Anti-Herpes Simplex Virus Activity of Substituted 1-Hydroxyacridones

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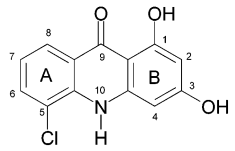
5-Chloro-1,3-dihydroxyacridone, **1**, is a potent and selective inhibitor of Herpes Simplex Virus Type-1 (HSV-1).[1] Substituted 1,3-dihydroxyacridones represent a new class of nonnucleoside HSV-1 inhibitors, and biochemical studies indicate a novel mechanism of action for **1,** although the target is not yet defined.[2] With the goal of lead optimization, analogues of **1** were synthesized in an effort to describe the structure−activity relationships between **1** and its hypothetical binding site. Modifications of key functional groups led to the identity of several features of **1** that were important for activity. In the process, a more expedient and reliable synthesis of **1** and its analogues was developed. Analogues were evaluated against HSV-1 and HSV-2 using a viral plaque-elimination assay for viral inhibition of HSV-1 and HSV-2, and effects on replication of the host cell were also measured in order to assess a therapeutic index (TI) of selectivity. Several new analogues with significant antiviral activity were identified, including 5-methoxy-1,3-dihydroxyacridone (**11**), which inhibits replication of several HSV-2 strains with a mean ED_{50} of 0.7 μM and a TI range of 25−60-fold.

Introduction

The *Herpesviridae* include member viruses that infect a variety of different animal species. Herpes viruses are characterized by virion structure, genome organization, and the ability to remain latent in host cells for extended periods of time. Eight distinct human herpes viruses have been identified and are subdivided into the alpha, beta, and gamma subfamilies. HSV-1 is a member of the alpha subfamily of herpes viruses and is commonly associated with cold sores. However, active viral replication can lead to more serious cytopathy in the immunocompromised.[3] Additionally, drug-resistant strains of HSV-1 have been isolated from laboratory and clinic settings. Most of the existing drugs used to treat HSV are nucleoside derivatives that inhibit viral replication after metabolism by acting as fraudulent building blocks for viral DNA synthesis. Due to the similarity in the mechanism of available drugs, cross-resistance is common among drug-resistant strains.[4,5] Thus, the development of nonnucleoside HSV inhibitors with novel mechanisms of action is an important focus area of antiviral research.

The lead molecule, 5-chloro-1,3-dihydroxyacridone, **1** (Figure 1), was originally synthesized to examine structure−activity relationship (SAR) of the regioisomer 7-chloro-1,3-dihydroxyacridone, **3**, an inhibitor of mammalian DNA topoisomerase II.[6] Compound **1** was found to be inactive against DNA topoisomerase II and only a weak inhibitor of cell growth in the original study.[7] Since DNA topoisomerase II has been implicated as candidate host cell target for antiviral drug development,[1,4] compound **3** and several analogues were tested as inhibitors of HSV-1 replication in cultured cells. Many of the analogues showed significant activity, but the activity did not appear to correlate with inhibition of DNA topisomerase II.



1

Figure 1. 5-Chloro-1,3-dihydroxyacridone (**1**), and acridone numbering scheme.

Compound **1** was identified as the most active and selective 1,3-dihydroxyacridone analogue with an ED_{50} of 4 ± 1 μM against the replication of HSV-1 in Vero cells and a CC_{50} of 52 ± 6 μM against Vero cell replication. Studies have shown that **1** indirectly interferes with the cleavage and packaging of HSV-1 DNA[2], while viral inhibition by **3** appears to be mediated via a different mechanism, possibly through the inhibition of cellular DNA topoisomerase II. Only one other compound series, a group of thioureas, has been reported to interfere with the cleavage and packaging of HSV-1 DNA, but the biochemical mechanism of thioureas and **1** with respect to the molecular target is clearly different.[2,8] Given the propensity of HSV to develop resistance toward current therapies, it is particularly attractive to develop lead series that act differently on a critical step of HSV replication that has only recently been exploited for drug discovery.

Chemistry

The synthesis of 1,3-dihydroxyacridones has been accomplished through a one-step procedure involving the coupling of substituted anthranilic acids or their esters with phloroglucinol in the presence of a mild Lewis acid.[9,10] Originally, the synthesis of **1** was per-

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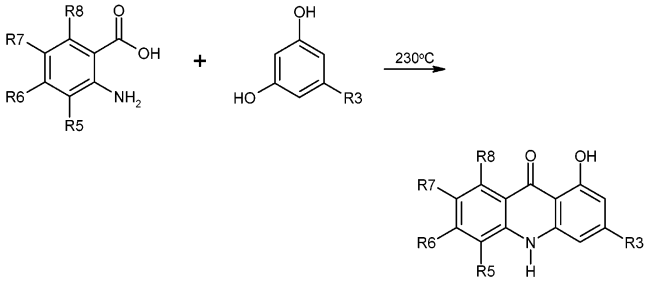
Scheme 1. Thermal Coupling



formed in this way with 3-chloroanthranilic acid and phloroglucinol in the presence of zinc chloride at reflux in *n*-butyl alcohol. This reaction is believed to go through a Friedel−Crafts-like mechanism for the initial electrophilic aromatic substitution and subsequently form the acridone system through hydrolytic cyclization occurring between the primary amine and the hydroxyl adjacent to the substitution.[11] The reaction gave only minimal yield after refluxing for 48 h and was not repeatable. The workup was difficult due to several factors, including removal of the *n*-butyl alcohol, removal of inorganic salts, and insolubility of both the desired product and the byproducts. Under these conditions, pure product material was obtained only once in a yield of 2% after repeated efforts. Other 3-substituted anthranilic acids also gave poor yields or failed to react under these conditions. As an alternative to this method, a thermal coupling was considered.

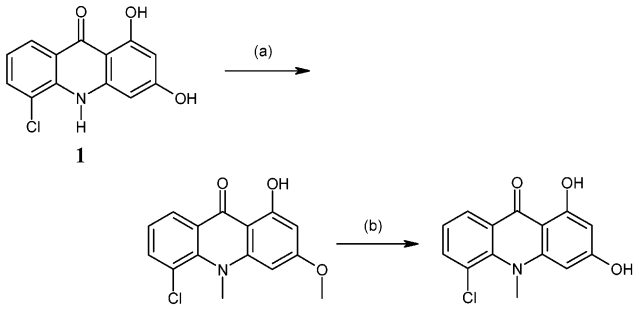
Initially, we only wished to obtain a reliable method for the synthesis of **1**, so the thermal coupling was attempted in this specific case using an equimolar mixture of the two starting materials and heating to 230 °C for 35 min. In several attempts, yields of 25−35% were consistently obtained. On the basis of the success of these initial results, the procedure was attempted on several other substituted anthranilic acids. Generally, yields were similar to those obtained from the traditional solvent-based procedures. The thermal coupling procedure was then extended to substituted resorcinol derivatives and resorcinol itself with varying levels of success. In the process of expanding this synthetic methodology, several important analogues of **1** were synthesized. While the yields were not always impressive using the thermal coupling, the main advantage was that the products could be obtained in one step and often with little time spent on purification. In addition to analogues obtained through thermal coupling and through the traditional procedure (Scheme 1), *N*-methylated analogues were also prepared from **1** (Scheme 2). Attempts to demethylate the 3-methoxy group of **16** with boron tribromide failed; however, demethylation with hydrobromic acid[12] was successful.

Biological Results

Analogues were evaluated for inhibition of HSV-1 (strain KOS) and HSV-2 (strain 186) replication in infected Vero cells using a standard viral plaque-elimination assay.[13] Results are shown in Table 1.

Initially, the 5-, 6-, 7-, and 8-chloro-1,3-dihydroxyacridone regioisomers (**1**, **2**, **3**, and **4,** respectively) were examined against HSV-1. All of these molecules were selective inhibitors of HSV-1, but the 5-chloro isomer

Scheme 2. Analogues Synthesized from **1**[a]



[a] (a) MeI, Cs_2CO_3 (b) HBr, reflux.

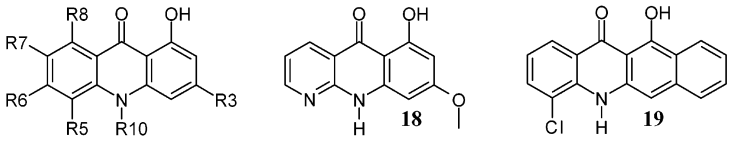
showed superior antiviral activity. On the basis of these results, it was determined that the first targets should involve substitution at the 5-position, leaving the bridge components and B-ring of the acridone scaffold constant. Accordingly, compounds **10**, **11**, and **12** were prepared. Compounds **10**, **11**, and **12** showed significant antiviral activity, but only **11**, with an ED_{50} value of 2.2 μM and therapeutic index (TI) of 13, showed a better activity profile than the lead compound **1**. Interestingly, the 5,7-dichloro compound, **12**, showed intermediate antiviral activity to the 5-chloro and 7-chloro monosubstituted analogues, and the inhibition of Vero cell growth was significantly reduced. Compound **18** exhibited low micromolar activity in the cell growth inhibition assay. Although this result eliminated **18** as an antiviral candidate, it was considered to be an interesting compound to examine further as an inhibitor of cell replication.

Next, variations at the 3-position and methylation of the secondary amine were examined. Variable substitution of the 3-position was studied with compounds **13** and **15**. Additionally, compound **19** (phenyl ring fused to the 2 and 3 aromatic carbons of the acridone ring system) was considered a distant member of this group due also to the elimination of the 3-hydroxyl substituent. The results of antiviral testing indicate that the 3-hydroxyl group is an important element in the pharmacophore of the lead compound. Although none of the 3-substituted compounds were inhibitors of HSV-1 replication, results obtained for **13** suggested that 3-methyl-substituted acridones may be interesting to examine as potential antitumor leads. Methylation of the secondary amine, as in compounds **16** and **17**, also eliminated HSV-1 activity, indicating that the secondary amine is another important feature of the pharmacophore.

In summary, it is likely that the 3-hydroxyl substituent and the secondary amine play an important role in the interaction of the lead molecule with its biochemical target. These two major pharmacophoric components may correspond to hydrogen-bond acceptor and donating sites at a hypothetical binding domain. A-Ring substitution appears to be necessary, and in the case of chloro-substitution, the 5-position appears to be the preferred position for anti-HSV-1 activity.

Since HSV-2 is also a significant human pathogen, experiments were conducted to examine analogues for activity against HSV-2 replication. ED_{50} values were initially determined for the inhibition of HSV-2 (strain 186) in Vero cells. The studies revealed that some

Table 1. Anti-HSV Activity of Acridone Analogues[a]



1-17, 20 **18** **19**

cmpd	R5	R6	R7	R8	R3	R10	Vero CC_{50}	HSV-1 ED_{50}	HSV-2 ED_{50}	HSV-1 TI	HSV-2 TI
1	Cl	H	H	H	OH	H	60 ± 3	4 ± 1	>30[c]	13	NA
2	H	Cl	H	H	OH	H	29 ± 4	10 ± 1	NT	3	NA
3	H	H	Cl	H	OH	H	52 ± 6	16 ± 4	15	3.5	3.5
4	H	H	H	Cl	OH	H	70 ± 4	10 ± 2	NT	7	
5	H	H	OH	H	OH	H	40 ± 2	15 ± 2	NT	2.7	
6	H	H	OH	H	OMe	H	25	NT	<10		<2.5
7	H	H	OMe	H	OH	H	NT	NT	NT		
8	H	H	OMe	H	OMe	H	>50 (7)	NT	>10		
9	H	H	H	Me	OH	H	60 ± 4	44 ± 2	NT	1.4	
10	Me	H	H	H	OH	H	32	8 ± 1	15	4	2.1
11	OMe	H	H	H	OH	H	30	2.2	0.6	13	50
12	Cl	H	Cl	H	OH	H	>100 (41)	12	20	>5.0	>8.3
13	Cl	H	H	H	Me	H	13	NA	NA		
14	OMe	H	H	H	OMe	H	>50 (30)	NT	>50 (35)		1.0
15	Cl	H	H	H	OMe	H	>100 (28)	>50 (6)	NA		
16	Cl	H	H	H	OMe	Me	>100 (15)	>100	>50 (36)		
17	Cl	H	H	H	OH	Me	50	>50	>30[c]		
18	b	*b*	*b*	*b*	*b*	*b*	5.5	NT	NA @ 50[d]		
19	*b*	*b*	*b*	*b*	*b*	*b*	>100	NA @ 100	NT		NT
20	H	H	H	H	OH	H	68	45	NT	1.5	NT
PAA							3500	240	NT	29	NT
ACV							>400 (18)	0.5	2.5	>800	>160

[a] TI = therapeutic index (Vero CC_{50}/HSV1 or HSV2 ED_{50}); parenthetical values = % inhibition at stated μM concentration; NA = not active; NT = not tested; PAA = phosphonoacetic acid; ACV = acyclovir; ED_{50} and CC_{50} values calculated using nonlinear regression from dose response data using sigmoidal fit. [b] Structure shown above. [c] Reduction in plaque size was observed. [d] No toxicity.

Table 2. Anti-HSV Activity of 1,3-Dihydroxy-5-methoxy Acridone (**11**)[a]

virus strain	ED_{50} (μM)	TI	ACV ED_{50} (μM)	ACV TI
HSV-1 (KOS)	2.2	14	0.5	>800
HSV-2 (186)	0.6	50	2.5	>160
HSV-2 (186, c2)	0.6	50	1.7	>235
HSV-2 (186, c4)	1.2	25	2.5	>160
HSV-2 (G)	0.5	60	NT	
HSV-2 (333)	0.7	43	NT	

[a] TI = therapeutic index (Vero CC_{50}/ED_{50}); NT = not tested.

analogues were active and quite selective inhibitors of HSV-2. With an ED_{50} of 600 nM and a TI value of 50, compound **11** was the best analogue of the series. In a side-by-side assay with acyclovir, **11** was considerably more active than acyclovir, although selectivity was about 10-fold lower. On the basis of these findings, compound **11** was further evaluated against additional HSV-2 laboratory strains including two plaque-purified stocks of strain 186, and results are summarized in Table 2. With a mean ED_{50} of 0.7 μM, compound **11** was about 3-fold more active than acyclovir. Selectivity of **11** against HSV-2 was the highest of the 1,3-dihydroxy-acridone series and only about 10-fold lower than acyclovir, the gold standard. Activity of **11** was better than that of phosphonoacetic acid (PAA), a pyrophosphate analogue closely related to the clinically used drug Foscarnet.

Discussion

The discovery and subsequent investigation of the lead molecule, **1**, was the product of several years of research on the biological properties of a series of related acridone analogues. The fact that these analogues have diverse and significant biological activities has been both beneficial and confounding throughout these studies. Antiviral drug research and development is generally challenging because of the need to maximize antiviral activity while keeping adverse cell toxicity to a minimum. In this study, the lead molecule was a relatively nontoxic member of a series that was originally explored as inhibitors of cell replication. Although subsequent studies revealed much about the novel effects of **1** on the HSV-1 viral life cycle,[2] the exact biochemical target and mechanism of action is currently unknown. Without knowledge of biochemical target and mechanism, synthetic targets in the early stage development of SAR around the lead could only be designed through a traditional ligand-based approach. Biological evaluation of target analogues led to a clearer picture of the structural requirements for activity, particularly for the HSV-1 series. In addition, several intriguing new biologically active molecules were discovered through these studies.

The acridone scaffold has provided a variety of molecules with interesting and significant biological activities. Perhaps this is not too surprising given that the scaffold itself provides a variety of drug-like features, including a secondary amine, a carbonyl group, and phenyl rings, in a compact form. However, the rigidity of the acridone scaffold makes it difficult to explore 3-dimensional space via the analogue approach. In this study, the tricyclic scaffold remained intact in all of the analogues. The positions of functional groups that have an impact on the activity of the lead compound were identified. It should be possible to use this

information to propose new molecules that contain these key functional groups in the correct positions with a less rigid scaffold.

Work on this series of antiviral acridones has yielded several significant inhibitors, but antiviral selectivity in general still remains an issue. It may be that a more radical change in the structure of the molecule is needed to reach a new tier in the potency and selectivity of these molecules. The impact of most of the important functional groups on the inhibition of cell growth and HSV were examined. Future work in the investigation of SAR will attempt to enhance antiviral activity by capitalizing on the current information. Since the 3-hydroxyl position has been identified as a possible site of interaction with the hypothetical receptor, it is important to try to replace that site with bioisosteres. The 5-position has been identified as another important site; however, it is not clear whether the electron-donating or -withdrawing potential of a 5-substituent has a significant impact on HSV activity. It may be that the primary contribution to the pharmacophore in this position is a hydrophobic effect that can be fulfilled by either electron-withdrawing or -donating groups. Reduction of cell growth inhibition in compound **12** (5,7-dichloro analog) with retention of HSV-1 activity may indicate that cell growth inhibition can be modulated through bis-substitution on the B-ring of the acridone scaffold.

Alteration of the carbonyl and 1-hydroxyl functionalities is clearly an area of interest that is in need of further exploration. This motif may be a component of the acridone scaffold that is important for cell toxicity, as suggested previously.[14] It is possible that the removal or replacement of the 1-hydroxyl group or the carbonyl may only reduce cell toxicity and not have an effect on the antiviral activity.

Although a viable synthesis for 5-subsituted 1,3-dihydroxyacridones has been developed, there is room for improvement in the yields. Yields of the thermal condensation might benefit from inert atmosphere to exclude oxygen from the reaction. A survey of Lewis acids could also lead to the discovery of a suitable solution-based synthesis to replace the neat thermal condensation reaction used in this study. In addition, the synthesis of 2- and 4-substituted acridones was not thoroughly examined. These analogues will likely require the development of a reliable multistep synthesis.

Conclusion

The research study encompassed herein focused on the early stages of the antiviral drug discovery process. Beginning with a potent and selective antiviral lead molecule and very limited sets of analogues, the goal was to validate future development of these lead molecules by constructing SAR. A viable and expedient synthetic method for the synthesis of the HSV-1 lead molecule, **1**, was established. A rationally designed synthetic effort was utilized to yield structurally novel series of selective HSV-1 and HSV-2 inhibitors. In the case of the HSV-1 inhibition by **1**, it is clear that a novel biochemical mechanism of inhibition is responsible for activity.[2] Target analogues were identified according to traditional medicinal chemistry principles such as bioisosteric replacement, deletion and modification of key functional groups, and the synthesis of regioisomers. Several key elements of the SAR surrounding the HSV-1 lead, **1**, were established. Finally, molecules with in vitro activity profiles approaching currently used drugs have been identified for HSV-1 and HSV-2.

Biological Experimental Section

Compounds were tested as inhibitors of HSV replication in Vero (African green monkey kidney; ATCC# CRL 1587) cells using a standard plaque-elimination assay.[13] One hundred thousand Vero cells per well were added in RPMI-1640 medium with 10% (v/v) fetal calf serum to 12-well culture plates and incubated overnight in a 37 °C humidified atmosphere of 5% carbon dioxide. Medium was aspirated, and the Vero cell sheet was infected with between 50 and 100 plaque-forming units of either HSV-1 (KOS strain) or HSV-2 (strain 186). After 1 h of incubation, to allow virus adsorption and penetration, the innoculate was aspirated and replaced with medium with 0.5% (v/v) fetal calf serum, with or without test compounds. Treatments were duplicated within each experiment. Plates were incubated for 2 days without movement, stained with crystal violet (0.8% v/v in 50% ethanol), and destained with water. Viral plaques were scored by eye, and the number was calculated as a percentage of the infected control in order to determine percent inhibition. Data were plotted and analyzed using statistical software (GraphPad Prizm, San Diego, CA). The ED_{50} value is the concentration of compound that inhibits viral replication by 50% relative to control. The effect of compound on Vero cell replication was measured using the Sulforhodamine B-dye binding assay, originally developed at the NCI for the in vitro anti-cancer drug discovery program. The method is standard and has been described in detail elsewhere by us and others.[15] The CC_{50} value is the concentration of compound that inhibits cell replication by 50% relative to control and after 2 days of continuous treatment. Antiviral selectivity is related to the therapeutic index (TI value), which is the ratio of the ED_{50}/CC_{50}. The higher the TI value, the better the activity profile. Additional testing of compound **11** against HSV-2 strains was accomplished using the same methods. HSV-2 strains 186, 333, and G were a generous gift of Dr. S. Bachenheimer (Microbiology, UNC−CH). The two clones of HSV-2 186 (c2 and c4) were obtained following plaque purification using standard virological techniques.

Chemistry Experimental Section

Compounds **1**−**20** were analyzed by proton NMR on a 300 MHz Varian instrument, and chemical shifts are presented in ppm. Elemental analysis was performed by Atlantic Microlabs (Norcross, GA). High-resolution mass spectral analysis was performed at the School of Pharmacy, University of North Carolina at Chapel Hill, on a Hitachi M-80 mass spectrometer. The syntheses of analogues 6-chloro-1,3-dihydroxyacridone (**2**), 7-chloro-1,3-dihydroxyacridone (**3**), 1,3,7-trihydroxyacridone (**5**), and 1,3-dihydroxyacridone (**20**) used in biological assays were previously described.[6]

5-Chloro-1,3-dihydroxyacridone (1). Into a 4 mL sealed vial was added 2-amino-3-chlorobenzoic acid (172 mg, 1.0 mmol) and phloroglucinol (110 mg, 1.0 mmol). The vial was then heated to 230 °C for 40 min. The resulting residue was suspended in acetone (20 mL). The suspension was filtered to yield a brown powder. The brown powder was then triturated in hot methanol (50 mL) and filtered to yield 83 mg of yellow solid, 32%. NMR (D_6 DMSO): δ 6.20 (1H, d, J = 2.3), 6.94 (1H, d, J = 2.0), 7.38 (1H, t, J = 8.1), 8.01 (1H, d, J = 7.3), 8.27 (1H, d, J = 7.3), 10.79 (1H, s), 11.12 (1H, s) 14.10 (1H, s). Anal.: $C_{13}H_8NO_3Cl$. HRMS: m/z (rel int. %) 261 (100) $(M)^+$. Calcd for $C_{13}H_8NO_3Cl$: 261.0193. Found: 261.0186.

8-Chloro-1,3-dihydroxyacridone (4). 6-Chloro-2-aminobenzoic acid (780 mg, 4.54 mmol), anhydrous phloroglucinol (573 mg, 4.54 mmol), and zinc chloride (818 mg, 6.00 mmol) were dissolved in anhydrous *n*-butanol (20 mL) in a flame-dried round-bottom flask with a reflux condenser under a nitrogen atmosphere and refluxed for 20 h. The cooled reaction

mixture was then filtered. The solids were then washed into a separate collection flask with acetone. The filtrate was concentrated and flash chromatographed to yield 484 mg of yellow solid, 41%. NMR (D_6 DMSO): δ 5.99 (1H, d, $J = 1.41$), 6.24 (1H, d, $J = 1.86$), 7.21 (1H, d, $J = 7.56$), 7.39 (1H, d, $J = 8.37$), 7.59 (1H, t, $J = 7.9$), 10.58 (1H, s), 11.83 (1H, s) 14.26 (1H, s). Anal.: $C_{13}H_8NO_3Cl \cdot 2H_2O$. HRMS: *m/z* (rel int. %) 261 (100) $(M)^+$. Calcd for $C_{13}H_8NO_3Cl$: 261.0193. Found: 261.0194.

1,7-Dihydroxy-3-methoxyacridone (6). Into a 20 mL vial was added 2-amino-5-hydroxybenzoic acid (281 mg, 1.83 mmol) and 5-methoxyrecorcinol (284 mg, 2.02 mmol). The vial was sealed and heated in an oil bath at 230 °C for 35 min. The vial was removed and allowed to cool to room temperature. The resulting solid was then triturated in hot ethyl acetate and filtered to yield the product as a yellow solid, 335 mg, 71%. NMR (D_6 DMSO): δ 3.96 (3H, s), 6.21 (1H, d, $J = 2.4$), 6.45 (1H, d, $J = 2.4$), 7.41 (1H, dd, $J = 2.8, 7.2$), 7.54 (1H, d, $J = 9.1$), 7.61 (1H, d, $J = 2.8$) 9.78 (1H, s), 11.97 (1H, s), 14.48 (1H, s). Anal.: $C_{14}H_{11}NO_4.0.5$. HRMS: *m/z* (rel int. %) 257 (7.5) $(M)^+$. Calcd for $C_{14}H_{11}NO_4$: 257.0688. Found: 257.0693.

1,3-Dihydroxy-7-methoxyacridone (7). Into a 20 mL vial was added 2-amino-5-methoxybenzoic acid (2.17 g, 6.00 mmol) and anhydrous phloroglucinol (1.64 g, 6.75 mmol). The vial was sealed and heated in an oil bath at 230 °C for 35 min. The vial was removed and allowed to cool to room temperature. The resulting solids were triturated in ethyl acetate and methyl alcohol and filtered. The combined filtrates were evaporated, dissolved in DMF (15 mL) and ethyl acetate (150 mL), and washed with 4:1 H_2O:saturated aqueous sodium bicarbonate (2 × 150 mL) followed by H_2O (2 × 150 mL). The organic phase was dried over sodium sulfate and filtered. The crude product was then flash chromatographed to yield 600 mg of yellow powder, 18%. NMR (D_6 DMSO): δ 3.97 (3H, s), 6.10 (1H, d, $J = 2.4$), 6.39 (1H, d, $J = 2.4$), 7.61 (3H, m) 10.55 (1H, s), 11.84 (1H, s), 14.43 (1H, s)/ Anal.: $C_{14}H_{11}NO_4.1.5$. HRMS: *m/z* (rel int %) 257 (7.5) $(M)^+$. Calcd for $C_{14}H_{11}NO_4$: 257.0691. Found: 257.0688.

3,7-Dimethoxy-1-hydroxyacridone (8). Into a 20 mL vial was added 2-amino-5-methoxybenzoic acid (1.00 g, 6.00 mmol) and 5-methoxyresorcinol (947 mg, 6.75 mmol). The vial was sealed and heated in an oil bath at 225 °C for 35 min. The vial was removed and allowed to cool to room temperature. The resulting solids were triturated in ethyl acetate and filtered to yield 780 mg of yellow powder, 48%. NMR (D_6 DMSO): δ 3.97 (6H, s), 6.25 (1H, s), 6.47 (1H, s), 7.55−7.67 (3H, m), 11.97 (1H, s), 14.41 (1H, s). Anal. $C_{15}H_{13}NO_4$. HRMS: *m/z* (rel int. %) 271 (100) $(M)^+$. Calcd for $C_{15}H_{13}NO_4$: 271.0845. Found: 271.0840.

1,3-Dihydroxy-8-methylacridone (9). 6-Methyl-2-aminobenzoic acid (2.05 g, 13.56 mmol), anhydrous phloroglucinol (1.72 g, 13.6 mmol), and zinc chloride (2.73 g, 20.0 mmol) were dissolved in anhydrous *n*-butanol (40 mL) in a flame-dried round-bottom flask with a reflux condenser under a nitrogen atmosphere and refluxed for 20 h. The cooled reaction mixture was then filtered. The solids were then washed into a separate collection flask with acetone. The filtrate was then concentrated and flash chromatographed to yield 60 mg of yellow solid, 2%. NMR (D_6 DMSO): δ 2.83 (3H, s), 5.94 (1H, d, $J = 1.8$), 6.22 (1H, d, $J = 1.8$), 6.94 (1H, d, $J = 7.1$), 7.52 (1H, t, $J = 7.8$), 10.42 (1H, s), 11.59 (1H, s) 14.60 (1H, s). Anal.: $C_{14}H_{11}NO_3 \cdot 2H_2O$.

1,3-Dihydroxy-5-methylacridone (10). Into a 4 mL vial was added 3-methyl-2-aminobenzoic acid (151 mg, 1.00 mmol) and anhydrous phloroglucinol (126 mg, 1.00 mmol). The vial was sealed and heated in an oil bath at 180−200 °C for 100 min. The crude product was triturated in acetone and filtered to yield 38 mg of yellow solid, 16%. NMR (D_6 DMSO): δ 2.55 (3H, s), 6.07 (1H, s), 6.70 (1H, s), 7.18 (1H, t, $J = 8.0$), 7.61 (1H, d, $J = 8.0$), 8.08 (1H, d, $J = 8.0$), 10.50 (1H, s), 10.64 (1H, s) 14.30 (1H, s). Anal.: $C_{14}H_{11}NO_3$. HRMS: *m/z* (rel int %) 241 (100) $(M)^+$. Calcd for $C_{14}H_{11}NO_3$: 241.0739. Found: 241.0740.

1,3-Dihydroxy-5-methoxyacridone (11). Into a 4 mL vial was added 3-methoxyanthranilic acid (334 mg, 2.00 mmol) and anhydrous phloroglucinol (270 mg, 2.14 mmol). The vial was sealed and heated in an oil bath at 230 °C for 40 min. The crude product was dissolved in methanol and filtered. The filtrate was absorbed onto silica gel, flash chromatographed, and recrystallized in ethyl acetate to yield 55 mg of yellow solid, 11%. NMR (D_6 DMSO): δ 4.15 (3H, s), 6.14 (1H, d, $J = 1.8$), 6.82 (1H, d, $J = 2.2$), 7.31 (1H, t, $J = 8.1$), 7.44 (1H, d, $J = 8.1$), 7.85 (1H, d, $J = 8.4$), 10.59 (1H, s), 11.38 (1H, s) 14.40 (1H, s). Anal.: $C_{14}H_{11}NO_4$. HRMS: *m/z* (rel int %) 257 (100) $(M)^+$. Calcd for $C_{14}H_{11}NO_4$: 257.0688. Found: 257.0682.

5,7-Dichloro-1,3-dihydroxyacridone (12). Into a 4 mL vial was added 3,5-dichloroanthranilic acid (412 mg, 2.00 mmol) and anhydrous phloroglucinol (270 mg, 2.14 mmol). The vial was sealed and heated in an oil bath at 230 °C for 40 min. The resulting solids were triturated in hexanes and filtered to yield a brown powder. The brown powder was then triturated in EtOAc to yield 195 mg of yellow solid, 33%. NMR (D_6 DMSO): δ 6.21 (1H, d, $J = 1.8$), 6.94 (1H, d, $J = 1.8$), 8.15−8.17 (2H, m), 10.87 (1H, s), 11.24 (1H, s) 13.83 (1H, s). Anal.: $C_{13}H_7Cl_2NO_3 \cdot 0.75H_2O$. HRMS: *m/z* (rel int %) 294 (100), 295 (70) $(M)^+$. Calcd for $C_{13}H_7NO_3Cl_2$: 294.9803. Found: 294.9817.

5-Chloro-1-hydroxy-3-methylacridone (13). Into a 20 mL vial was added 2-amino-3-chlorobenzoic acid (500 mg, 2.91 mmol) and orcinol (620 mg, 5.00 mmol). The vial was sealed and heated in an oil bath at 230 °C for 28 min. The resulting solids were dissolved in 2:1 ethyl acetate:hexanes (9 mL) and filtered. The filtrate was concentrated in vacuo and dissolved in ethyl acetate (75 mL). The solution was washed with 0.1 M KOH in H_2O (2 × 50 mL). The organic phase was dried with sodium sulfate and filtered and concentrated in vacuo. The resulting solid was triturated in hexanes and filtered to yield a yellow solid, 21 mg, 3%. NMR (D_6 DMSO): δ 3.47 (3H, s), 6.60 (1H, d, $J = 0.7$), 7.39−7.42 (2H, m), 8.08 (1H, dd, $J = 7.7, 1.5$), 8.26 (1H, d, $J = 8.0$), 8.60 (1H, d, $J = 7.7$), 11.23 (1H, s), 13.73 (1H, s). Anal.: $C_{14}H_{10}NO_2Cl.0$. HRMS: *m/z* (rel int %) 259 (49) $(M)^+$. Calcd for $C_{14}H_{10}NO_2Cl$: 259.0400. Found: 259.0400.

3,5-Dimethoxy-1-hydroxyacridone (14). Into a 4 mL vial was added 3-methoxy-2-aminobenzoic acid (334 mg, 2.00 mmol) and 5-methoxyresorcinol (308 mg, 2.20 mmol). The vial was sealed and heated in an oil bath at 230 °C for 35 min. The resulting solids were dissolved in ethyl acetate (100 mL) and washed with 0.1 M KOH in H_2O (2 × 100 mL). The organic phase was dried with sodium sulfate, filtered, and flash chromatographed to yield 70 mg of yellow solid, 13%. NMR (D_6 DMSO): δ 3.96 (3H, s), 4.17 (3H, s), 6.28 (1H, d, $J = 2.0$), 7.03 (1H, d, $J = 2.4$), 7.34 (1H, t, $J = 8.0$), 7.47 (1H, d, $J = 7.9$), 7.87 (1H, d, $J = 8.3$), 11.48 (1H, s), 14.35 (1H, s). Anal.: $C_{15}H_{13}NO_4 \cdot 0.75H_2O$. HRMS: *m/z* (rel int %) 271 (8.7) $(M)^+$. Calcd for $C_{15}H_{13}NO_4$: 271.0845. Found: 271.0851.

5-Chloro-1-hydroxy-3-methoxyacridone (15). Into a 4 mL vial was added 3-chloroanthranilic acid (171 mg, 1.00 mmol) and 5-methoxyresorcinol (195 mg, 1.39 mmol). The vial was sealed and heated in an oil bath at 230 °C for 40 min. The resulting material was dissolved in ethyl acetate (50 mL) and washed with 0.1 M KOH in H_2O (2 × 50 mL). The organic phase was dried with sodium sulfate, filtered, and concentrated in vacuo. The crude product was then recrystallized in 3:1 hexanes:ethyl acetate to yield 10 mg of yellow solid, 4%. NMR (D_6 DMSO): δ 3.99 (3H, s), 6.35 (1H, s), 7.16 (1H, s), 7.41 (1H, t, $J = 7.7$), 8.05 (1H, d, $J = 6.6$), 8.30 (1H, d, $J = 8.0$), 11.25 (1H, s), 14.04 (1H, s). HRMS: *m/z* (rel int %) 275 (30) $(M)^+$. Calcd for $C_{14}H_{10}NO_3Cl$: 275.0349. Found: 275.0354.

5-Chloro-1-hydroxy-3-methoxy-N-methylacridone (16). Into a stirring solution of **1** (710 mg, 2.71 mmol) in DMF (15 mL) was added cesium carbonate (2.83 g, 8.67 mmol) and methyl iodide (1.68 mL, 27.1 mmol). The solution was heated in an oil bath at 40 °C for 6 h. The reaction was cooled to room temperature, and ethyl acetate (50 mL) was added. The resulting mixture was filtered and washed with ethyl acetate (100 mL). The filtrate was washed with H_2O (4 × 100 mL). The organic phase was dried with sodium sulfate, filtered, and flash chromatographed to yield 550 mg of yellow solid, 70%.

NMR (D_6 DMSO): δ 4.04 (3H, s), 4.06 (3H, s), 6.44 (1H, d, J = 2.0), 6.66 (1H, d, J = 2.0), 7.48 (1H, t, J = 7.9), 8.05 (1H, dd, J = 7.5, 1.6), 8.35 (1H, dd, J = 7.9, 1.6), 14.16 (1H, s). Anal.: $C_{15}H_{12}NO_3Cl$. MS: m/z (rel int %) 289 (100) $(M)^+$. Calcd for $C_{15}H_{12}NO_3Cl$: 289.0506. Found: 289.0508.

5-Chloro-1,3-dihydroxy-N-methylacridone (17). 16 (395 mg, 1.36 mmol) was suspended in 48% aqueous HBr (23 mL). The suspension was heated with an oil bath at reflux for 18 h. The reaction was allowed to cool to RT and stored at 0 °C for 2 days. The resulting yellow solid was filtered and then dissolved into H_2O (100 mL) and ethyl acetate (100 mL) with vigorous stirring over a period of 10 min. The organic phase was then isolated, dried with sodium sulfate, filtered, and concentrated to yield 285 mg of yellow solid, 76%. NMR (D_6 DMSO): δ 4.04 (3H, s), 4.06 (3H, s), 6.44 (1H, d, J = 2.0), 6.66 (1H, d, J = 2.0), 7.48 (1H, t, J = 7.9), 8.05 (1H, dd, J = 7.5, 1.6), 8.35 (1H, dd, J = 7.9, 1.6), 14.16 (1H, s). Anal.: $C_{14}H_{10}ClNO_3$. HRMS: m/z (rel int %) 275 (20) $(M)^+$. Calcd for $C_{14}H_{10}NO_3Cl$: 275.0349. Found: 275.0354.

6-Hydroxy-8-methoxy-10H-benzo[b][1,8]naphthyridin-5-one (18). Into a 4 mL vial was added 2-aminonicotinic acid (552 mg, 4.00 mmol) and 5-methoxyresorcinol (616 mg, 4.40 mmol). The vial was sealed and heated in an oil bath at 230 °C for 40 min. The resulting crude material was dissolved in ethyl acetate (100 mL) and a small quantity of DMF. The solution was then washed with 0.10 M aqueous KOH (2 × 100 mL). The organic phase was dried with sodium sulfate, filtered, and concentrated to yield 204 mg of light brown solid 22%. NMR (D_6 DMSO): δ 3.98 (3H, s), 6.38 (1H, d, J = 1.2), 6.63 (1H, d, J = 1.2), 7.42−7.45 (1H, m), 8.62 (1H, dd, J = 7.0, 1.0), 8.89−8.92 (1H, m), 12.41 (1H, s), 13.90 (1H, s). Anal.: $C_{13}H_{10}N_2O_3$. HRMS: m/z (rel int %) 242 (12) $(M)^+$. Calcd for $C_{13}H_{10}N_2O_3$: 242.0691. Found: 242.0691.

4-Chloro-11-hydroxy-5H-benzo[b]acridin-12-one (19). Into a 4 mL vial was added 2-amino-3-chlorobenzoic acid (171 mg, 1.00 mmol) and 1,3-dihydroxynaphthalene (320 mg, 2.0 mmol). The vial was sealed and heated in an oil bath at 230 °C for 40 min. The resulting crude material was triturated in ethyl acetate and filtered. The filtrate was flash chromatographed to yield a red solid, 25 mg, 8%. NMR (D_6 DMSO): δ 7.36 (1H, t, J = 7.5), 7.52 (1H, t, J = 7.9), 7.76 (1H, t, J = 9.1), 7.76 (1H, t, J = 9.1), 7.89 (1H, s), 7.95 (1H, d, J = 7.6), 8.08 (1H, t, J = 6.8), 8.33−8.41 (2H, m), 11.14 (1H, s), 15.43 (1H, s). Anal. ($C_{17}H_{10}NO_2Cl \cdot 1.0\ H_2O$) C, N; H: calcd 3.86; found, 3.30. HRMS: m/z (rel int %) 295 (100) $(M)^+$. Calcd for $C_{17}H_{10}NO_2Cl$: 295.0400. Found: 295.0403.

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