br s, 1H), 8.06 (d, *J* = 9.0 Hz, 1H), 7.81 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.84 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.80 (d, *J* = 2.3 Hz, 1H), 4.72 (br s, 1H), (2.89–2.81 (m, 1H), 2.72 (t, *J* = 6.0 Hz, 2H), 2.69–2.61 (m, 1H), 2.42 (s, 3H), 2.34–1.90 (m, 6H), 1.77–1.74 (m, 1H); HRMS (ESI) calcd for C₂₀H₂₁Cl₂N₂O₂ (M + H)⁺ 391.0975, found 391.0973.

1,2-Dichloro-6-(2-morpholinoethoxy)acridin-9(10H)-one (77).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.76 (s, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 6.89 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.83 (d, *J* = 2.2 Hz, 1H), 4.22 (t, *J* = 5.5 Hz, 2H), 3.59 (t, *J* = 4.4 Hz, 4H), 3.32 (t, *J* = 4.5 Hz, 4H), 2.76 (t, *J* = 5.5 Hz, 2H); HRMS (ESI) calcd for C₁₉H₁₉Cl₂N₂O₃ (M + H)⁺ 393.0767, found 393.0764.

6-(2-(tert-Butylamino)ethoxy)-1,2-dichloroacridin-9(10H)-one (78).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.76 (br s, 1H), 8.07 (d, *J* = 9.3 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.45 (d, *J* = 9.0 Hz, 1H), 6.89–6.81 (m, 2H), 4.10 (t, *J* = 5.8 Hz, 2H), 2.91 (t, *J* = 6.5 Hz, 2H), 1.07 (s, 9H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0977.

6-(3-(tert-Butylamino)propoxy)-1,2-dichloroacridin-9(10H)-one (79).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.07 (d, *J* = 8.5 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 6.87–6.82 (m, 2H), 4.17 (t, *J* = 6.3 Hz, 2H), 2.65 (t, *J* = 6.7 Hz, 2H), 1.89–1.85 (m, 2H), 1.03 (s, 9H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1135.

6-(3-(Butylamino)propoxy)-1,2-dichloroacridin-9(10H)-one (80).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.09 (d, *J* = 8.9 Hz, 1H), 7.83 (d, *J* = 9.1 Hz, 1H), 7.46 (d, *J* = 9.2 Hz, 1H), 6.89–6.83 (m, 2H), 4.17 (t, *J* = 6.9 Hz, 2H), 2.70 (t, *J* = 6.8 Hz, 2H), 1.86 (t, *J* = 6.4 Hz, 2H), 1.45–1.27 (m, 6H), 0.89 (t, *J* = 6.4 Hz, 3H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1135.

6-(2-((Adamantan-1-yl)amino)ethoxy)-1,2-dichloroacridin-9(10H)-one (81).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.07 (d, *J* = 9.0 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 6.88 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.81 (d, *J* = 2.1 Hz, 1H), 4.08 (t, *J* = 5.6 Hz, 2H), 3.17 (d, *J* = 4.8 Hz, 3H), 2.93 (t, *J* = 5.3 Hz, 2H), 2.08–2.00 (m, 2H), 1.64–1.54 (m, 10H); HRMS (ESI) calcd for C₂₅H₂₇Cl₂N₂O₂ (M + H)⁺ 457.1444, found 457.1443.

2,3-Dichloro-6-(3-chloropropoxy)acridin-9(10H)-one (82).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.78 (s, 1H), 8.25 (s, 1H), 8.11 (d, *J* = 8.9 Hz, 1H), 7.69 (s, 1H), 6.92 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 4.24 (t, *J* = 6.0 Hz, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 2.28–2.22 (m, 2H); HRMS (ESI) calcd for C₁₆H₁₃Cl₃N₁O₂ (M + H)⁺ 356.0006, found 356.0007.

2,3-Dichloro-6-(2-(diethylamino)ethoxy)acridin-9(10H)-one (83).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.23 (s, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.68 (s, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.78 (dd, *J* = 9.0, 2.1 Hz, 1H), 4.13 (t, *J* = 6.1 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0980.

2,3-Dichloro-6-(3-(dibutylamino)propoxy)acridin-9(10H)-one (84).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.78 (br s, 1H), 8.25 (s, 1H), 8.10 (d, *J* = 8.9 Hz, 1H), 7.69 (s, 1H), 6.89 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.85 (d, *J* = 2.4 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.55 (t, *J* = 6.7 Hz, 2H), 2.41–2.35 (m, 4H), 1.92–1.87 (m, 2H), 1.38–1.22 (m, 8H), 0.83 (t, *J* = 7.2 Hz, 6H); HRMS (ESI) calcd for C₂₄H₃₁Cl₂N₂O₂ (M + H)⁺ 449.1757, found 449.1755.

6-(3-(tert-Butylamino)propoxy)-2,3-dichloroacridin-9(10H)-one (85).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.24 (s, 1H), 8.10 (d, *J* = 8.9 Hz, 1H), 7.69 (s, 1H), 6.90 (dd, *J* = 9.2, 2.9 Hz, 1H), 6.86 (d, *J* = 3.1 Hz, 1H), 4.19 (t, *J* = 6.4 Hz, 2H), 2.73 (t, *J* = 6.7 Hz, 2H), 1.93–1.82 (m, 2H), 1.07 (s, 9H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1130.

6-(3-(Butylamino)propoxy)-2,3-dichloroacridin-9(10H)-one (86).

¹H NMR (DMSO-d₆, 400 MHz) δ 8.24 (s, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 7.68 (s, 1H), 6.89–6.81 (m, 2H), 4.16 (t, *J* = 5.7 Hz, 2H), 2.67 (t, *J* = 6.6 Hz, 2H), 1.93–1.87 (m, 2H), 1.41–1.27 (m, 6H), 0.87 (t, *J* = 7.0 Hz, 3H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 393.1131, found 393.1130.

6-(2-(Diethylamino)ethoxy)-2,3-difluoroacridin-9(10*H*)-one (92).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 8.08 (d, *J* = 9.0 Hz, 1H), 8.01 (dd, *J* = 11.1, 9.2 Hz, 1H), 7.44 (dd, *J* = 11.9, 6.9 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.82 (dd, *J* = 8.9, 2.3 Hz, 1H), 4.13 (t, *J* = 6.1 Hz, 2H), 2.83 (t, *J* = 6.1 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁F₂N₂O₂ (M + H)⁺ 347.1566, found 347.1570.

3,6-Bis(2-(diethylamino)ethoxy)-2-fluoroacridin-9(10*H*)-one (93).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.54 (br s, 1H), 8.07 (d, *J* = 9.2 Hz, 1H), 7.80 (d, *J* = 11.6 Hz, 1H), 7.03 (d, *J* = 7.1 Hz, 1H), 6.85–6.82 (m, 2H), 4.20 (t, *J* = 5.9 Hz, 2H), 4.15 (t, *J* = 6.0 Hz, 2H), 2.88 (t, *J* = 5.9 Hz, 2H), 2.83 (t, *J* = 6.0 Hz, 2H), 2.62–2.55 (m, 8H), 0.99 (t, *J* = 7.0 Hz, 12H); HRMS (ESI) calcd for C₂₅H₃₅F₁N₃O₃ (M + H)⁺ 444.2657, found 444.2651.

Synthesis of methyl 4-(2-(diethylamino)ethoxy)-2-hydroxybenzoate (95a).

A suspension of **56** (1.0 g, 5.95 mmol), K₂CO₃ (4.10 g, 29.76 mmol) and tetrabutylammonium chloride (TBAC) (574 mg, 1.78 mmol) in anhydrous acetone (50 mL) was stirred at room temperature. After 30 min, a solution of 2-bromo-*N,N*-diethylethanamine hydrobromide (1.86 g, 7.14 mmol) in acetone (5 mL) was added dropwise and the mixture was then heated at reflux for 4 h. The mixture was diluted with acetone (100 mL), filtered through a pad of Celite. The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure product **95a** as a clear oil (1.35 g, 85%). ¹H NMR (CDCl₃, 400 MHz) δ 10.96 (s, 1H), 7.73 (d, *J* = 9.5 Hz, 1H), 6.45 (m, 2H), 4.07 (t, *J* = 6.2 Hz, 2H), 3.92 (s, 3H), 2.88 (t, *J* = 6.2 Hz, 2H), 2.64 (q, *J* = 7.2 Hz, 4H), 1.07 (t, *J* = 7.2 Hz, 6H). HRMS (ESI) calcd for C₁₄H₂₂N₁O₄ (M + H)⁺ 268.1543, found 268.1552.

Synthesis of methyl 4-((2-(diethylamino)ethyl)amino)-2-hydroxybenzoate (95b).

To a stirred solution of methyl 4-iodosalicylate (**94**, 5.0 g, 17.98 mmol) in 50 mL of dry DMF at room temperature were added 2-diethylaminoethylamine (2.5 g, 21.58 mmol), Cs₂CO₃ (11.69 g, 35.97 mmol), 2-acetylcyclohexanone (200 mg, 1.43 mmol) and CuI (170 mg, 0.89 mmol). The reaction mixture was stirred at 100 °C for 4 h. The mixture was filtered through a pad of Celite and the filtrate was diluted with ethyl acetate (250 mL). The organic layer was washed with saturated NH₄Cl, water and brine and dried over anhydrous Na₂SO₄. The solvent was removed by rotary evaporation and the product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the pure **95b** as a thick syrup (3.97 g, 83%). ¹H NMR (CDCl₃, 400 MHz) δ 11.01 (s, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 6.12 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.07 (d, *J* = 2.4 Hz, 1H), 4.97 (br s, 1H), 3.88 (s, 3H), 3.15 (m, 2 H), 2.69 (t, *J* = 6.2 Hz, 2H), 2.57 (q, *J* = 7.2 Hz, 4H), 1.04 (t, *J* = 7.2 Hz, 6H). HRMS (ESI) calcd for C₁₄H₂₃N₂O₃ (M + H)⁺ 267.1703 found 267.1711.

Synthesis of methyl 4-(2-(diethylamino)ethoxy)-2-(((trifluoromethyl)sulfonyl)oxy)-benzoate (96a), and methyl 4-((2-(diethylamino)ethyl)amino)-2-(((trifluoromethyl)sulfonyl)oxy)benzoate (96b).

Compounds **96a** (1.47 g, 99%) and **96b** (3.3 g, 74%) were synthesized by the same procedure as described for **17**.

96a: ^1H NMR (CDCl_3 , 400 MHz) δ 8.05 (d, J = 8.9 Hz, 1H), 7.05 (dd, J = 8.9, 2.4 Hz, 1H), 6.85 (d, J = 2.4 Hz, 1H), 4.50 (t, J = 4.7 Hz, 2H), 3.91 (s, 3H), 3.59 (t, J = 4.7 Hz, 2H), 3.32 (q, J = 7.3 Hz, 4H), 1.41 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{21}\text{F}_3\text{N}_1\text{O}_6\text{S}_1$ ($\text{M} + \text{H}$) $^+$ 400.1036 found 400.1044. **96b:** ^1H NMR (CDCl_3 , 400 MHz) δ 7.88 (d, J = 8.7 Hz, 1H), 7.07 (br s, 1H), 6.73 (dd, J = 8.7, 2.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 3.88 (s, 3H), 3.58 (m, 2H), 3.23 (t, J = 5.6 Hz, 2H), 3.14 (q, J = 7.3 Hz, 4H), 1.36 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{22}\text{F}_3\text{N}_2\text{O}_5\text{S}_1$ ($\text{M} + \text{H}$) $^+$ 399.1196 found 399.1195.

Representative procedure for the synthesis of methyl 4-(2-(diethylamino)ethoxy)-2-((4-fluoro-3-methoxyphenyl)amino)-benzoate (97a).

To a degassed solution of **96a** (1.00 g, 2.50 mmol) and 4-fluoro-3-methoxyaniline (**88**) (389 mg, 2.75 mmol) in toluene (50 mL) at room temperature were added palladium acetate (34 mg, 0.15 mmol), XPhos (143 mg, 0.30 mmol) and Cs_2CO_3 (1.62 g, 50.12 mmol). The mixture was again degassed and heated at reflux for 5 h. After cooling to room temperature, the mixture was filtered through a pad of Celite and washed with dichloromethane (100 mL). The filtrate was concentrated under reduced pressure and the crude product was chromatographed on silica gel, with ethyl acetate/hexanes as eluent, to afford the desired product **97a** as a brown syrup (811 mg, 83%). ^1H NMR (CDCl_3 , 400 MHz) δ 9.52 (s, 1H), 7.93 (d, J = 8.9 Hz, 1H), 7.08 (dd, J = 8.5, 2.4 Hz, 1H), 6.84 (m, 2H), 6.51 (d, J = 2.5 Hz, 1H), 6.30 (dd, J = 8.9, 2.5 Hz, 1H), 4.20 (t, J = 5.2 Hz, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.20 (t, J = 5.2 Hz, 2H), 3.00 (q, J = 7.2 Hz, 4H), 1.25 (t, J = 7.2 Hz, 6H). HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{28}\text{F}_1\text{N}_2\text{O}_4$ ($\text{M} + \text{H}$) $^+$ 391.2028 found 391.2034.

Synthesis of methyl 4-((2-(diethylamino)ethyl)amino)-2-((4-fluoro-3-methoxyphenyl)amino)benzoate (97b).

Compound **97b** (693 mg, 71%) was synthesized by the same procedure as described for **97a**. ^1H NMR (CDCl_3 , 400 MHz) δ 9.54 (s, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.06 (dd, J = 8.6, 2.5 Hz, 1H), 6.89 (dd, J = 7.6, 2.4 Hz, 1H), 6.82 (m, 1H), 6.20 (d, J = 2.2 Hz, 1H), 6.04 (dd, J = 8.8, 2.2 Hz, 1H), 4.90 (br s, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.15 (t, J = 5.9 Hz, 2H), 2.74 (t, J = 5.9 Hz, 2H), 2.66 (q, J = 7.2 Hz, 4H), 1.07 (t, J = 7.2 Hz, 6H). HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{29}\text{F}_1\text{N}_3\text{O}_3$ ($\text{M} + \text{H}$) $^+$ 390.2187 found 390.2191.

Synthesis of acridones 98 and 99.

Compounds **98** (298 mg, 65%) and **99** (238 mg, 52%) were synthesized by the same procedure as described for **5a** and purified by recrystallization from a mixture of acetone and methanol (1:1).

6-(2-(Diethylamino)ethoxy)-2-fluoro-3-methoxyacridin-9(10H)-one (98).

^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 8.05 (d, J = 8.9 Hz, 1H), 7.76 (d, J = 12.2 Hz, 1H), 7.15 (d, J = 7.6 Hz, 1H), 6.91 (d, J = 2.4 Hz, 1H), 6.72 (dd, J = 8.9, 2.4 Hz, 1H), 4.09 (t, J = 6.2 Hz, 2H), 3.93 (s, 3H), 2.81 (t, J = 6.2 Hz, 2H), 2.56 (q, J = 7.1 Hz, 4H), 0.99 (t, J = 7.1 Hz, 6H). HRMS (ESI) calcd for $\text{C}_{20}\text{H}_{24}\text{F}_1\text{N}_2\text{O}_3$ ($\text{M} + \text{H}$) $^+$ 359.1765 found 359.1766.

6-((2-(Diethylamino)ethyl)amino)-2-fluoro-3-methoxyacridin-9(10H)-one (99).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.42 (s, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.73 (d, *J* = 11.9 Hz, 1H), 7.03 (d, *J* = 7.3 Hz, 1H), 6.65 (br s, 1H), 6.60 (dd, *J* = 6.9, 2.0 Hz, 1H), 6.37 (d, *J* = 1.7 Hz, 1H), 3.94 (s, 3H), 3.34 (2H are merzed), 2.84–2.75 (m, 6H), 1.07 (t, *J* = 7.0 Hz, 6H). HRMS (ESI) calcd for C₂₀H₂₅F₁N₃O₂ (M + H)⁺ 358.1925 found 358.1923.

Synthesis of acridones 100–102.

By use of our standardized procedures, as described above for **19** (Buchwald-Hartwig cross coupling (Pd(dba)₂, DPPF, KO^{*t*}Bu)), **5a** (Eaton's acid-mediated cyclization), **6a** (demethylation (HI/phenol)), **7** (*O*-alkylation by (alkylamino)-alkyl chlorides, the acridones **100–102** were synthesized in good yields (67–79%) from **17** and commercially available 2,5-dichloroaniline, 2,4-dichloroaniline and 2,3-dichloroaniline, respectively.

1,4-Dichloro-6-(3-(diethylamino)propoxy)acridin-9(10H)-one (100).

¹H NMR (DMSO-d₆, 400 MHz) δ 10.68 (s, 1H), 8.06 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 6.88 (dd, *J* = 8.8, 2.1 Hz, 1H), 4.13 (t, *J* = 5.5 Hz, 2H), 2.85 (t, *J* = 5.8 Hz, 2H), 2.60 (q, *J* = 6.7 Hz, 4H), 0.99 (t, *J* = 6.8 Hz, 6H); HRMS (ESI) calcd for C₂₀H₂₃Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0979.

2,4-Dichloro-6-(2-(diethylamino)ethoxy)acridin-9(10H)-one (101).

¹H NMR (DMSO-d₆, 400 MHz) δ 10.94 (br s, 1H), 8.11–8.08 (m, 2H), 8.03 (d, *J* = 2.5 Hz, 1H), 7.52 (d, *J* = 2.2 Hz, 1H), 6.91 (dd, *J* = 9.0, 2.3 Hz, 1H), 4.13 (t, *J* = 6.2 Hz, 2H), 2.86 (t, *J* = 6.0 Hz, 2H), 2.59 (q, *J* = 7.1 Hz, 4H), 1.00 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0980.

3,4-Dichloro-6-(2-(diethylamino)ethoxy)acridin-9(10H)-one (102).

¹H NMR (DMSO-d₆, 400 MHz) δ 10.91 (br s, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.10 (d, *J* = 8.9 Hz, 1H), 7.55 (s, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 4.13 (t, *J* = 5.4 Hz, 2H), 2.86 (t, *J* = 5.5 Hz, 2H), 2.58 (q, *J* = 6.8 Hz, 4H), 1.00 (t, *J* = 6.9 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₁Cl₂N₂O₂ (M + H)⁺ 379.0975, found 379.0979.

Synthesis of acridones 109–113.

By use of our standardized procedures, as described above for **31** (*O*-alkylation by bromo-chloro alkanes), **32** (amination by alkyl amines), **17** (Tf₂O/pyridine), **97a** (Buchwald-Hartwig cross coupling: Pd(OAc)₂, XPhos, Cs₂CO₃), and **5a** (Eaton's acid-mediated cyclization), the acridones **109–113** were synthesized in good yields (40–75%) via the appropriate intermediates **104–106** and **108** from commercially available methyl 2,5-dihydroxybenzoate (**103**) and anilines **107**, **34** and **60**.

7-(2-(Dipropylamino)ethoxy)-1,3-difluoroacridin-9(10H)-one (109).

¹H NMR (DMSO-d₆, 400 MHz) δ 11.90 (s, 1H), 7.57 (d, *J* = 2.7 Hz, 1H), 7.47 (d, *J* = 9.0 Hz, 1H), 7.39 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.04 (dd, *J* = 10.4, 2.4 Hz, 1H), 6.96 (ddd, *J* = 11.9, 9.6, 2.3 Hz, 1H), 4.09 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 7.1 Hz, 2H), 2.46 (t, *J* = 6.7 Hz, 4H),

1.45–1.40 (m, 4H), 0.86 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{21}H_{25}F_2N_2O_2$ ($M + H$)⁺ 375.1879, found 375.1883.

7-(2-(Dipropylamino)ethoxy)-1-fluoro-3-methoxyacridin-9(10*H*)-one (110).

¹H NMR (DMSO- d_6 , 400 MHz) δ 11.62 (s, 1H), 7.54 (d, J = 2.6 Hz, 1H), 7.41 (d, J = 9.0 Hz, 1H), 7.33 (dd, J = 9.0, 2.7 Hz, 1H), 6.68 (d, J = 1.7 Hz, 1H), 6.57 (dd, J = 13.5, 2.0 Hz, 1H), 4.07 (t, J = 5.9 Hz, 2H), 3.88 (s, 3H), 2.81 (t, J = 5.8 Hz, 2H), 2.45 (t, J = 7.2 Hz, 4H), 1.43–1.41 (m, 4H), 0.85 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{28}F_1N_2O_3$ ($M + H$)⁺ 387.2078, found 387.2079.

7-(2-(Dipropylamino)ethoxy)-3-fluoro-1-methoxyacridin-9(10*H*)-one (111).

¹H NMR (DMSO- d_6 , 400 MHz) δ 11.49 (s, 1H), 7.54 (d, J = 2.8 Hz, 1H), 7.38 (d, J = 8.9 Hz, 1H), 7.31 (dd, J = 8.9, 2.8 Hz, 1H), 6.70 (dd, J = 10.3, 2.3 Hz, 1H), 6.56 (dd, J = 11.9, 2.3 Hz, 1H), 4.06 (t, J = 6.0 Hz, 2H), 3.86 (s, 3H), 2.81 (t, J = 5.9 Hz, 2H), 2.45 (t, J = 7.1 Hz, 4H), 1.45–1.39 (m, 4H), 0.85 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{28}F_1N_2O_3$ ($M + H$)⁺ 387.2078, found 387.2083.

1-Chloro-7-(2-(dipropylamino)ethoxy)-3-methoxyacridin-9(10*H*)-one (112).

¹H NMR (DMSO- d_6 , 400 MHz) δ 11.52 (s, 1H), 7.53 (d, J = 2.7 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.33 (dd, J = 8.9, 2.8 Hz, 1H), 7.03 (d, J = 1.6 Hz, 1H), 6.67 (d, J = 1.6 Hz, 1H), 4.07 (t, J = 5.8 Hz, 2H), 3.87 (s, 3H), 2.83 (t, J = 5.6 Hz, 2H), 2.46 (t, J = 7.0 Hz, 4H), 1.47–1.38 (m, 4H), 0.85 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{28}Cl_1N_2O_3$ ($M + H$)⁺ 403.1783, found 403.1784.

3-Chloro-7-(2-(dipropylamino)ethoxy)-1-methoxyacridin-9(10*H*)-one (113).

¹H NMR (DMSO- d_6 , 400 MHz) δ 11.61 (s, 1H), 7.55 (d, J = 2.9 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.33 (dd, J = 8.9, 2.9 Hz, 1H), 6.83–6.82 (m, 2H), 4.07 (t, J = 6.0 Hz, 2H), 3.88 (s, 3H), 2.82 (t, J = 6.0 Hz, 2H), 2.45 (t, J = 7.2 Hz, 4H), 1.47–1.37 (m, 4H), 0.85 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{22}H_{28}Cl_1N_2O_3$ ($M + H$)⁺ 403.1783, found 403.1782.

Synthesis of acridones 119 and 120.

By use of our standardized procedures, as described above for **97a** (Buchwald-Hartwig cross coupling: Pd(OAc)₂, XPhos, Cs₂CO₃), **5a** (Eaton's acid-mediated cyclization), **22** (demethylation: BBr₃), and **7** (*O*-alkylation by (alkylamino)alkyl chloride), the acridones **119** and **120** were synthesized in good yields (60–65%) via the appropriate intermediates **115–118** from commercially available methyl 2-bromo-5-methoxybenzoate (**114**) and 3,4-dichloroaniline (**64**).

1,2-Dichloro-7-(2-(dipropylamino)ethoxy)acridin-9(10*H*)-one (119).

¹H NMR (DMSO- d_6 , 400 MHz) δ 7.80 (d, J = 9.1 Hz, 1H), 7.58 (d, J = 2.7 Hz, 1H), 7.52–7.47 (m, 2H), 7.38 (dd, J = 9.0, 2.7 Hz, 1H), 4.08 (t, J = 6.0 Hz, 2H), 2.82 (t, J = 6.0 Hz, 2H), 2.45 (t, J = 7.2 Hz, 4H), 1.47–1.37 (m, 4H), 0.85 (t, J = 7.3 Hz, 6H); HRMS (ESI) calcd for $C_{21}H_{25}Cl_2N_2O_2$ ($M + H$)⁺ 407.1288, found 407.1286.

2,3-Dichloro-7-(2-(dipropylamino)ethoxy)acridin-9(10*H*)-one (120).

¹H NMR (DMSO-*d*₆, 400 MHz) δ 11.92 (s, 1H), 8.28 (s, 1H), 7.73 (s, 1H), 7.58 (d, *J* = 2.3 Hz, 1H), 7.51 (d, *J* = 9.0 Hz, 1H), 7.42 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.09 (t, *J* = 5.7 Hz, 2H), 2.82 (t, *J* = 5.7 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 4H), 1.45–1.38 (m, 4H), 0.85 (t, *J* = 7.3 Hz, 6H); HRMS (ESI) calcd for C₂₁H₂₅Cl₂N₂O₂ (M + H)⁺ 407.1288, found 407.1287.

Synthesis of 2-chloro-7-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (123).

By use of our standardized procedures, as described above for **97a** (Buchwald-Hartwig cross coupling: Pd(OAc)₂, XPhos, Cs₂CO₃), **5a** (Eaton's acid-mediated cyclization), **22** (demethylation: BBr₃) and **7** (*O*-alkylation by (alkylamino)alkyl chloride), the acridone **123** was synthesized in a good yield (71%) via the appropriate intermediates **121** and **122** from commercially available methyl 2-bromo-5-methoxybenzoate (**114**) and 4-chloroaniline (**25**). ¹H NMR (DMSO-*d*₆, 400 MHz) 8.14 (d, *J* = 2.5 Hz, 1H), 7.71 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.61 (d, *J* = 2.9 Hz, 1H), 7.57 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.42 (dd, *J* = 9.0, 2.9 Hz, 1H), 4.10 (t, *J* = 6.1 Hz, 2H), 2.82 (t, *J* = 6.0 Hz, 2H), 2.57 (q, *J* = 7.1 Hz, 4H), 0.99 (t, *J* = 7.1 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₂ClN₂O₂ (M + H)⁺ 345.1364, found 345.1370.

Synthesis of 6-chloro-2-(2-(diethylamino)ethoxy)acridin-9(10*H*)-one (128).

By use of our standardized procedures, as described above for **3a** (copper-catalyzed amination reaction), **5a** (Eaton's acid-mediated cyclization), **22** (demethylation (BBr₃)) and **7** (*O*-alkylation by (alkylamino)alkyl chloride), the acridone **128** was synthesized in a good yield (77%) via the appropriate intermediates **125–127** from commercially available **1d** and 4-methoxyaniline (**124**). ¹H NMR (DMSO-*d*₆, 400 MHz) 12.15 (s, 1H), 9.98 (s, 1H), 8.22 (d, *J* = 8.7 Hz, 1H), 7.70 (d, *J* = 2.4 Hz, 1H), 7.62–7.59 (m, 2H), 7.49 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 1H), 4.46 (t, *J* = 4.7 Hz, 2H), 3.56 (q, *J* = 4.5 Hz, 2H), 3.29–3.21 (m, 4H), 1.26 (t, *J* = 7.2 Hz, 6H); HRMS (ESI) calcd for C₁₉H₂₂ClN₂O₂ (M + H)⁺ 345.1364, found 345.1369.

Biological Experiments.

Culture Conditions.—*P. falciparum* strains D6, Dd2, 7G8, and TM90-C2B were cultured in human erythrocytes at 2% hematocrit in RPMI 1640 containing 0.5% Albumax, 45 μg/L hypoxanthine, and 50 μg/L gentamicin, as previously described.

In Vitro Blood Stage Antimalarial Activity.—In vitro antimalarial activity was determined by the Malaria SYBR Green I-based Fluorescence (MSF) assay described previously with minor modifications as previously described, and expressed as the compound concentration inhibiting growth by 50% (IC₅₀).

In Vitro Liver Stage Antimalarial Activity.—In vitro liver stage activity of each new acridone was assessed utilizing luciferase-expressing *P. berghei* sporozoite infected HepG2 hepatocyte cells (a hepatoma cell line that allows higher rates of sporozoites invasion and full development of *P. berghei*)^{33, 34}. Briefly, luciferase-expressing *P. berghei* (ANKA-676m1c11) sporozoites were extracted from salivary glands of infected *Anopheles*

stephensi mosquitoes. HepG2-A16 cells were cultivated and seeded in 96-well plates and grown for 24 h then 10000 sporozoites were incubated with the HepG2 cells for 3 h; after which, the wells were washed with media to remove non-invaded sporozoites. Drug solutions then were added and plates were placed in 37 °C and 5% CO₂ incubator for 45 h. Luciferin was added and the plates were incubated for 30 minutes; after which, luciferase activity was determined in a luminometer.

In Vivo Blood Stage Efficacy against Murine Malaria: The in vivo activity of selected acridones was assessed against the blood stages using a modified 4-day test^{23, 31, 32}. A 4- to 5-week-old female CF1 mice (Charles River Laboratories) were infected intravenously with 2.5×10^5 *P. yoelii* (Kenya strain, MR4 MRA-428) parasitized erythrocytes from a donor animal. Drug administration commenced the day after the animals were inoculated (day 1). The test compounds were dissolved in PEG-400 and administered by oral gavage once daily for four successive days; chloroquine phosphate was used as a positive control. Blood for blood film analysis and body weights were obtained on the day following the last dose and then at weekly intervals through day 28. Blood films were Giemsa stained and examined microscopically to determine the levels of parasitemia. These blood samples were collected from the tail vein with the aid of a syringe-needle. All mice were observed daily to assess their clinical signs, which were recorded. Animals with observable parasitemia following the experiment were euthanized; animals cleared of parasites from their bloodstream were observed daily with assessment of parasitemia performed weekly until day 28 at which point we score the animal(s) as cured of infection, and the animals were euthanized. All treated mice with a negative smear on day 28 were considered cured (100% protection). ED₅₀ and ED₉₀ values (mg/kg/day) were derived graphically from the dose required to reduce parasite burden by 50% and 90%, respectively, relative to drug-free controls.

Animals and Ethic Statement at Portland VA Medical Center (PVAMC).—In vivo blood stage testing was carried out at PVAMC, and the procedures were conducted under protocols approved by PVAMC Institutional Animal Care and Use Committee (Protocol number 3072). Research was conducted in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

In Vivo Liver Stage Efficacy against Murine Malaria—Real-time in vivo imaging system (IVIS) was used to determine the in vivo liver stage antimalarial efficacy of selected acridone candidates^{33, 35, 36}. Luciferase-expressing *P. berghei* sporozoites were extracted from salivary glands of *A. stephensi* mosquitoes. C57BL/6 male albino mice were infected *i.v.* in the tail vein with 50000 sporozoites on day 0. Drugs were administered orally on days -1, 0, +1. All drug solutions were prepared fresh before drug administration by dissolving the needed quantity of drug in cold (4°C) 0.5% (w/v) hydroxyethyl cellulose and 0.2% (v/v) Tween-80 (0.5% HECT) or PEG400. If needed, drugs were ground using a ProScientific 300D homogenizer and the particle size was measured using a Horiba LA-950V2 particle size analyzer. Formulations of acridones were prepared in PEG 400 and administered orally

(PO) at 10, and/or 40 mg/kg on days -1, 0, and 1. A vehicle control (PEG400) group was included as a negative control in all the studies. Positive control consisted on three doses of 4-methyl primaquine which was dissolved in 0.5% HECT and was given at 5 mg/kg on days -1, 0, and 1 post infections. All dosing solutions were given based on mice body weight measured on the day before infections took place.

P. berghei parasite load in the liver and blood (approximately at 24 and 48 and 72 h post animal infections) was assessed using an IVIS Spectrum instrument. Mice were injected IP with 150 μ L D-luciferin solution (Gold Biotechnology, St. Louis, MO) at a concentration of 200 mg/kg body weight. Three minutes after luciferin injection, the mice were placed in the induction chamber and anesthetized. As soon as the mice were asleep, they were transferred in the IVIS chamber and positioned ventral side down on the heated platform with their noises positioned inside the nose cones. The IVIS camera exposure times were 1 and 5 minutes for the 24, 48, and 72 h time points with a large binning setting and an f-stop = 1. The IVIS Living Image software (version 4.0) was used to quantitate the bioluminescence in photons per second observed from the liver region or whole animal surface area respectively for the liver and blood stage efficacy studies. Starting at 6 days after infection, all mice were assessed for blood stage parasitemia which was quantitated using flow cytometry conducted using a FC500 MPL flow cytometer (Beckman Coulter, Fullerton, CA). The green photomultiplier tube and filter setting were used in these studies (520–555 nm filter settings for the green PMT and greater than 580 nm settings for the red PMT). Infected erythrocytes, uninfected erythrocytes, and leukocytes were gated on logarithmic forward/side dot plots. The method of FCM sample preparation has been previously described previously. In brief, a small 3 μ L sample of blood was obtained from the tails of all mice. The blood was transferred into 0.3 mL of 1% heparinized isotonic buffer (PBS saline). 1 mM of 0.04% of glutaraldehyde was added to fix each sample, and samples were incubated for 60 minutes at 4 °C followed by centrifugation at 450 g for 5 minutes. The supernatant was removed by aspiration, and the cells were re-suspended in 0.5 mL PBS buffer supplemented with 0.25% (v/v) Triton X-100 for 10-minute incubation at room temperature. After centrifugation, the permeabilized cells were re-suspended in 0.5 mL of RNAase at 1 mg/mL concentrations and incubated for at least 2 h at 37 °C to ensure complete digestion of reticulocytes which are at high concentrations due to anemia associated with *P. berghei* infection. 20 μ L of YOYO-1 dye at a concentration of 2500 ng/mL was added to the 0.5 mL sample volume to create a final dye concentration of 100 ng/mL of YOYO-1. Parasitemia was monitored for up to 31-days post infections. Blood samples for parasitemia determination were taken every week or prior to euthanizing the animals sick with malaria that were being removed from the study. Mice that tested negative for parasitemia on day 31 post infection were considered cured. The minimum curative dose (ED₁₀₀) in 100% of animals dosed was defined as the lowest dose which cured all animals for the entire 31-days follow-up period.

Animals and Ethic Statement at Walter Reed Army Institute of Research (WRAIR).—Male C57BL/6 Albino mice aged 6 weeks old (Charles River) were used in this study. The mice were left to acclimatise for 7 days prior to the beginning of research studies. All mice were assigned a study number with an individual ear tag. Cards attached to each mouse cage were also used to identify the study groups. All animals were quarantined for

stabilization for 7 days prior to infection. Mice were housed in a designated room with food and water supplied ad libitum and a 12:12 light: dark cycle.

The animal protocol for this study was approved by the Walter Reed Army Institute of Research, Institutional Animal Care and Use Committee (Protocol number 18-ET-12) in accordance with national and Department of Defence guidelines. Research was conducted in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

HepG2 Cytotoxicity Assay.—To identify toxicity to mammalian cells, an MTT assay using hepatic HepG2 cells was employed^{39, 49}. Drugs were dissolved in DMSO to make 10 mM stock solutions. Human hepatocarcinoma cells (HepG2) were maintained on RPMI-1640 medium supplemented with 10% fetal bovine serum at 37 °C in a humidified 5% CO₂ atmosphere. Cells were seeded at a density of 2×10^4 per well in 96-well flat-bottom tissue culture plates containing complete medium in a total volume of 160 μ L/well. The cells were allowed to attach at 37 °C overnight. On the following day, drug solutions (40 μ L/well) were serially diluted with complete culture medium across the plate. The plates were then incubated at 37 °C and 5% CO₂ for another 24–36 h. Afterward, the medium was aspirated and replaced with complete RPMI medium (200 μ L/well), and the plates were incubated for an additional 24 h at 37 °C and 5% CO₂. An aliquot of a stock solution of resazurin (Alamar Blue, prepared in 1 $\times$ PBS) was then added at 20 μ L per well (final concentration 10 μ M), and the plates were returned to the incubator for 3 h. After this period, fluorescence in each well, indicative of cellular redox activity was measured in a Gemini EM plate reader with excitation wavelength at 560 nm and emission wavelength at 590 nm. IC₅₀ values were determined by nonlinear regression analysis of logistic concentration–fluorescence intensity curves (GraphPad Prism software).

In Vitro Metabolic Profile.—The half-life of lead acridones in mouse and human liver microsomes were determined as described previously^{37–39}. All samples were tested in human and mouse liver microsomal preparations. Sample stocks at 10 or 20 μ M (depending on solubility) in DMSO were diluted to a final concentration of 1 μ M with a mixture containing 0.5 mg/mL of prewarmed pooled human or mouse liver microsomes (BD Gentest), 1.3 μ M NADP (Sigma), 3.3 μ M MgCl₂ (Sigma), and 0.1 M pH 7.4 PBS using a TECAN Genesis robotic liquid handler. The reaction was started with the addition of 1U/mL glucose-6-phosphate dehydrogenase (G6PD). The mixture was incubated on a shaking platform at 37 °C, and aliquots were taken and quenched with the addition of an equal volume of cold acetonitrile at 0, 10, 20, 30, and 60 min. Samples were centrifuged at 3700 rpm for 10 min at 20 °C to remove debris. Sample quantification was carried out by LC/MS, and metabolic half-life was calculated by fitting the data to a first-order decay model, and intrinsic clearance ($C_{L_{int}}$) is dependent on half-life and concentration of protein, calculated as: $C_{L_{int}} = \ln 2 / (T_{1/2} [\text{microsomal protein}])$.

In Vivo Pharmacokinetic (PK) Studies.—PK studies were conducted using the previously established methods^{33, 39}. Male 7-week-old ICR mice weighing from 23 to 28 g

(Charles River Laboratories, Inc. Raleigh, NC) were used for the PK evaluations. On arrival, the animals were acclimated for 7 days in quarantine. The animals were housed in a cage maintained in a room with a temperature range of 64–79 °F, 34–68% relative humidity and a 12-h light/dark cycle. Food and water were provided ad libitum during quarantine and throughout the study. The animals were fed a standard rodent maintenance diet. All animal studies were performed under IACUC-approved protocols. These protocols detail the experimental procedures and designs as well as the number of animals that were used. All animal use, care, and handling were performed in accordance with the current “Guide for the Care and Use of Laboratory Animals” (8th Edition, 2011). PK studies were performed using intragastric (IG) administration. For each time point to be acquired, three male ICR mice per time-point were dosed at single *p.o.* dosing of 80 mg/kg. The drug vehicle was DD water, administered at 100 μ L/20 g. At each time point, blood and plasma samples were collected. Whole blood was collected by cardiac puncture. Blood samples were collected in lithium heparin tubes. Following the separation of appropriate aliquots, plasma was obtained from the whole blood via centrifugation. Liver tissues were isolated. All liquid and tissue samples were immediately preserved on dry ice and later stored at –80 °C until analytical work was performed. Drug concentrations were generated for each sample taken from animals dosed with test compounds. A measured plasma or liver drug concentration vs. time curve was produced, in graphic and tabular form, for each subject on both linear/linear and log/linear scales, for the parent compound. Mean plasma drug concentration vs time curves were also prepared separately. Maximum plasma concentration (C_{max}), and time to maximum concentration (t_{max}) were obtained from the plasma drug concentration–time curves. The elimination half-life ($t_{1/2}$) was calculated from $(\ln 2)/k_{el}$, which is the elimination rate constant calculated from the log concentration–time plot. The area under the curve (AUC) was determined by the linear trapezoidal rule with extrapolation to infinity based on the concentration of the last time point divided by the terminal rate constant. Mean residence time (MRT) was determined by dividing the area under the first moment curve (AUMC) by AUC. The volume of the central compartment (V_z) and volume of the tissue compartment (V_z/F) were calculated as the product of CL and MRT.

In Vitro Cardiotoxicity.—The measurement of hERG channel inhibitory activity of the test compounds was performed by Essen BioScience Inc., Ann Arbor, MI. Basic principles and operation of the IonWorks platform have been described by Schroeder et al⁵⁰.

In Vitro Mutagenicity.—Mutagenicity evaluation was assessed using the Ames assay^{51, 52} (EBPI, Ontario, Canada) against *Salmonella typhimurium* TA100 and TA98, with and without S9 activation. Tester strain *S. typhimurium* (TA100 or TA98) cultures were inoculated from the lyophilized pellets and grown in nutrient broth provided by EBPI. Each inoculated flask (125 or 150 ml volume) was placed in the incubator at 37 °C overnight. The positive controls for the assay were 5 μ g/mL 2-aminoanthracene for incubations with S9 mix and a mixture of 1 μ g/mL 4-nitroquinoline N-oxide and 2 μ g/mL 2-nitrofluorene for incubations without S9 mix. The tester strains (2.5 mL) were mixed with exposure medium and, where appropriate, 2.0 mL S9 to give a final volume of 18 mL. An aliquot (0.2 mL) of the mixture was then added to each well of a 96-well plate. Test chemical (10 μ L/well) was then added to each well. The 96-well plates were covered and sealed in zip-lock bags and

incubated for 96 h at 37°C. Plates were scored visually and the positive wells against background mutations were recorded.

Culture of *P. falciparum* for Drug Selection Studies.—Malaria parasites were maintained in human O+ erythrocytes (Interstate Blood Bank) at 5% hematocrit in RPMI-1640 (Invitrogen) medium supplemented with 0.5% AlbuMAX I (Life Technologies), 30 mM sodium bicarbonate, 25 mM HEPES, 0.37 mM hypoxanthine, and 10 µg/mL gentamicin and maintained at 37 °C under an atmosphere of 90% nitrogen, 5% carbon dioxide, and 5% oxygen. Cultures were monitored by Giemsa-stained blood smears and maintained at a parasitemia of 1–5%. *P. falciparum* strains were obtained from the Malaria Research and Reference Reagent Resource Center.

In Vitro Selection of *P. falciparum* With Reduced Susceptibility to Acridone 73.—Cloned lines of Dd2 and 3D7 were used for drug selections, with reference stocks cryopreserved for whole genome sequencing. Independent clones of parasites in 50 mL cultures were exposed to incrementally increasing 73 concentrations for several months, beginning at 10 nM and ending at up to 900 nM. After cultures persisted at 300 nM 73, or ~10-fold the IC₅₀ of the control lines (Table 11), the parasites were assessed for acridone susceptibility by 72 h growth inhibition assays. 73-resistant parasites that emerged from drug pressure were expanded, cryopreserved and cloned by limiting dilution for genomic DNA isolation and further phenotypic characterizations.

In Vitro Blood Stage Antimalarial Activity of Acridone Resistant Parasites.—3D7 and Dd2 lines of *P. falciparum* were obtained from the Malaria Research and Reference Reagent Resource Center (BEI Resources). Acridone stocks were dissolved in DMSO at 10 mM and maintained at –20 °C. Drug susceptibility was assessed using a 72 h SYBR Green I fluorescence microplate as described elsewhere in this manuscript, except that parasites were stage-synchronized using sorbitol and introduced into the assays at ring stage. IC₅₀ values were derived from fitting of data using a variable Hill slope function with the aid of GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). IC₅₀ values represent means of two or three biological replicates, run at 48 h intervals to preserve ring-stage specificity of the assay.

Whole Genome Sequencing.—Genomic *P. falciparum* DNA was isolated with either a QIAamp DNA extraction kit (QIAGEN; Redwood City, CA) or ZYMO Quick gDNA Miniprep kit (ZYMO Research; Irvine, CA) according to manufacturer's instructions. Whole genome sequencing of Dd2 control and 73-resistant parasite DNA was performed using 1 µg gDNA to generate libraries with the Illumina TruSeq DNA library prep kit following manufacturer's instructions. DNA libraries were sequenced on an Illumina MiSeq using 250 base pair paired-end reads with a 2% PhiX spike-in. The PhiX spike-in served as a sequencing control and to maintain sequence diversity. Reads were trimmed to remove adapter sequences using Cutadapt v1.2.1 prior to read mapping. Reads were aligned to the *P. falciparum* PlasmoDB v9.3 3D7 reference genome using the bwa-mem algorithm of bwa v0.7.5a-r405. varScan v2.3.2 was used to identify candidate SNP loci with 8x read coverage with 87.5% of the reads supporting the SNP genotype.

***P. falciparum* Cytochrome B Gene (*pfcytb*) Sequencing.**—Genomic DNA was used for direct Sanger sequencing of *pfcytb* (mal_mito_3). Initial PCR was performed using the *pfcytb* flanking forward and reverse primers 5'-TTCCTGATTATCCAGACGCT-3' and 5'-TGTTCCGCTCAATACTCAGA-3'. Sanger sequencing (performed by Genewiz, South Plainfield, NJ) utilized the aforementioned primers and two additional internal primers: 5'-CACTCACAGTATATCCTCCACA-3' and 5'-GAGTTATTGGGGTGCAA CTG-3' for complete gene coverage. Sequences were assembled and aligned using MacVector Software 9.5 (MacVector Inc., Apex, NC). All chromatograms were inspected visually to confirm SNPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CQ	chloroquine
ATV	atovaquone
SAR	structure–activity relationship
IC₅₀	half maximal inhibitory concentration
nM	nanomolar
MDR	multidrug-resistant
EC₅₀	50% effective concentrations
EC₉₀	90% effective concentrations
PK	pharmacokinetic
TBAC	tetrabutylammonium chloride
IVIS	in vivo imaging system
i.v	intravenously
p.o.	oral route
hERG	human-ether-a-go-go-related gene

G6PD	glucose-6-phosphate dehydrogenase
ELQ	endochin-like quinolone

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Figure 1.

Transition from dual-function acridone to broad-spectrum acridone chemotype.

Figure 2.
Structures of 1,4/2,4/3,4-dichloro-acridones **100–102**.

Figure 3. Acridones efficacy in the in vivo liver stage model in C57BL/6 male Albino mice infected with 50000 luciferase-expressing *P. berghei* sporozoites.

Scheme 1.

Synthesis of acridones **7–15**, **23**, **24**, and **29–32** with a substitution at the 2/3 position of ring-A and (dialkylamino)alkoxy/chloroalkoxy moiety at the 6 position of ring-B^a

^aReagents and conditions: (i) Cu powder, K₂CO₃, pentanol, reflux, 5 h; (ii) Eaton's acid, 90 °C, 5–24 h; (iii) HI, phenol, reflux, 3 h; (iv) Cl-(CH₂)_n-R¹/R². HCl, K₂CO₃, acetone, reflux, 5–12 h; (v) Tf₂O, pyridine, CH₂Cl₂, 0 °C–rt, 5 h; (vi) Pd(dba)₂, DPPF, KO^tBu, toluene, 100 °C, 8–12 h; (vii) BBr₃, CH₂Cl₂, –78 °C–rt, 12 h; (viii) Br-(CH₂)₃-Cl, K₂CO₃, acetone, reflux, 24 h; (ix) *tert*-butylamine, NaI, DMSO, 145 °C, 2 h.

Scheme 2.

Synthesis of acridones **38–55**, **62** and **63** with disubstitutions at the 1 and 3 positions of ring-A, and alkoxy/(dialkylamino)alkoxy/chloroalkoxy moiety at the 6 position of ring-B^a

^aReagents and conditions: (i) $\text{Pd}(\text{dba})_2$, DPPF, KO^tBu , toluene, 100°C , 8-12 h; (ii) Eaton's acid, 90°C , 5-24 h; (iii) HI, phenol, reflux, 3 h; (iv) $\text{Br}-(\text{CH}_2)_n-\text{Cl}$, K_2CO_3 , acetone, reflux, 24 h; (v) alkylamine, NaI, DMSO, 145°C , 2 h; (vi) $\text{Cl}-(\text{CH}_2)_n-\text{R}^3.\text{HCl}$, K_2CO_3 , acetone, reflux, 3-12 h; (vii) $\text{Br}-(\text{CH}_2)_2-\text{Br}$, KI, K_2CO_3 , acetone, reflux, 24 h; (viii) dipropylamine, KI, K_2CO_3 , acetone, reflux, 18 h; (ix) Tf_2O , pyridine, CH_2Cl_2 , 0°C -rt, 5 h.

Scheme 4.

Synthesis of acridones **109–113**, **119**, **120**, **123** and **128** with mono/di-substitutions on ring-A and (dialkylamino)alkoxy moiety at the 7 position of ring-B^a

^aReagents and Conditions: (i) Br-(CH₂)₂-Cl, K₂CO₃, acetone, reflux, 10 h; (ii) dipropyl amine, NaI, DMSO, 145 °C, 3 h; (iii) Tf₂O, pyridine, CH₂Cl₂, 0 °C-rt, 5 h; (iv) Pd(OAc)₂, XPhos, Cs₂CO₃, toluene, 110 °C, 5–8 h; (v) Eaton's acid, 90 °C, 5–24 h; (vi) BBr₃, CH₂Cl₂, –78 °C-rt, 12 h; (vii) Cl-(CH₂)₂-N(Et)₂.HCl, K₂CO₃, acetone, reflux, 10 h; (viii) Cl-(CH₂)₂-N(Pr)₂.HCl, K₂CO₃, acetone, reflux, 10 h; (ix) Cu powder, K₂CO₃, pentanol, reflux, 5 h.

Table 1.In Vitro Blood Stage Antimalarial Activity and Cytotoxicity of Acridones (T3.5, **7–15**, **23**, **24** and **29–32**)

compd	R ¹	R ²	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
			D6	Dd2	7G8	Tm90-C2B		
T3.5	-	-	49	77	86	71	> 40000	> 816
7	3-Cl		32	41	38	589	36389	1137
8	3-Br		25	34	37	968	> 40000	> 1600
9	3-F		377	485	353	1669	47872	127
10	3-H		1347	1189	1187	2531	96774	72
11	3-Cl		56	63	70	563	59023	1054
12	3-Cl		2.5	11	9.1	1542	40631	16252
13	3-Cl		529	781	959	1500	25485	48
14	3-Cl		272	254	294	1890	19653	72
15	3-Cl		540	511	517	1044	31279	58

compd	R ¹	R ²	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
			D6	Dd2	7G8	Tm90-C2B		
23	3-CF ₃		816	555	1537	1394	38594	47
24	3-CF ₃		26	0.44	5.6	1535	> 100000	> 3846
29	2-Cl		212	351	304	1163	> 100000	> 473
30	2-Cl		3.6	15	9.2	317	>100000	> 27777
31	2-Cl		24	38	51	371	> 100000	> 4167
32	2-Cl		674	1023	> 2500	2135	75225	112
ATV	-	-	0.10	0.10	0.20	8256	23160	231600
CQ	-	-	15	163	171	208	37577	2505

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

In order to compare results run on different days and with different batches of each stain, D6: CQ sensitive; Dd2: MDR strain with Old World genetic background; 7G8: MDR strain with New World genetic background; Tm90-C2B: Atovaquone resistant clinical isolate; ATV: Atovaquone; CQ: Chloroquine.

^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6).

Table 2.In Vitro Blood Stage Antimalarial Activity and Cytotoxicity of Acridones (**38–55**, **61** and **63**) **38-55, 62 and 63**

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
38	Cl	Cl		14	12	44	409	> 100000	> 7143
39	F	F		5.0	7.0	22	46	> 200000	> 40000
40	Cl	Cl		2.2	2.9	6.6	70	> 100000	> 45454
41	F	F		1.4	0.30	1.9	89	> 100000	> 71428
42	F	F		0.12	0.16	0.50	40	> 100000	> 100000
43	F	F		0.027	0.033	0.50	40	> 200000	> 200000
44	F	F		0.026	0.041	0.050	5.4	> 100000	> 100000
45	Cl	Cl		8.2	22	21	465	13365	1630
46	F	F		7.8	16	12	1979	77586	9947
47	Cl	Cl		0.0050	0.0030	0.0060	153	37194	7438

38-55, 62 and 63

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
48	F	F		0.00030	0.00030	0.00030	6.8	42821	> 100000
49	F	F		0.0020	0.015	0.016	2.0	> 100000	> 100000
50	Cl	Cl		19	45	114	228	130000	6842
51	F	F		32	7.8	44	705	38601	1206
52	F	F		0.35	0.49	2.1	440	61461	175602
53	Cl	Cl		101	186	294	82	11087	110
54	F	F		115	8.9	627	2500	41492	361
55	F	F		75	265	14	> 2500	101000	1346
62	Cl	OMe		0.65	4.2	6.2	81	> 200000	> 307692

38-55, 62 and 63

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
63	OMe	Cl		251	184	251	> 2500	45000	179
ATV			-	0.10	0.10	0.20	8256	23160	231600
CQ			-	15	163	171	208	37577	2505

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

In order to compare results run on different days and with different batches of each stain, *D6*: CQ sensitive; *Dd2*: MDR strain with Old World genetic background; *7G8*: MDR strain with New World genetic background; *Tm90-C2B*: Atovaquone resistant clinical isolate; *ATV*: Atovaquone; *CQ*: Chloroquine.

^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6).

Table 3.In Vitro Blood Stage Antimalarial Activity and Cytotoxicity of Acridones (**70–81**)

compd	R ¹	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a Vs HepG2	selectivity index (SI) ^b (D6)
		D6	Dd2	7G8	Tm90-C2B		
70		2.5	27	45	1377	>100000	> 40000
71		3.2	17	3.1	1045	> 100000	> 31250
72		393	2000	1250	> 2500	> 100000	> 254
73		10	12	17	1259	14431	1443
74		0.17	0.050	0.30	142	41600	244706
75		0.022	0.041	0.043	228	54510	2478
76		48	172	440	368	47608	992
77		65	38	273	196	> 100000	> 1538
78		106	679	295	> 2500	38367	362

compd	R ¹	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a Vs HepG2	selectivity index (SI) ^b (D6)
		D6	Dd2	7G8	Tm90-C2B		
79		196	1359	1634	> 2500	24830	127
80		46	37	106	68	22654	492
81		30	77	176	163	10000	333
ATV	-	0.10	0.10	0.20	8256	23160	231600
CQ	-	15	163	171	208	37577	2505

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

In order to compare results run on different days and with different batches of each stain, *D6*: CQ sensitive; *Dd2*: MDR strain with Old World genetic background; *7G8*: MDR strain with New World genetic background; *Tm90-C2B*: Atovaquone resistant clinical isolate; *ATV*: Atovaquone; *CQ*: Chloroquine.

^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6).

Table 4.

In Vitro Blood Stage Antimalarial Activity and Cytotoxicity of Acridones (**82–86**, **92**, **93** and **98–102**) **82-86, 92, 93, 98-102**

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
82	2-Cl	3-Cl		179	161	2332	945	> 100000	> 558
83	2-Cl	3-Cl		44	132	73	507	> 100000	> 2273
84	2-Cl	3-Cl		3.4	1.7	11	356	49945	1469
85	2-Cl	3-Cl		123	589	421	551	6099	49
86	2-Cl	3-Cl		36	145	161	177	75244	2490
92	2-F	3-F		146	187	110	270	> 200000	> 1370
93	2-F			116	209	774	386	> 200000	> 12500
98	2-F	3-OMe		156	448	533	622	> 200000	> 1282

82-86, 92, 93, 98-102

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
99	2-F	3-OMe		526	1169	1295	1512	126000	239
100	1-Cl	4-Cl		506	1301	848	> 2500	77000	152
101	2-Cl	4-Cl		928	> 2500	1293	> 2500	35000	38
102	3-Cl	4-Cl		749	2442	> 2500	> 2500	46000	61
ATV	-	-	-	0.10	0.10	0.20	8256	23160	231600
CQ	-	-	-	15	163	171	208	37577	2505

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

In order to compare results run on different days and with different batches of each stain, D6: CQ sensitive; Dd2: MDR strain with Old World genetic background; 7G8: MDR strain with New World genetic background; Tm90-C2B: Atovaquone resistant clinical isolate; ATV: Atovaquone; CQ: Chloroquine.

^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6).

Table 5.In Vitro Blood Stage Antimalarial Activity and Cytotoxicity of Acridones (**109–113**, **119**, **120**, **123** and **128**) **109-113, 119, 120, 123, and 128**

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
109	1-F	3-F		0.40	1.0	3.2	270	20230	50575
110	1-F	3-OMe		0.70	0.13	0.42	47	26418	37740
111	1-OMe	3-F		35	105	175	> 250	55733	1592
112	1-Cl	3-OMe		79	92	288	558	> 100000	> 1266
113	1-OMe	3-Cl		30	11	32	96	36757	1225
119	1-Cl	2-Cl		99	17	32	274	12654	128
120	2-Cl	3-Cl		26	17	14	58	> 100000	> 3846
123	H	2-Cl		159	399	521	297	> 100000	> 629
128	H	3-Cl		110	132	81	97	29970	272

109-113, 119, 120, 123, and 128

compd	R ¹	R ²	R ³	blood stage IC ₅₀ (nM) ^a vs <i>P. falciparum</i>				cytotoxicity IC ₅₀ (nM) ^a vs HepG2	selectivity index (SI) ^b (D6)
				D6	Dd2	7G8	Tm90-C2B		
ATV	-	-	-	0.10	0.10	0.20	8256	23160	231600
CQ	-	-	-	15	163	171	208	37577	2505

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

In order to compare results run on different days and with different batches of each stain, *D6*: CQ sensitive; *Dd2*: MDR strain with Old World genetic background; *7G8*: MDR strain with New World genetic background; *Tm90-C2B*: Atovaquone resistant clinical isolate; *ATV*: Atovaquone; *CQ*: Chloroquine.

^bSI (selectivity index) = IC₅₀ (cytotoxicity)/IC₅₀ (D6).

Table 6.

In Vitro Liver Stage Antimalarial Activity of Acridones

compd	liver stage IC ₅₀ (nM) ^a Vs <i>P. berghei</i>
7	113
8	100
9	119
10	503
12	14.8
13	456
14	436
15	202
29	221
30	12.5
40	523
42	17
43	48
44	72
47	3.7
48	1.4
49	0.090
50	138
52	3.4
55	362
62	2.9
70	183
73	5.5
74	1.1
75	0.50
81	86
84	7.8
109	0.75
110	0.65
128	793
ATV	6.5
CQ	> 31262

^aIC₅₀ values are the average of at least three determinations, each carried out in triplicate (±10%).

Table 7.In Vivo Antimalarial Efficacy in a Murine *P. yoelii*

compd	code name	Blood stage efficacy vs <i>P. yoelii</i> (4day)	
		ED ₅₀ (mg/kg/d)	ED ₉₀ (mg/kg/d)
7	T2	48	72
44	T42	1.09	12.7
45	T27	3.09	50.2
46	T26	1.62	9.91
47	T49	0.21	3.54
48	T36	1.12	8.90
49	T41	0.66	2.53
50	T50	0.43	24.1
52	T43	0.60	2.48
73	T13	1.80	8.80
74	T35	1.04	1.14
75	T31	1.03	1.64
CQ	-	1.46	3.28

Table 8.

hERG Activity of Lead Acridones

compd	hERG activity (% inhibition at 10 uM)
44	20
47	38
48	65
49	17
50	59
52	102
73	78
74	25
75	57
CQ	39

Table 9.

In Vitro Metabolic Stability of Acridones

compd	metabolic stability vs microsomes $t_{1/2}$ (min)	
	human	mouse
7	> 60	> 60
8	> 60	> 60
9	> 60	> 60
12	> 60	> 60
14	> 60	34
15	> 60	>60
30	12	16
40	> 60	55
42	14	6.0
43	> 60	59
47	> 60	> 60
48	16	15
49	20	33
50	9.0	16
52	> 60	> 60
55	> 60	> 60
62	7.0	10
70	> 60	> 60
73	> 60	50
74	11	17
75	12	21
84	12	30
128	> 60	34

Table 10.

PK Parameters of Lead Acridones in Plasma and Liver Following Single Oral Dose of 80 mg/kg Administrations in Mice.

compd	Plasma/ Liver	C_{max} (ng/ml)	T_{max} (hr)	AUC_{last} (ng.h/ml)	AUC_{inf} (ng.h/ml)	$t_{1/2}$ (h)	CL/F (ml/hr/kg)	Vz/F (ml/kg)	MRT b (h)
7 (T2)	Plasma	1355	2.00	5309	5807	1.90	13774	37689	3.41
	Liver	193452	1.00	1457315	1457562	6.70	-	-	5.59
48 (T36)	Plasma	99	7.00	7558	56485	54.24	24466	657604	10.18
	Liver	3927	7.00	63900	101502	13.80	932	13970	8.90
52 (T43)	Plasma	526	7.78	3434	5075	7.78	15914	175302	11.82
	Liver	19711	4.00	236372	237386	6.59	348	3478	13.26
74 (T35)	Plasma	631	5.50	3841	4516	2.29	17714	58684	4.69
	Liver	42344	7.00	546818	546889	1.84	147	392	6.07
75 (T31)	Plasma	226	2.75	3049	3239	6.00	25879	223865	6.96
	Liver	21269	2.75	167981	169389	4.63	537	3605	10.08
Primaquine	Plasma	531	0.50	1256	1314	1.84	16415	43801	2.30
	Liver	8148	0.50	27450	27665	4.27	724	4418	4.50

C_{max} : maximum plasma or hepatic concentration; T_{max} : time to C_{max} ; AUC_{last} : area under the concentration-time curve from 0 up to the last sampling time at which a quantifiable concentration is found; AUC_{inf} : area under the concentration-time curve from 0 up to infinity; $t_{1/2}$: apparent elimination half-life; CL/F: apparent oral clearance; Vz/F: apparent volume of distribution after oral dose; MRT b: mean residence time, body.

Table 11.IC₅₀ Values of Standard Antimalarials and Acridones Against Selected *P. falciparum* Parasites.

compd	Dd2 IC ₅₀ (nM) ^a	Dd2 ^{V259L} IC ₅₀ (nM) ^a	Dd2 ^{M133I+A138T} IC ₅₀ (nM) ^a	Dd2 ^{M133I} IC ₅₀ (nM) ^a	3D7 IC ₅₀ (nM) ^a	3D7 ^{A82T+V259L} IC ₅₀ (nM) ^a
73	40 ± 5.5	1439 ± 49	1678 ± 78	242 ± 18	28 ± 1.7	1518 ± 358
7	117 ± 12	2122 ± 83	1067 ± 57	nt ^b	84 ± 5.7	1645 ± 132
45	31 ± 1.4	668 ± 32	1077 ± 67	nt	16 ± 0.1	612 ± 38
46	20 ± 1.6	525 ± 20	60 ± 1.7	nt	15 ± 1.3	1150 ± 98
48	0.23 ± 0.03	5.5 ± 0.2	0.86 ± 0.08	nt	0.3 ± 0.02	17 ± 3.4
71	22 ± 1.5	551 ± 13	324 ± 29	nt	11.1 ± 1.3	605 ± 46
74	9.0 ± 1.0	216 ± 8.2	50 ± 13	nt	nt	79 ± 7.9
75	14 ± 1.4	140 ± 4.5	38 ± 3.6	nt	nt	57 ± 3.5
76	616 ± 17	3113 ± 81	nt	nt	nt	nt
78	139 ± 3.9	3070 ± 381	nt	nt	nt	nt
81	390 ± 15	1103 ± 48	nt	nt	nt	nt
84	212 ± 7.6	1321 ± 58	602 ± 115	nt	178 ± 12	750 ± 46

compd	Dd2 IC ₅₀ (nM) ^a	Dd2 ^{V259L} IC ₅₀ (nM) ^a	Dd2 ^{M133I+A138T} IC ₅₀ (nM) ^a	Dd2 ^{M133I} IC ₅₀ (nM) ^a	3D7 IC ₅₀ (nM) ^a	3D7 ^{A82T+V259L} IC ₅₀ (nM) ^a
128	366 ± 14	355 ± 7.9	340 ± 7.1	nt	262 ± 8.4	284 ± 11
ELQ300	13.0 ± 0.4	11.0 ± 0.3	6.7 ± 0.4	9.8 ± 0.3	11.0 ± 0.3	5.4 ± 0.3
ATV	0.5 ± 0.04	4.1 ± 0.2	1.4 ± 0.1	6.9 ± 1.0	0.5 ± 0.03	1.9 ± 0.2
CQ	181 ± 4.3	260 ± 17	186 ± 8.0	182 ± 5.6	9.6 ± 0.4	9.6 ± 0.8
Quinine	254 ± 9.8	215 ± 7.5	199 ± 13	197 ± 3.5	nt	nt
Mefloquine	18 ± 1.3	23 ± 1.0	13 ± 0.6	14 ± 0.7	nt	nt
Lumefantrine	5.7 ± 0.2	6.5 ± 0.3	3.6 ± 0.2	3.6 ± 0.3	3.0 ± 0.3	4.5 ± 0.1
Piperaquine	9.0 ± 0.2	7.1 ± 0.3	7.4 ± 0.3	10 ± 0.3	7.2 ± 1.3	5.0 ± 0.4
Quinacrine	29 ± 0.1	18 ± 0.7	29 ± 0.7	23 ± 0.6	nt	nt

^aIC₅₀ values ± SEM; N=3

^bnt: not tested;

CQ: Chloroquine; ATV: Atovaquone; ELQ-300: currently MMV preclinical candidate, cytochrome b Q_i site inhibitor