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One-pot synthesis and negative ion mass spectrometric investigation of a densely functionalized cinnoline

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ABSTRACT

Known, densely substituted 3-amino-5,7,8-trichloro-6-hydroxycinnoline-4-carbonitrile was synthesized using a one pot synthetic protocol under base-mediated conditions in a polar medium. Condensation of excess malononitrile with chloranil in ethanol at reflux gave quinone methide—2-(2,4,5-trichloro-3-hydroxy-6-oxocyclohexa-2,4-dien-1-ylidene)malononitrile which was isolated as the triethylamine salt. This represents an atom efficient, simple, and effective procedure for the preparation of a highly substituted cinnolines that may serve as relay materials for antimalarial prototypes.

An impressive range of bicyclic heterocyclic compounds have been studied as prototypes for antimalarial drugs of which only a handful have made the arduous and costly transition to clinically useful chemotherapeutic agents (Fig. 1).[1] Historically, they have been based on their natural product counterparts and among these, quinolines, such as quinine **1**, have continued to feature prominently in antimalarial drug research.[2] Additionally, there are acridine drugs, such as quinacrine **2**, originating from unnatural vat dyes which were themselves used to stain and quantify parasites within mammalian cells.[3] As part of our ongoing antimalarial program aimed at uncovering undiscovered or neglected chemo-types, we noted that cinnolines were under-represented within natural products (an exception is the symmetrical compound 4849F **3**.[4] It was of interest to study whether lead substances incorporating such heterocycles (**4** where X = N) could act as surrogates for the quinoline ring system, especially for drugs such as chloroquine (**4** where X = CH) and, more importantly, could evade induction and persistence of drug resistance.[5]

Cinnolines, in general, have been rarely studied in terms of their pharmacokinetic (ADMET) properties (especially pK_a values) whereas pharmacodynamic investigations, especially against our malaria receptor of interest, heme, are unknown.[2,6] Our studies have focused on understanding how additional nitrogen groups decorating bi- and tri-cycles modulate the lipophilicity, pharmacokinetics, and drug receptor binding,[7] especially in the 1,5-naphthyridine ring system, which is present in pyronaridine **5** (Fig. 1), a Chinese drug currently being fast tracked for global dissemination by MMV/WHO.[8]

The criteria for selecting drugs according to a generalized pharmacophore have been published[2,6,9] and subsequently, we have accumulated evidence of additional requirements that can be used to select potentially useful antiparasitic compounds.[10] These features include: (a) the capacity to hydrogen bond to the two heme propionic acid side chains **6** and/or (b) orient in a geometry[11] that can initiate redox reactions by electron/hydrogen atom transfer, facilitating conversion of the inert, stable, Fe(III) to a reactive Fe(II) state.[12] This type of oxidative stress is known to initiate and propagate damage to areas that traffic and accumulate such drug–receptor complexes,[13] including sensitive proteins (e.g., hemoglobin),[14] lipids[15] and DNA.[16]

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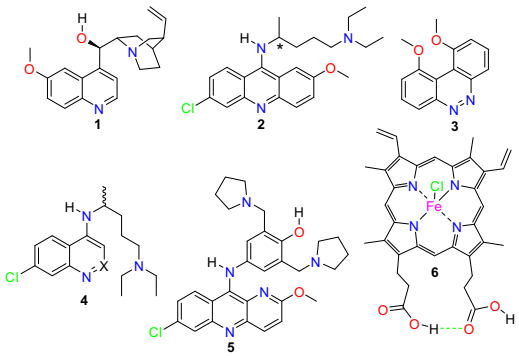
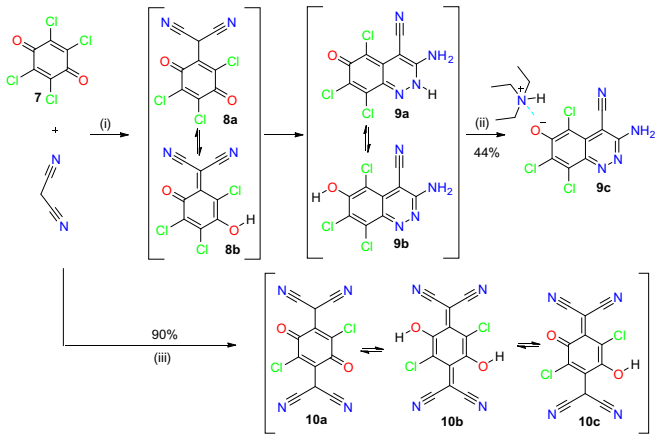


Figure 1. Quinine **1**, mepacrine (quinacrine) **2**, 4849F **3**, X = N **4a**, chloroquine (X = CH) **4b**, pyronaridine **5**, hemin chloride **6**.



Scheme 1. Synthesis of: 3-amino-5,7,8-trichloro-4-cyano-cinnolin-6-olate trimethylamine salt **9c** using a one pot reaction. Reagents and conditions: (i) wet EtOH, Et_3N (2 equiv), reflux; (ii) $H_2N–NH_2$ (wet), reflux, 10 min, 44% over 2 steps. 2′-(2,5-Dichloro-3,6-dihydroxycyclohexa-2,5-diene-1,4-diylidene)dimalononitrile, **10a–c** (inseparable mixture), (iii) malononitrile (2 equiv) dry EtOH, Et_3N (trace), reflux, 30 min, then evaporate, −12 °C, 15 h, 90%.

Our strategy has been to identify compounds that initiate antiparasitic drug action from distonic anion radical cascades,[17] using an automated, non-biological screen that could be converted into a high-throughput mode using robotic auto-samplers.[18] We have previously reported that pulsed radiolysis[19] and subsequent negative ion electrospray mass spectrometry[20] were ideally suited to this task and have now identified selected cinnolines as potential antiparasitic agents.[21]

In general, densely substituted cinnolines are difficult to construct in a reproducible manner, and to the best of our knowledge, one-pot methods from commercially available raw materials are rare.[22] Ideally, cinnolines are required to contain both hydrogen atom donor and acceptor sites and either nitrile and/or halogen groups. A literature search identified one suitable set of compounds which Gomaa[23] used to outline a synthesis of a densely substituted halogenated cinnoline. This type of compound is easily accessible in a two-step procedure from the antifungal compound chloranil.[24]

Notably, in the first part of the synthesis, Gomaa[23] employed 3 equiv of malononitrile to produce an apparently stable quinone-methide, which was isolated by preparative thin-layer chromatography. Upon close examination of this Letter, the following questions arose regarding the quinone-methide formation step. Firstly: (a) why were the reported mass ions 4 Da higher than the expected mass ions?[23] (b) did the use of excess malononitrile imply inherent low reactivity of chloranil under the conditions employed?[21] and, if not; (c) why were products resulting from multiple dehalo-alkylation not reported?[23]

We were also curious to determine whether this two-step process could be converted to a ‘one-pot’ method, eliminating the intermediate preparative TLC step[23] and whether robust analytical methods could be developed to rapidly monitor this and similar reactions?

Herein, we describe a scalable one-pot method for accessing highly halogenated cinnolines which avoids expensive chromatographic purification and displays the expected spectrometric profile. To the best of our knowledge, spectrometric monitoring of such reactions using negative ion electrospray mass spectrometry remains unexplored and, consequently, the methodology outlined here may prove useful for optimizing this type of cinnoline synthesis.

Results and discussion

During initial experiments it was observed that mixtures of compounds were produced using Gomaa’s route (Scheme 1).[18] Use of the stepwise synthesis as outlined by Gomaa, and also our one-pot modification (without isolation of the unusually stable quinone-methides), afforded a complex mixture of products (using DMF as solvent) that were each identified either by isolation or by Collision Induced Dissociation (CID) studies of negative ion electrospray mass spectrometry. In the first part of the synthesis, upon addition of one equivalent of triethylamine to the chloranil/malononitrile solution in either methanol or ethanol, it was noted that the pale green color rapidly transformed to an intense blue species, suggesting formation of charge transfer complexes or radical formation. The yield of semi-quinone radical ($^{\cdot}QH/Q^{\cdot-}$) of halogenated benzoquinones in polar media, such as acetonitrile–water (1:1), has been suggested to be low (<20%) at low substrate concentration but significantly increased upon addition of an H-atom donor, for example, 2-propanol (see ESI). It is probable that the deep blue color observed in the reactions in methanol also involved a semi-quinone radical. Gorner and Sonntag[25] have suggested that other mechanisms involving $^{\cdot}QH/Q^{\cdot-}$ radicals, including quenching of the triplet state at enhanced halo-benzoquinones concentrations and H-atom abstraction from an organic solvent in mixtures with water, must also be considered. Interestingly, these were stable and could be observed on TLC (see ESI).

When 2 equiv of malononitrile were used in anhydrous ethanol, and the solution heated at reflux before being concentrated then left overnight, symmetrical compounds, represented by an inseparable mixture of tautomers **10a–10c** were isolated in 90% yield (as the free bases). These were characterized by spectroscopy and spectrometry.[26] Some of the impurities detected within this crude mixture corresponded to compounds **13a–13c** formed by the addition of hydrazine to the tautomeric mixture of **10a–10c** (Scheme 2).

Addition of hydrazine

Using a modified method utilizing an aqueous quench rather than recrystallization from DMF, gave a black precipitate that could be further purified by washing with chloroform, thus avoiding chromatographic purification. This aqueous work-up removed many of the impurities that may have led Gomaa[23] to suggest an unusual mass spectral pattern. Investigation of the aqueous layer using negative ion electrospray suggested the presence of various species including **10a–10c**. DEPT-Q ^{13}C NMR spectroscopy[23,26] of the purified fraction confirmed the presence of the expected

number of ^{13}C peaks, however the presence of two additional species suggested that the isolated compound was salt **9c** (Fig. 2).

Various solvents were used to improve yields of **9a**/**9b** of which ethanol gave the highest yield (Table 1). The reaction could be accelerated using microwave irradiation (entry 7, Table 1), however, this also resulted in the formation of impurities that were difficult to remove from the final product (Scheme 2).

^{13}C INADEQUATE NMR spectroscopy was considered as being useful in the characterization of similar compounds,[27] but we found this to be an inefficient option for our anticipated drug discovery program. Thus, alternative analytical methods were sought to characterize **9a**/**9b** and also lay groundwork for the preparation of libraries of cinnoline compounds. Consequently, before a detailed pharmacological study of our cinnoline lead substance **9c**, could be undertaken, a study of its synthesis and behavior under both positive and negative ion electrospray mass spectrometry was thought desirable to further explore the subsequent action of **9a**/**9b** at the heme-drug receptor.

Positive ion electrospray mass spectroscopic analyses of the salt revealed a nominal base peak at 102 Da, suggesting the abundance of triethylamine. In contrast, negative ion electrospray MS of the crude material (ESI) showed that the anion radical was formed, (287 Da). Preliminary calculations suggested that formation of the distonic radical anion was thermodynamically favored (MM2 calculations, not shown) which was then ejected to form a tri-aza-substituted compound. Although, other structures could be invoked, involving di-aza-cumulenes,[29] none were capable of losing N_2. Any mechanism must also account for the loss of HCl, which suggested that both substituents were in close proximity and, since the molecule was planar, by necessity appended upon the same ring ejecting N_2. Consequently, the current investigation employed Collision Induced Dissociation (CID) mass spectra to determine the fragmentation. To the best of our knowledge, electrospray mass spectra of substituted cinnolines in the negative ion mode are unknown, although some generalizations involving electron ionization of benzo[*c*]cinnoline derivatives were reported. Bowie et al. noted that loss of nitrogen (from either the molecular ion or a fragment ion) featured in all of their spectra, confirming the presence of a —N=N— group.[30] In spectra involving 1-, 2-, or 3-substituted benzo[*c*]cinnolines, the M-N_2, process precedes fragmentation through Me, Cl, NH_2, NMe_2, or CO_2H substituents, but for those possessing either MeO, EtO, NEt_2, CO_2Me or CO_2Et substituents, fragmentation proceeds via loss of the substituent and, subsequently, ejection of N_2.[30]

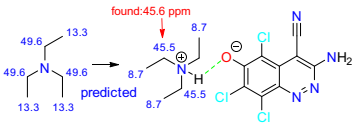
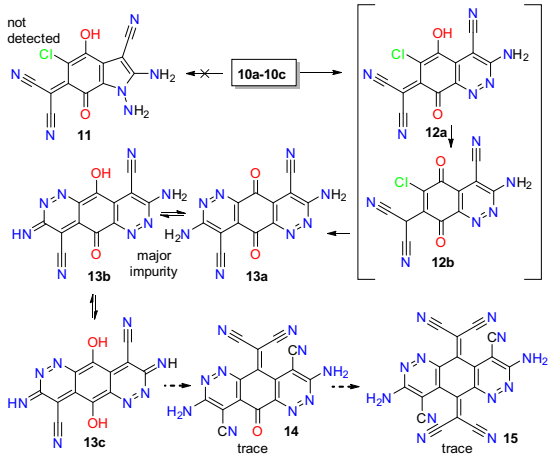


Figure 2. Selected ^{13}C NMR data 3-amino-5,7,8-trichloro-6-((triethyl-l5-azanyl) oxy)cinnoline-4-carbonitrile (in DMSO-d_6).

Table 1
Synthesis of **9c** under different reactions conditions

Entry	Solvent	Temp (°C)	**9a**/**9b** (%)
1	Water	Reflux	0
2	Water	25	0
3	Ethanol	25	0
4	Ethanol	Reflux	44
5	THF	25	0
6	THF	Reflux	0
7	Solvent free	Melt	30



Scheme 2. Proposed mechanism for the formation of impurities **13a**–**13c** (inseparable tautomeric species) observed in the crude mixture using mass spectrometry.

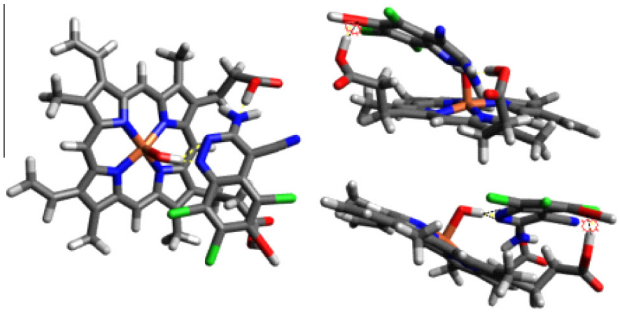


Figure 3. Various views of the **9a**/**9b** cinnoline interacting with heme on the same side as the axial substituent[6] by hydrogen bonding. Molecular mechanics rendered using Avogadro.[28] Yellow (or black) dotted lines indicate hydrogen bonding (see ESI for larger figure on a black background).

Preliminary mass spectrometry and molecular modeling (see Figs. 2 and 3, ESI) identified that this compound could potentially interact with heme by hydrogen bonding. In summary, the reported protocol is a simple, novel and versatile one-pot method for the synthesis of cinnolines which are isolated as a salt. Further investigations are currently underway in our laboratory to explore the scope of this procedure using other nitriles suitable for evaluation in pharmacological assays and dehalogenation reactions of **9a**/**9b** (using excess Mg/*t*-BuOH) for the preparation of simpler halo-cinnolines related to chloroquine **4a** (Fig. 1).

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Supplementary data

Supplementary data (spectral data and molecular modeling figures for **9a**/**9b**, and MS data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.10.104. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Lotharius, J.; Gamo-Benito, F.; Angulo-Barturen, I.; Clark, J.; Connelly, M.; Ferrer-Bazaga, S.; Parkinson, T.; Viswanath, P.; Bandodkar, B.; Rautela, N.;

Bharath, S.; Duffy, S.; Avery, V.; Mahrle, J.; Guy, R.; Wells, T. *Malar. J.* **2014**, *13*, 143.
2. (a) Dascombe, M. J.; Drew, M. G. B.; Evans, P. G.; Ismail, F. M. D. *Front. Drug Des. Disc.* **2007**, *3*, 559–560; (b) Ismail, F. M. D.; Dascombe, M. J.; Carr, P.; North, S. E. *J. Pharm. Pharmacol.* **1996**, *48*, 841–850; (c) Ismail, F. M. D.; Dascombe, M. J.; Carr, P.; Merette, S. A. M.; Rouault, P. *J. Pharm. Pharmacol.* **1998**, *50*, 483–492.
3. Schlitzer, M. *ChemMedChem* **2007**, *2*, 944–986.
4. a) Blair, L.; Sperry, J. *J. Nat. Prod.* **2013**, *76*, 794–812; b) Wang, K.; Guo, L.; Zou, Y.; Li, Y.; Wu, J. *J. Antibiot.* **2007**, *60*, 325–327.
5. Keneford, J.; Simpson, J. *J. Chem. Soc.* **1947**, 917–920.
6. Dascombe, M.; Drew, M. G. B.; Morris, H.; Wilairat, P.; Auparakkitanon, S.; Moule, W.; Alizadeh-Shekalgourabi, S.; Evans, P.; Lloyd, M.; Dyas, A.; Carr, P.; Ismail, F. M. D. *J. Med. Chem.* **2005**, *48*, 5423–5436.
7. Cheng, C. *J. Theor. Biol.* **1976**, *59*, 497–501.
8. Croft, S.; Duparc, S.; Arbe-Barnes, S.; Craft, J.; Shin, C.; Fleckenstein, L.; Borghini-Fuhrer, I.; Rim, H. *Malar. J.* **2012**, *11*, 270.
9. Ismail, F. M. D.; Drew, M. G. B.; Dascombe, M. J. *Chim. Oggi: Chem. Today* **2009**, *27*, 18–22.
10. Ismail, F. M. D.; Cox, A. H.; Dempster, N. M.; Ford, J. L.; Dascombe, M. J.; Drew, M. G. B. *J. Pharm. Pharmacol.* **1998**, *62*, 1489–1490.
11. Hydrogen bonding to both propionic groups is a favoured interaction: Ismail, F. M. D.; Dempster, N. M.; Edwards, G.; Morris, H.; Saleem, I.; Alrawahi, A.; Azam, M.; Dascombe, M. J.; Smith, M.; Parker, A. W.; Clark, I. P.; Alizadeh-Shekalgourabi, S.; Drew, M. G. B. *Invited Lecture: Quantification of Haem: Anti-malarial Drug Receptor Geometry by Crystallography, DFT, Spectroscopic and Spectrometric Methods in vitro and in silico*, 31st BMSS 3-Day Meeting Mass Spectrometry for a Changing World Sunday to Wednesday: 5–8th September, 2010; Cardiff City Hall.
12. Monti, D.; Vodopivec, B.; Basilico, N.; Olliaro, P.; Taramelli, D. *Biochemistry* **1999**, *38*, 8858.
13. Sugioka, Y.; Suzuki, M.; Sugioka, K.; Nakano, M. A. *FEBS Lett.* **1987**, *223*, 251–254.
14. Balaji, S.; Trivedi, V. *J. Basic Clin. Physiol. Pharmacol.* **2013**, *24*, 105–114.
15. Berman, P.; Adams, P. *Free Radical Biol. Med.* **1997**, *22*, 1283–1288.
16. Yu, X.; Ramiandrasoa, F.; Guetzoyan, L.; Pradines, B.; Quintino, E.; Gadelle, D.; Forterre, P.; Cresteil, T.; Mahy, J.; Pethe, S. *Chem. Med. Chem.* **2012**, *7*, 587–605.
17. Marchi, S.; Giorgi, C.; Suski, J.; Agnoletto, C.; Bononi, A.; Bonora, M.; De Marchi, E.; Missiroli, S.; Patergnani, S.; Poletti, F.; Rimessi, A.; Duszynski, J.; Wieckowski, M.; Pinton, P. *J. Signal Transduct.* **2012**, *2012*, 1–17.
18. Luippold, A.; Arnhold, T.; Jorg, W.; Sussmuth, R. *Int. J. Mass Spectrom.* **2010**, *296*, 1–9.
19. Ismail, F. M. D.; Drew, M. G. B.; Navaratnam, S.; Bisby, R. H. *Res. Chem. Intermed.* **2009**, *35*, 363–377.
20. Rosu, F.; De Pauw, E.; Gabelica, V. *Biochimie* **2008**, *90*, 1074–1087.
21. Notably, very few cinnolines have been screened for antimalarial activity: most of the studies were conducted when this type of pharmacological testing used rather unreliable assays conducted under war-time conditions and in vivo, but without constructing dose response curves: see Simpson, J. C. E.; Schofield, K. *Nature* **1946**, *157*, 439–440.
22. Simpson, J. C. *The Condensed Pyridazine and Pyrazine Rings (Cinnolines, Phthalazines, and Quinoxadines)—Chemistry of Heterocyclic Compounds: A Series of Monographs*; Wiley-Blackwell, 1953.
23. Gomaa, M. *Tetrahedron Lett.* **2003**, *44*, 3493–3496.
24. Amani, A. M. *Drug Res.* **2014**, *64*, 420–423.
25. Gorner, H.; von Sonntag, C. *J. Phys. Chem. A* **2008**, *112*, 10257–10263.
26. *Chloranil Mass Spectra:* The mass spectrum of the chloranil standard in acetonitrile (see ESI) exhibits the expected four-chlorine isotope cluster at *m*/*z* 244 and an unexpected three chlorine isotope cluster at *m*/*z* 225. The latter is known to arise from dehalohydroxylation from reactions occurring during analysis and not due to contamination of the standard [Sarr, D. H.; Kazunga, C.; Charles, M. J.; Pavlovich, J. G.; Aitken, M. D. *Environ. Sci. Technol.* **1995**, *29*, 2735–2740]. *Synthetic procedure for cinnoline preparation:* To a solution of chloranil **7** (1.0 mmol) in ethanol (10 ml), a malononitrile solution (3.0 mmol, 0.198 g) in ethanol (5.0 ml) and triethylamine (1.0 mmol) were added dropwise with rapid stirring. The mixture was heated at reflux for 3–6 h forming an intense blue solution after 30 min. The reaction was monitored by TLC (ethyl acetate) until chloranil was consumed (see ESI). To the crude mixture of ylidene–malononitriles, **8a**/**10a**, 3 equiv of hydrazine were added by syringe pump and the mixture heated at reflux for 10 min. The mixture was poured onto ice water (100 g) and the resulting solid filtered (at the pump) and further purified by washing with chloroform. The insoluble dark fraction was retained to give the product. The red solution contained two materials, one of which was also the desired cinnoline **9c** and another, as yet, an unidentified compound. Reactions were repeated using various solvents. [See Table 1 for yields.] *3-Amino-5,7,8-trichloro-6-((triethyl-l5-azanyl)oxy)cinnoline-4-carbonitrile* (**9c**): NMR ^{13}C (DMSO-d_6): 8.54 (CH_3), 45.64 (CH_2), 60.96, 109.19, 112.18, 113.81, 114.28, 125.70, 137.56; 147.22, 167.37 (4 °C). *Synthesis of 2-(2,5-dichloro-4-dicyanomethyl-3,6-dioxo-cyclohexa-1,4-dienyl)-malononitrile* (**10a**–**10c**): A mixture of chloranil **7** (0.2459 g, 1 mmol) and malononitrile (0.1260 g, 2 mmol) in absolute ethanol (50 mL) containing 2 drops of triethylamine was heated at reflux for 30 min. whereupon the reaction color gradually changed from yellow to green. The reaction mixture was cooled and evaporated to 15 ml. After refrigeration for 15 h the crystalline green product was filtered at the pump, washed with excess deionized water and air dried in the dark conditions. Purification by recrystallization from dioxane gave dark green plates; yield 90%; mp 240–241 °C. Compare with the literature mp.[24]
27. Schmidt, A.; Namyslo, J.; Mordhorst, T. *Tetrahedron* **2006**, *62*, 6893–6898.
28. Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. *J. Cheminform.* **2012**, *4*, 17.
29. Diazacumulenes with an even number of atoms are calculated to have a higher configurational stability than those with an odd number of atoms. *cis*-Diimides are thermodynamically more stable than the corresponding *trans*-isomers. Gordon, M. S.; Fischer, M. S. *J. Am. Chem. Soc.* **1968**, *90*, 2471–2476.
30. Bowie, J. H.; Lewis, G. E.; Reiss, J. A. *Aust. J. Chem.* **1968**, *21*, 1233–1245.