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Bioorganic & Medicinal Chemistry 14 (2006) 334–343
Bioorganic & Medicinal Chemistry

Parallel synthesis of 9-aminoacridines and their evaluation against chloroquine-resistant *Plasmodium falciparum*

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Received 1 July 2005; revised 4 August 2005; accepted 5 August 2005
Available online 10 October 2005

Abstract—A parallel synthetic strategy to the 9-aminoacridine scaffold of the classical anti-malarial drug quinacrine (**2**) is presented. The method features a new route to 9-chloroacridines that utilizes triflates of salicylic acid derivatives, which are commercially available in a variety of substitution patterns. The route allows ready variation of the two diversity elements present in this class of molecules: the tricyclic aromatic heterocyclic core, and the disubstituted diamine sidechain. In this study, a library of 175 compounds was designed, although only 93 of the final products had purities acceptable for screening. Impurity was generally due to incomplete removal of 9-acridones (**18**), a degradation product of the 9-chloroacridine synthetic intermediates. The library was screened against two strains of *Plasmodium falciparum*, including a model of the drug-resistant parasite, and six novel compounds were found to have IC_{50} values in the low nanomolar range.

1. Introduction

Malaria is a devastating infectious disease caused by the protozoan parasite *Plasmodium*.[1] Four species of *Plasmodium* are known to infect humans: *P. vivax*, *P. malariae*, *P. ovale*, and *P. falciparum*, the latter being the most deadly. While the controversial use of polychlorinated biphenyl pesticides (e.g., DDT) has generally led to the eradication of malaria in North America and most European countries,[2] the disease is still widespread in Africa, Central and South America, and Southeast Asia. Malaria continues to affect 200–500 million people and cause 1–2 million fatalities annually.[3] As a result, malaria has produced devastating social and economic burdens on the countries most afflicted by it.[1]

The development of anti-malarial compounds continues to be a major focus of many laboratories,[4] and as such, a variety of compounds have been explored in the prophylaxis and treatment of this disease (Fig. 1). The efficacy of quinine (**1**), a derivative of cinchona-bark, as an anti-malarial compound was recognized as early as the 1600 s. The earliest synthetic anti-malarial agent was quinacrine (**2**). Quinacrine was supplemented by chloroquine (**3**),[5] along with various other 4- and 8-substituted quinolines, including mefloquine (**4**).[6] Chloroquine was

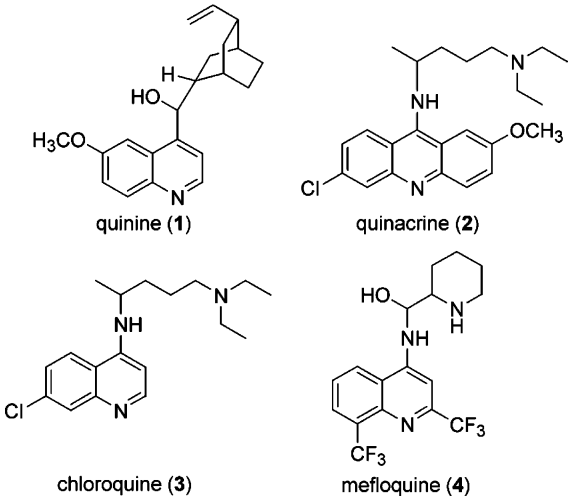

Figure 1. Structures of common quinoline and acridine anti-malarial compounds.

Keywords: Malaria; Acridine; Parallel synthesis.
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doi:10.1016/j.bmc.2005.08.017

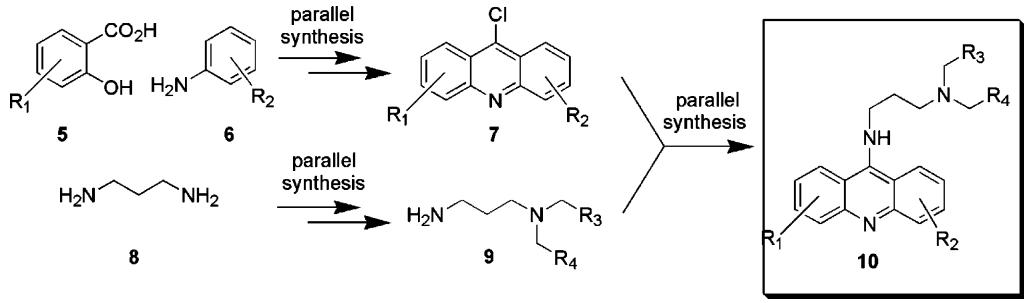


Figure 2. A parallel synthetic strategy to generate libraries of 9-aminoacridines.

found to be highly effective against all four human-infecting species of *Plasmodium*, and its inexpensive production led it to quickly become a standard anti-malarial treatment.[5] Unfortunately, over several decades this resulted in the development of chloroquine-resistant strains of *Plasmodium*, particularly of *P. falciparum*.[7,8] Certain substituted quinolines, including mefloquine (**4**), have been efficacious against chloroquine-resistant *P. falciparum* (CRPF), but this drug is also associated with a variety of neuropsychiatric side-effects.[9] Another promising class of compounds with activity against CRPF are the artemisinins.[10–12] The continuing exploration of compounds active against CRPF is an active pursuit of our laboratory.[13,14]

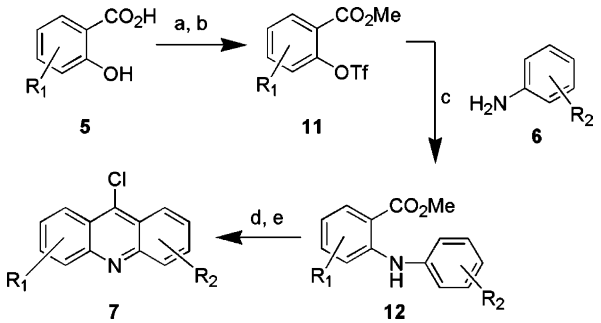
In the context of exploring the 9-aminoacridine scaffold of quinacrine (**3**) to target the propagation of prions,[15–17] we became interested in re-evaluating this class of molecules for anti-malarial activity, particularly against CRPF. Herein we report a parallel synthetic strategy to generate libraries of 9-aminoacridines based on the general structure of quinacrine, along with in vitro cell-based screening results against drug-sensitive and drug-resistant strains of *P. falciparum*. In addition to having anti-prion and anti-malarial properties, 9-aminoacridines are interesting for other types of biological activity. For example, 9-aminoacridines are known DNA intercalators,[18] inhibitors of mammalian topoisomerase I[19] and acetylcholinesterase,[20] and these compounds are also active against African trypanosomes.[21–23] Cancer chemotherapeutics based on the 9-aminoacridine scaffold have been developed[24] (e.g., Ledakrin and Amascrine). Additionally, 9-aminoacridines have been explored as photoaffinity labels[25] and as fluorescent probes used to detect cancer cells.[26]

Our general synthetic strategy to 9-aminoacridines is summarized in Figure 2. Derivatives of substituted salicylic acid chemset **5** were coupled to aniline chemset **6** to generate 9-chloroacridine chemset **7**, as was previously introduced.[27] The use of salicylic acid precursors, activated as triflates, is novel and deviates from an earlier approach to 9-chloroacridines based on the Ulmann coupling of anilines with 2-bromoaryl carboxylic acids,[28–30] of which much fewer are commercially available. To approach the amine sidechain, 1,3-diaminopropane (**8**) was dually functionalized to generate a library of requisite diamine building block chemset **9**. Finally, parallel coupling of 9-chloroacridines with the diamines was done to generate the desired 9-aminoacridine chemset **10**. A notable feature of this strategy is that it allows quick access to variations of the tricyclic acridine heterocycle as well as the amine sidechain, which will allow probing of the cooperativity of these two structural features.

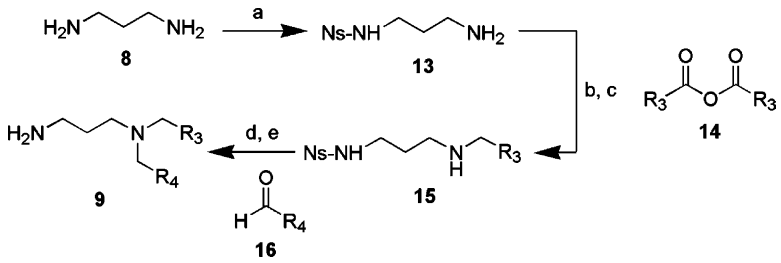
2. Results

2.1. Chemistry

The synthesis of the 9-chloroacridine heterocycles is illustrated in Scheme 1. The salicylic acid chemset (**5**) was methylated and then activated for cross-coupling as the corresponding aryl triflate chemset (**11**), generally in near quantitative yield giving compounds of high purity. These species were coupled to substituted aniline chemset **6** to generate diarylamine chemset **12**, using conditions developed by Buchwald et al.,[31] which proceeded smoothly. Next, hydrolysis of the methyl ester groups was performed using non-aqueous conditions designed for parallel synthesis,[27] followed by Friedel–Crafts cyclization with $POCl_3$ to generate the 9-chloroacridine chemset (**7**). The transformation of **11** to **7** was accomplished in a parallel 3-step, one-pot procedure, without intermediate workup, using a 12-place reaction carrousel (Radleys Discovery Technologies). The 9-chloroacridine products (**7**) were isolated in



Scheme 1. Synthesis of 9-chloroacridines. *Reagents and conditions:* (a) MeI (1.05 equiv), Cs_2CO_3 (0.5 equiv) DMF (90–100% yield); (b) Tf_2O (1.1 equiv), Et_3N (2.0 equiv), CH_2Cl_2, −78 °C to rt (95–100% yield); (c) substituted anilines **6** (1.05 equiv), $Pd(OAc)_2$ (0.05 equiv), BINAP (0.08 equiv), Cs_2CO_3 (1.4 equiv), toluene; (d) $Ba(OH)_2$–$8H_2O$ (2.5 equiv), CH_3OH, 80 °C overnight; (e) $POCl_3$ (neat) 120 °C, 1 h (5–50% yield).

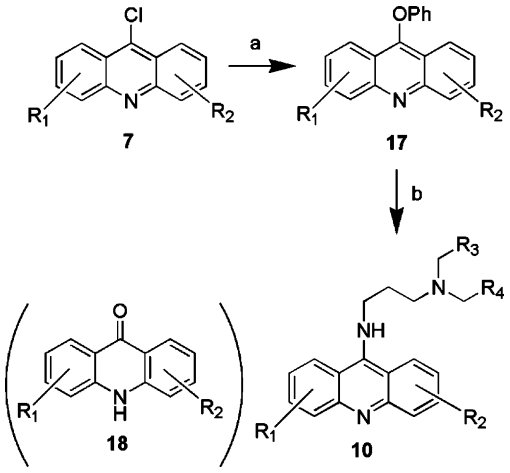


Scheme 2. Synthesis of diamines. *Reagents and conditions:* (a) 2-nitrobenzenesulfonyl chloride (0.1 equiv), CH_2Cl_2, °C to rt, overnight (70 g scale, 85% yield); (b) Carboxylic anhydrides (**14**) (1.2 equiv), pyridine (1.1 equiv), THF (90–100% yields); (c) BH_3dimethylsulfide complex (4.0 equiv), THF, 60 °C, 30 min (67–77% yields); (d) Aldehydes **16** (1.5 equiv), sodium triacetoxyborohydride (1.5 equiv), THF, *sonicated*, 35 °C, 1 h; (e) Benzenethiol (5 equiv), Cs_2CO_3 (2.5 equiv), degassed CH_3CN *under argon*.

acceptable yield and >95% purity after automated flash column purification using a CombiFlash system (Isco).

The synthesis of the diamine building blocks is illustrated in Scheme 2. 1,3-diaminopropane (**8**) was mono-sulfonylated under dilute conditions to attach the thiolate-labile 2-nitrobenzenesulfonyl (nosyl = Ns) protecting group[32,33] to generate **13** in high yield and purity. Amidation with carboxylic anhydride reagents (**14**), followed by reduction with borane–dimethylsulfide complex,[34] generated the corresponding secondary amine chemset **15** in good yield and excellent purity. An alternate acylation reaction using acid chloride reagents formed a byproduct with an additional acyl group, apparently attached to the sulfonamide nitrogen atom. Lastly, reductive amination with aldehyde chemset **16** and sodium triacetoxyborohydride,[35] followed by deprotection of nosyl groups by treatment with cesium benzenethiolate,[32,33] afforded the free amine chemset **9**. Steps a–c were carried out in large scale (>60 g initially) using traditional glassware, while steps d and e (1.5 g/each) were done in parallel using the Radleys carrousel apparatus. The final two heterogeneous reactions proceeded slowly in the 12-place Radleys carrousel, presumably due to poor mixing; magnetic stirring using the 6-place carrousel alleviated this problem. The reductive amination reaction (step d) could alternatively be performed using the smaller reaction vessels of the 12-place carrousel, but agitated in a sonicating wash bath rather than by magnetic stirring. The products of the last two steps were isolated in high purity, either by aqueous workup, or in parallel using solid-phase extraction (SPE) with Dowex 50WX2-400 ion-exchange resin. Chromatographic purification was not required in any of the steps of the sequence.

The coupling of 9-chloroacridines with diamines to generate the desired 9-aminoacridine products is illustrated in Scheme 3. 9-Chloroacridine chemset **7** was converted to the reactive intermediate 9-phenoxyacridine chemset **17** by treatment with phenol in DMSO. Subsequent reaction with the diamine chemset **9** afforded the desired 9-aminoacridine products **10**. Direct treatment of 9-chloroacridines with diamines, that is, omitting the activation step, afforded little or no product. Furthermore, in some of the coupling reactions, the formation of 9-acridone byproducts (chemset **18**) was observed, presumably due to hydrolysis of 9-chloroacridines.[36] This side reaction could be partially suppressed



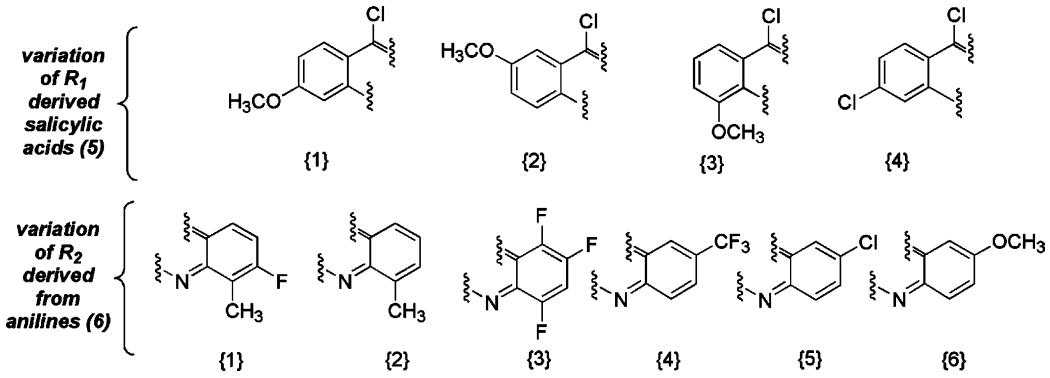
Scheme 3. Coupling of 9-chloroacridines with diamines. *Reagents and conditions:* (a) phenol (15 equiv), Cs_2CO_3 (1 equiv), 3Å molecular sieves, DMSO, 100 °C, 2 h; (b) Diamines (**9**) (4 equiv), DMSO, 100 °C, 4 h; then methyl isocyanate polystyrene resin (NovaBiochem, 8 equiv), overnight, rt.

by the addition of 3 Å molecular sieves during the coupling, although decomposition also occurred during ambient storage of the precursor chloroacridines.

The coupling reactions are carried out in 48-position parallel synthesis blocks (Bohdan) and agitated with the Bohdan MiniBlock High Capacity Shaking and Washing Station (600 RPM, 100 °C). After coupling was achieved, the remaining diamine was then scavenged by treatment with electrophilic methyl isocyanate resin. Subsequent workup was performed by SPE with Dowex 50WX2-400 ion-exchange resin. A three-step parallel SPE protocol was devised to purify the 9-aminoacridine products: first, the crude product was loaded on the column and washed with TFA (5% in MeOH) to elute DMSO and phenol; next, the resin was washed with pyridine (5% in MeOH) to elute unreacted 9-phenoxyacridines (**17**) and 9-acridones (**18**); finally, the desired 9-aminoacridine product chemset (**10**) was eluted using Et_3N (5% in MeOH).

2.2. Synthesis of 9-aminoacridine and diamine components

A selection of 15 diversely substituted 9-chloroacridine components and 16 diamine components was defined.



Scheme 4. Composition of 9-chloroacridine (**7**) chemset.

The precursor components of the 9-chloroacridine library are defined in Scheme 4. Various substituent patterns were chosen to test the scope of the chemistry, specifically electronic effects in the Buchwald coupling and cyclization steps. The synthesis results of the 9-chloroacridine component are outlined in Table 1. The purified yields after the three-step sequence ranged from moderate to fairly low. A degree of degradation was observed in nearly all of the 9-chloroacridines synthesized, namely hydrolysis to form 9-acridones (**18**).

The design of the disubstituted diamine chemset (**9**) is outlined in Figure 4. All amines in this library were based on a 1,3-diaminopropane linker. Two carboxylic anhydrides (acetic and propionic) were used to vary R_3, while the majority of variation was achieved with a selection of aldehydes to vary R_4. The results of the synthesis of the diamines are outlined in Table 2. Of the products, the volatile low molecular weight dialkyl-substituted diamines (entries 1–2, 9–10) were isolated as trifluoroacetate salts. Overall, yields were moderate to very good in the reductive amination and nosyl deprotection reactions. Each of the diamine compounds was produced in very high purity, as assessed by NMR.

Table 1. 9-chloroacridine (**7**) chemset synthesis results

Entry	Salicylic acid	Aniline	Product	Yield[a] (%)
1	**5**{1}	**6**{1}	**7**{1,1}	48
2	**5**{1}	**6**{2}	**7**{1,2}	26
3	**5**{1}	**6**{3}	**7**{1,3}	21
4	**5**{1}	**6**{4}	**7**{1,4}	44
5	**5**{2}	**6**{1}	**7**{2,1}	42
6	**5**{2}	**6**{2}	**7**{2,2}	37
7	**5**{2}	**6**{3}	**7**{2,3}	6
8	**5**{2}	**6**{4}	**7**{2,4}	31
9	**5**{3}	**6**{1}	**7**{3,1}	25
10	**5**{3}	**6**{2}	**7**{3,2}	30
11	**5**{3}	**6**{3}	**7**{3,3}	31
12	**5**{4}	**6**{1}	**7**{4,1}	18
13	**5**{4}	**6**{2}	**7**{4,2}	40
14	**5**{4}	**6**{4}	**7**{4,4}	23
15	**5**{1}	**6**{5}	**7**{1,5}	49

[a] Purified yield of the final three-step one-pot sequence.

2.3. Proof-of-concept library

The coupling of 9-chloroacridines with disubstituted diamines to generate a proof-of-concept library of 9-aminoacridines (**10**) is outlined in Table 3. In the first set of entries (1–15), the synthesized 9-chloroacridines were coupled with commercially available sidechain **9**{1,9}, while in the second set of entries (16–31), the commercially available 9-chloroacridine heterocycle **7**{4,6} of quinacrine was coupled with the set of synthesized diamines. Yields and purities of the coupled products were highly variable. Notable degradation of the synthesized 9-chloroacridine starting materials to 9-acridones (**18**) accounts for the overall lower yields in the first set of entries. Impurity was generally accounted for by the presence of 9-acridones not effectively separated during the SPE purification. We suspect that the second set of entries was less prone to impurity problems due to the shelf stability of the commercially available quinacrine 9-chloroacridine **7**{4,6} starting material. Products where final purity was less than 50% by RP-HPLC analysis were indicated as failed entries and constituted 20% of this library.

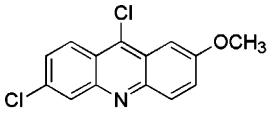
2.4. Cross-coupled library

Having established our parallel synthesis methodology in a proof-of-concept library, we then prepared a cross-coupled library of synthesized 9-chloroacridines with synthesized diamines. The proof-of-concept library showed some classes of 9-chloroacridines achieving better yields and purities than others during the coupling reaction, with some being particularly more prone to hydrolysis to the 9-acridone, possibly due to electron-withdrawing substitutuents increasing electrophilicity at C_9. Thus, we designed a library utilizing a subset of 9-chloroacridines (**7**), specifically compounds **7**{1,1}, **7**{1,2}, **7**{1,4}, **7**{2,1}, **7**{2,2}, **7**{2,4}, **7**{4,1}, **7**{4,2}, and **7**{1,5}, that appeared be the most promising and likely to generate compounds acceptable for screening. These species were cross-coupled with the entire series of synthesized amines (**9**), to generate a library of 144 compounds (Table S1, Supporting information). Using the same criteria established for the proof of concept library, 53% of the compounds were marked as failed entries, due to final purities being less than 50% after the initial SPE purification. While the number of failed couplings is significant, it is notable that the relative success

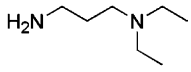
Table 2. Disubstituted diamine chemset (**9**) synthesis results

Entry	Anhydride	Aldehyde	Product	Yield: reductive amination (%)	Yield: nosyl deprotection (%)
1	**14**{1}	**16**{1}	**9**{1,1} · 2TFA	46	81
2	**14**{1}	**16**{2}	**9**{1,2} · 2TFA	82	85
3	**14**{1}	**16**{3}	**9**{1,3}	84	91
4	**14**{1}	**16**{4}	**9**{1,4}	71	87
5	**14**{1}	**16**{5}	**9**{1,5}	70	91
6	**14**{1}	**16**{6}	**9**{1,6}	87	93
7	**14**{1}	**16**{7}	**9**{1,7}	71	33
8	**14**{1}	**16**{8}	**9**{1,8}	58	94
9	**14**{2}	**16**{1}	**9**{2,1} · 2TFA	75	89
10	**14**{2}	**16**{2}	**9**{2,2} · 2TFA	83	92
11	**14**{2}	**16**{3}	**9**{2,3}	73	100
12	**14**{2}	**16**{4}	**9**{2,4}	79	97
13	**14**{2}	**16**{5}	**9**{2,5}	69	74
14	**14**{2}	**16**{6}	**9**{2,6}	66	84
15	**14**{2}	**16**{7}	**9**{2,7}	63	73
16	**14**{2}	**16**{8}	**9**{2,8}	42	79

Table 3. Proof library: coupling reactions to generate a library of 9-aminoacridines (**10**)



7{4,6}



9{1,9}

Entry	9-Chloroacridine component	Diamine component	Product	Yield (%) (purity, %)[a]
1	**7**{1,1}	**9**{1,9}	**10**{1,1,1,9} · 2HCl	52 (100)
2	**7**{1,2}	**9**{1,9}	**10**{1,2,1,9} · 2HCl	16 (83)
3	**7**{1,3}	**9**{1,9}	**10**{1,3,1,9} · 2HCl	Failed
4	**7**{1,4}	**9**{1,9}	**10**{1,4,1,9} · 2HCl	19 (100)
5	**7**{2,1}	**9**{1,9}	**10**{2,1,1,9} · 2HCl	22 (95)
6	**7**{2,2}	**9**{1,9}	**10**{2,2,1,9} · 2HCl	4 (81)
7	**7**{2,3}	**9**{1,9}	**10**{2,3,1,9} · 2HCl	Failed
8	**7**{2,4}	**9**{1,9}	**10**{2,4,1,9} · 2HCl	21 (92)
9	**7**{3,1}	**9**{1,9}	**10**{3,1,1,9} · 2HCl	8 (56)
10	**7**{3,2}	**9**{1,9}	**10**{3,2,1,9} · 2HCl	15 (50)
11	**7**{3,3}	**9**{1,9}	**10**{3,3,1,9} · 2HCl	Failed
12	**7**{4,1}	**9**{1,9}	**10**{4,1,1,9} · 2HCl	22 (81)
13	**7**{4,2}	**9**{1,9}	**10**{4,2,1,9} · 2HCl	22 (73)
14	**7**{4,4}	**9**{1,9}	**10**{4,4,1,9} · 2HCl	Failed
15	**7**{1,5}	**9**{1,9}	**10**{1,5,1,9} · 2HCl	10 (73)
16	**7**{4,6}	**9**{1,1} · 2TFA	**10**{4,6,1,1} · 2HCl	32 (81)
17	**7**{4,6}	**9**{1,2} · 2TFA	**10**{4,6,1,2} · 2HCl	18 (100)
18	**7**{4,6}	**9**{1,3}	**10**{4,6,1,3} · 2HCl	48 (100)
19	**7**{4,6}	**9**{1,4}	**10**{4,6,1,4} · 2HCl	34 (100)
20	**7**{4,6}	**9**{1,5}	**10**{4,6,1,5} · 2HCl	52 (100)
21	**7**{4,6}	**9**{1,6}	**10**{4,6,1,6} · 2HCl	32 (95)
22	**7**{4,6}	**9**{1,7}	**10**{4,6,1,7} · 2HCl	Failed
23	**7**{4,6}	**9**{1,8}	**10**{4,6,1,8} · 2HCl	19 (68)
24	**7**{4,6}	**9**{2,1} · 2TFA	**10**{4,6,2,1} · 2HCl	34 (100)
25	**7**{4,6}	**9**{2,2} · 2TFA	**10**{4,6,2,2} · 2HCl	60 (100)
26	**7**{4,6}	**9**{2,3}	**10**{4,6,2,3} · 2HCl	69 (100)
27	**7**{4,6}	**9**{2,4}	**10**{4,6,2,4} · 2HCl	62 (100)
28	**7**{4,6}	**9**{2,5}	**10**{4,6,2,5} · 2HCl	73 (100)
29	**7**{4,6}	**9**{2,6}	**10**{4,6,2,6} · 2HCl	47 (90)
30	**7**{4,6}	**9**{2,7}	**10**{4,6,2,7} · 2HCl	Failed
31	**7**{4,6}	**9**{2,8}	**10**{4,6,2,8} · 2HCl	51 (87)

[a] Purity determined by RP-HPLC-MS integration detected at 254 nm.

of certain 9-chloroacridines in the proof-of-concept library translated quite well to success in the cross-coupled library. Specifically, certain 9-chloroacridines (**7**) that worked relatively well in the proof library achieved a reasonable degree of yield and purity in the cross-coupled library: **7**{1,1}, **7**{2,1}, **7**{2,4}, **7**{4,1}, and **7**{4,2}.

2.5. Screening and biological evaluation

The compounds of the proof-of-concept and cross-coupled libraries were screened for in vitro anti-malarial activity against *P. falciparum* cultured in human erythrocytes. Growth inhibition of the parasite was measured using a fluorescent-active cell sorting (FACS) assay.[13,14] Two strains of the parasite were examined: 3D7 and W2, which are established models of chloroquine-sensitive and chloroquine, mefloquine-resistant *P. falciparum*, respectively. Initially, activity was screened at a fixed concentration of 30 nM (Figure 3), with data represented as parasite growth relative to untreated controls. Chloroquine (**3**) serves as a positive control for the 3D7 strain and a negative control for the W2 strain. Quinacrine (**2**) also serves as a positive control for

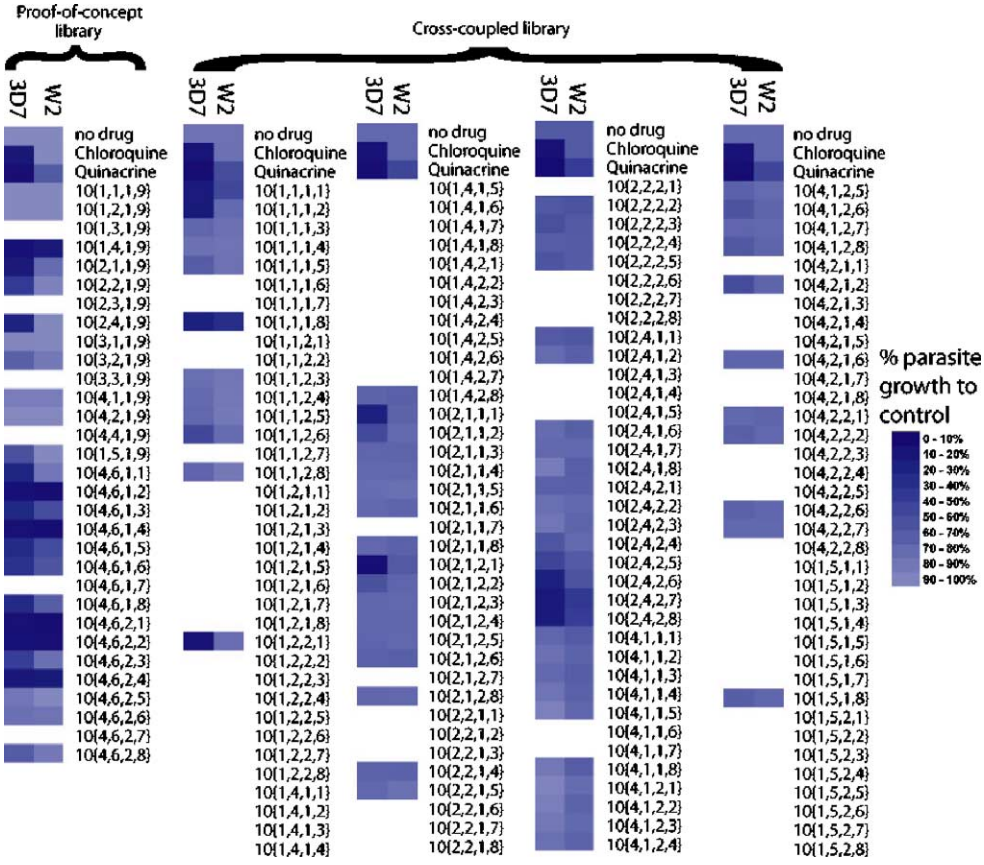


Figure 3. Relative activity of the library defined in Table 3 against *P. falciparum* strains 3D7 and W2 cultured in human erythrocytes (measured at fixed concentration: 30 nM). Data are represented in blue scale, as percent parasite growth relative to untreated controls, with darker squares indicating a higher activity against the parasites. Omitted entries are due to failure during synthesis.

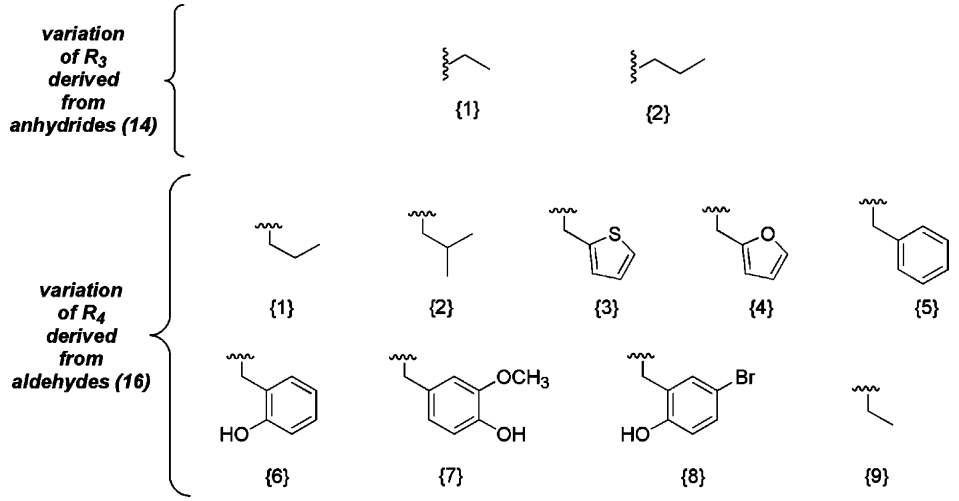


Figure 4. Composition of disubstituted diamine (**9**) chemset.

3D7, while this compound also has partial activity against the W2 strain. In this assay, several compounds of the proof-of-concept library were found to be highly active against both strains of the parasite, with notable decrease in parasitic growth. The 9-aminoacridines in the proof-of-concept library that appeared the most interesting, particularly in inhibiting the growth of the chloroquine-resistant W2 strain, were **10**{1,4,1,9}, **10**{4,6,1,2}, **10**{4,6,1,4}, **10**{4,6,2,1}, **10**{4,6,2,2}, and **10**{4,6,2,4}. Of the products derived from novel 9-chloroacridines, only **10**{1,4,1,9}, derived from **7**{1,4}, appeared to have significant activity at 30 nM. Notably, use of the commercially available acridine heterocycle scaffold **7**{4,6} with variation of the amine (Table 3, entries 16–31) appears to be a promising strategy for optimizing 9-aminoacridines for anti-malarial activity. Unfortunately, fewer compounds in the cross-coupled library appear as interesting, as many entries in this library failed in synthesis and thus were not screened. Several compounds were active against the 3D7 strain, but only one, **10**{1,1,1,8}, was found to have promising activity against the chloroquine-resistant W2 strain.

Structures and 50% effective inhibitory concentration (EC_{50}) values of the most promising 9-aminoacridines **10**{1,4,1,9}, **10**{4,6,1,2}, **10**{4,6,1,4}, **10**{4,6,2,1}, **10**{4,6,2,2}, and **10**{4,6,2,4} from the initial screen are presented in Figure 5. Of this set, compounds **10**{1,4,1,9} and **10**{4,6,2,1} appear to be the most potent, having sub-nanomolar EC_{50} values. The slightly less potent compounds can be grouped by their amine substitution patterns: **10**{4,6,1,2} and **10**{4,6,2,2} (both containing an isobutyl substituent), as well as **10**{4,6,1,4} and **10**{4,6,2,4} (both containing a furylmethyl substituent). The tolerance for fairly different styles of amine substituents, for example, branched alkyl groups and a furyl heterocyclic group, is an interesting feature of the pharmacophore. EC_{50} values were also determined for quinacrine (**2**) and chloroquine (**3**). Notably all of the synthesized compounds had improved potencies, when compared to **2** and **3**, against both strains of *P. falciparum*, and none of the compounds have previously been described in the literature.

3. Conclusion

A parallel synthetic strategy to generate 9-aminoacridines is described. The method features a new route to 9-chloroacridines that utilizes triflates of salicylic acid derivatives, which are commercially available in fairly diverse substitution patterns. Additionally, a procedure for the synthesis of disubstituted diaminoalkanes was presented. Two libraries were designed totaling 175 compounds, although only 93 of the final products had purities suitable for in vitro screening. Low purity was generally attributed to contamination by 9-acridones (**18**), a hydrolytic degradation product of

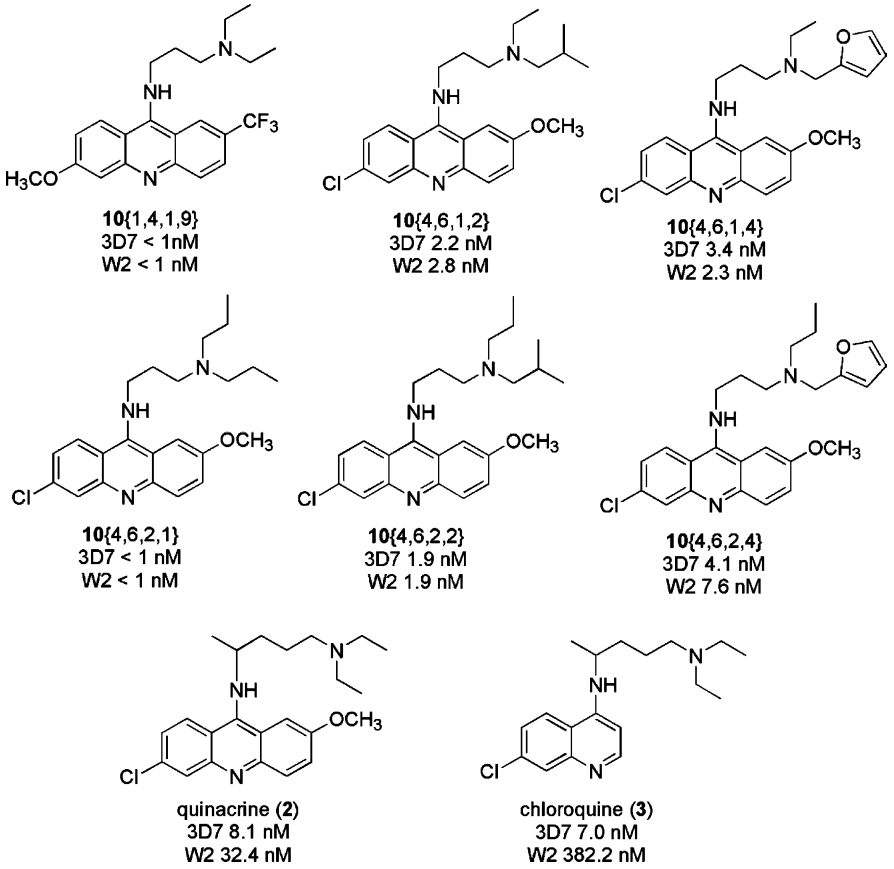


Figure 5. Structures and EC_{50} values of the most active compounds against *P. falciparum* strains 3D7 and W2 in cultured human erythrocytes, as well as for quinacrine (**2**) and chloroquine (**3**). Activity at a series of 12 concentrations was determined as percent parasite growth relative to untreated controls, EC_{50} values were determined with SigmaPlot.

the 9-chloroacridine synthetic intermediates. The compounds were screened initially at fixed concentration (30 nM), with the most active hits re-analyzed to determine dose–response. Six previously undescribed compounds were found to have nanomolar EC_{50} values and were particularly interesting due to improved potency against the W2 parasite strain, relative to quinacrine (**2**) and chloroquine (**3**). Of this set, most of them were derived from the commercially available acridine heterocycle **7**{4,6} with novel substituents on the amine sidechain, suggesting that further modification of this component may be a viable strategy for future optimizations.

4. Experimental

4.1. General

NMR spectra were recorded on a Varian Model AS 400-MHz machine. The following abbreviations are used to describe peak splitting when appropriate: s, singlet, d, doublet, t, triplet, q, quartet, br s, broad singlet, and mult, multiplet. Reactions were carried out under an atmosphere of argon. Reagents were of commercial quality unless otherwise indicated. Analytical thin layer chromatography (TLC) was carried out using plastic plates coated with silica gel 60 F254 (Whatman Part #4420 222). Developed plates were visualized using short wave UV light (254 nm) and were typically stained in an iodine/silica chamber and/or using a staining dip (e.g., *p*-anisaldehyde or ceric ammonium molybdate) followed by heating with a heat gun.

4.2. Measurement of in vitro anti-malarial activity

Growth inhibition of *P. falciparum* cultured in human erythrocytes was measured with flow cytometry.[37] Synchronized cultures of ring-stage parasites (0.8% parasitemia, 0.5% hematocrit) were grown in 96-well tissue culture plates (Falcon) in the presence of compounds for screening, at fixed concentration of 30 nM or serially diluted for dose–response. Cultures were grown in atmospherically regulated (6% CO_2, 5% O_2) incubators (Sanyo) at 37 °C for 72 h. Following drug incubation, cultures were fixed with 1% paraformaldehyde for 1 h at RT and subsequently stained with 50 nM YOYO-1 (Molecular Probes) in 1× PBS for 18 h at RT in the dark. Fluorescence of each sample was then obtained on a Becton–Dickenson LSR2 flow cytometer. YOYO-1 is a DNA intercalator that allows for distinction between parasitized and unparasitized red blood cells (RBCs), as RBCs lack DNA. Percent of parasitized RBCs was determined from fluorescent signal due to YOYO-1, and growth inhibition values were then calculated as the fraction of parasitized RBCs relative to cultures without drug. All screening was done in triplicate. Compounds were screened initially at a fixed concentration (30 nM). The most promising compounds were then evaluated for dose–response at 12 concentrations: 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6, 15.6, 7.8, 3.9, 2.0, and 0.9 nM. Fifty percent effective inhibitory concentrations (EC_{50} values) were determined using SigmaPlot.

4.3. General procedure for the synthesis of salicyl triflate chemset (11)

A solution of substituted salicylic acid **5** (11.7 mmol) in DMF (100 ml) was treated with Cs_2CO_3 (0.5 equiv) and iodomethane (1.05 equiv). The solution was stirred for 1.5 h, taken up in Et_2O/EtOAc (2:1) (100 ml), and washed with H_2O (50 ml), $NaHCO_3$ (10% aq, 50 ml), and NaCl (satd aq) (25 ml). The organic product layer was dried over $MgSO_4$ and concentrated in vacuo to yield methyl esters in excellent purity (80–100% yield).

The methyl esters (5.5 mmol) were dissolved in CH_2Cl_2 (50 ml), cooled to −78 °C, and stirred under argon. Treatment with Et_3N (2 equiv) was followed by the dropwise addition of trifluoromethanesulfonic anhydride (1.1 equiv). The mixture was stirred at −78 °C for 30 min and then warmed to RT, whereupon TLC indicated quantitative conversion. The solution was taken up in Et_2O (100 ml) and washed with HCl (1 M aq) (25 ml). The aqueous layer was back-extracted with additional Et_2O (25 ml), and the combined organic product layer was washed with NaCl (satd aq) (25 ml), dried over $MgSO_4$, and concentrated in vacuo to yield salicyl triflate chemset (**11**) in excellent purity (95–100% yield).

4.4. General procedure for the parallel synthesis of the diarylamine chemset (12)

Following the general procedure of Buchwald,[31] each of the vessels of a Radleys 12-place carrousel was loaded with a solution of salicyl triflate chemset **11** (0.728 mmol) in toluene (7 ml). The vessels were purged under argon, and each one of them was then treated with anilines (**6**) (1.2 equiv), Cs_2CO_3 (1.4 equiv), *rac*-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (BINAP) (0.08 equiv), followed by $Pd(OAc)_2$ (0.05 equiv). The carrousel was heated to 120 °C for 16 h, after which TLC of reaction mixtures generally indicated complete conversion to the diarylamine products (**12**). The products were concentrated in vacuo using a Genevac HT-4 parallel evaporation system and then used directly in the next step without purification or workup.

4.5. General procedure for the parallel synthesis of the 9-chloroacridine chemset (7)

The crude diarylamines (**12**) (ca. 0.728 mmol), loaded into the vessels of a Radleys 12-place carrousel, were redissolved in methanol (7 ml) and treated with barium hydroxide octahydrate (1.5 equiv). The carrousel was heated to 90 °C overnight, after which TLC showed complete ester hydrolysis. The crude carboxylic acid species were concentrated in vacuo using a Genevac Parallel Evaporation System and used directly in the next step without purification or workup. To effect cyclization, the species were treated with $POCl_3$ (7 ml) and heated to 120 °C under air for 2 h. Parallel removal of $POCl_3$ was accomplished by heating the Radleys carrousel to 160 °C, while water (60 °C) was pumped through the Radleys upper water jacket using a Julabo MB-5 heated water circulator, and while house vacuum was

applied to the carrousel (with an in-line dry ice/ethanol vacuum trap). After the removal of $POCl_3$, the crude 9-chloroacridines were azeotropically dried with EtOAc and then dry-loaded onto silica for flash column chromatography. Automated flash column purification using the CombiFlash system by Isco was done to yield the 9-chloroacridine products (**7**) in high purity determined by homogeneous ^1H NMR spectra. A general solvent gradient for this class of compounds was devised: 5:95 → 55:45 ethyl acetate/hexanes (2% Et_3N) over 25 min.

4.6. *N*-(3-Aminopropyl)-2-nitrobenzenesulfonamide (13)

1,3-Diaminopropane (236 ml, 2807 mmol) was cooled to 0 °C under argon, and then a solution of 2-nitrobenzenesulfonyl chloride (62.2 g, 280.7 mmol) in CH_2Cl_2 (2400 ml) was slowly added by pressure-equalizing dropping funnel, over a period of ca. 2 h. The reaction mixture was stirred overnight and then split into two equal portions for workup. Each portion was cooled to 0 °C, followed by dropping funnel addition of H_2O (300 ml) and HCl (concd aq) (150 ml) (*Caution: both additions are exothermic!*). The CH_2Cl_2 layers were extracted and disposed. The aqueous layers were then cooled to 0 °C, treated directly with solid NaOH until the mixtures were visibly heterogeneous, and then extracted with CH_2Cl_2 (6× 500 ml). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to yield the title compound (**13**) (60.27 g, 83% yield), which can also be purchased commercially (TCI America).

4.7. General procedure for amidation/reduction to generate the secondary amine chemset (15)

A solution of *N*-(3-aminopropyl)-2-nitrobenzenesulfonamide (**13**) (31.0 g, 120 mmol) in THF (400 ml) was stirred at 25 °C under argon in an ambient water bath and treated with pyridine (11 ml, 131 mmol, 1.1 equiv) followed by carboxylic anhydride reagents (**14**) (1.2 equiv). The solution was stirred at 25 °C for 1.5 h, after which RP-HPLC analysis indicated quantitative conversion to the amide. The reaction mixture was quenched with ammonium hydroxide (30% aq) (70 ml, 598 mmol, 5 equiv), stirred an additional 15 min, and then concentrated in vacuo. The residue was treated with ammonium chloride (satd aq): NaCl (satd aq) (2:1, 300 ml), and acidified with HCl (1 M aq) to pH 1, and then the product was extracted with Et_2O/EtOAc (1:1) (2× 200 ml). The organic layer was washed with HCl (1 M aq):NaCl (satd aq) (1:1, 100 ml), dried over Na_2SO_4, and concentrated in vacuo to yield the crude amide intermediate, used in the next step without further purification.

The subsequent borane-methylsulfide reduction step required an apparatus to remove dimethylsulfide by distillation:[34] the reaction was carried out in a 2000 ml single-neck round-bottomed flask attached to a 2-way Claisen adaptor. The adaptor was equipped with a rubber septum for reagent addition and a vacuum distillation head with a trap for distilled dimethylsulfide. The system was charged with a solution of crude amide (ca. 120 mmol) in anhydrous THF (400 ml), stirred at 25 °C under argon, and treated dropwise with borane–dimethylsulfide complex (46 ml, 478 mmol, 4 equiv). The solution was heated in an oil bath at 60 °C for 30 min, with concomitant distillation of dimethylsulfide from the reaction mixture. RP-HPLC analysis showed the reaction to be complete. The distillation head was replaced with a condenser, and H_2O was *slowly and carefully* added over 20 min., until major bubbling ceased. Next, HCl (1 M aq, 100 ml) was added and the solution was heated to 60 °C for 45 min. The solution was cooled to 0 °C, treated with Et_2O (500 ml), and extracted with HCl (1 M aq, 2× 300 ml). The combined aqueous layers were washed with Et_2O (200 ml), cooled to 0 °C, treated directly with solid NaOH until the mixture was visibly heterogeneous, and then extracted with EtOAc (3 × 200 ml), dried over Na_2SO_4, and concentrated in vacuo to yield the desired secondary amines (**15**) in high purity (50–71% yield).

4.8. General procedure for parallel reductive amination and nosyl deprotection (9)

Secondary amines (**14**) generated in the previous step were loaded into the vessels of a Radleys 12-place reaction carrousel as 0.3 M stock solutions in THF (12 ml each, 3.5 mmol), followed by aldehydes (**16**) (1.5 equiv), and sodium triacetoxyborohydride (1.5 equiv). The vessels were purged under argon and then agitated by placement in a Fisher Scientific FS20 sonicating water bath for 30–60 min., after which RP-HPLC showed the reductive amination reaction to be quantitative. Parallel solvent removal in vacuo was accomplished using a GeneVac HT-4 instrument. *Parallel workup by SPE:* initially columns (Teledyne Isco Inc., # 69-3873-146) were loaded with Dowex 50WX2-400 ion-exchange resin (~15 g), and the resin was then pre-conditioned with MeOH (1% CF_3CO_2H) (50 ml). The concentrated reaction mixtures were redissolved in MeOH (5% CF_3CO_2H) (50 ml) and loaded onto the columns. The resin was washed with MeOH and then the products were eluted with MeOH (10% Et_3N) (50 ml). Finally, the products of reductive amination were obtained after parallel solvent removal in vacuo using the GeneVac HT-4 instrument. *Alternative aqueous workup:* The crude reaction mixture in THF was carefully quenched with HCl (1 M aq) (25 ml), treated with Et_2O (150 ml), and the aqueous layer was extracted followed by an additional aqueous extraction with HCl (1 M aq) (25 ml). The aqueous layers were combined and washed with Et_2O (2 × 50 ml), basified with NaOH (1 M aq.) until visibly heterogeneous, and the products were extracted with Et_2O (3 × 30 ml). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to yield the products.

The crude tertiary amine products (ca. 3.5 mmol) were dissolved in CH_3CN (35 ml) and loaded into the vessels of a Radleys 6-place reaction carrousel. The vessels were purged of air by applying three iterations of house vacuum followed by positive pressure of argon. Each vessel was then treated with Cs_2CO_3 (2.5 equiv) followed by benzenethiol (5.0 equiv), and then the vessels were stirred vigorously under argon for 1 h at rt, after which RP-HPLC showed near quantitative (>80%) conversion. More benzenethiol (5.0 equiv) was added, and the

vessels were stirred for 1 h, whereupon RP-HPLC analysis showed the reactions were complete. An SPE workup identical to that described above for reductive amination was performed. *Alternative aqueous workup:* The crude reaction mixture in CH_3CN was taken up in EtOAc (150 ml) and extracted with HCl (1 M aq) (2× 50 ml). The aqueous layer was washed with EtOAc (50 ml), basified with NaOH (1 M aq) to pH 14, and extracted with EtOAc (3× 30 ml). The combined organic layer was dried over Na_2SO_4, and for volatile alkyl-substituted diamine products, CF_3CO_2H (2 equiv) was added. Finally, the products were obtained after concentration in vacuo.

4.9. General procedure for the parallel synthesis of the 9-aminoacridine chemset (10)

DMSO stock solutions of 9-chloroacridines (**7**), diamines (**9**), and phenol were prepared at 0.077, 0.60, and 2.9 M, respectfully. The 2 ml polypropylene vessels of a 48-position Bohdan MiniBlock were loaded with 9-chloroacridines (**7**) (250 μl DMSO stock, 19.3 μ mol), phenol (100 μl DMSO stock, 288 μmol, 15 equiv), Cs_2CO_3 (5 mg, 19.3 μmol, 1 equiv), and 3 Å molecular sieves (ca. 0.010 g). The latter two items were conveniently added in parallel via the Bohdan Resin Dispenser accessory. The block was simultaneously heated to 100 °C and agitated at 600 rpm using the Bohdan MiniBlock High Capacity Shaking and Washing Station for 2 h. RP-LCMS usually showed quantitative conversion to the 9-phenoxyacridine chemset (**17**). Next, diamines (**9**) were added (125 μL DMSO stock, 4 equiv) and the blocks were heated again to 100 °C at 600 rpm for 4 h. RP-LCMS analysis showed the presence of 9-aminoacridine product (**10**), as well as varying amounts of 9-acridone (**17**). Unreacted primary amine was scavenged by treatment with methyl isocyanate polystyrene resin (NovaBiochem, 1.40 mmol/g loading, 8 equiv) followed by agitation at 600 rpm overnight at ambient temperature. *Parallel workup by SPE:* pre-packed SPE columns (Bohdan SCX #13511218) were conditioned with H_2O (1% CF_3CO_2H). The reaction mixtures (in DMSO) were treated with H_2O (1% CF_3CO_2H) (1 ml) and loaded onto the columns. The resin was sequentially washed with H_2O (1% CF_3CO_2H) (2 ml), MeOH (2 ml), and MeOH (1% pyridine) (8 ml); followed by product elution with EtOAc (10% Et_3N) (3 ml). The desired 9-aminoacridine chemset (**10**) was finally obtained after parallel solvent removal in vacuo using the GeneVac HT-4 instrument.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmc.2005.08.017

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