ACS Medicinal Chemistry Letters

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Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00013 • Publication Date (Web): 16 Feb 2017

ACS Publications

ACS Medicinal Chemistry Letters is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Antimalarial Properties of Simplified Kalihinol Analogues

Mary Elisabeth Daub[a] , Jacques Prudhomme[b], Choukri Ben Mamoun[c*], Karine G. Le Roch[b*], and Christopher D. Vanderwal[a*]

[a]1102 Natural Sciences II, Department of Chemistry, University of California, Irvine, CA 92697

[b]Department of Cell Biology & Neuroscience, 900 University Avenue, University of California, Riverside, CA 92521

[c]Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT 06520

KEYWORDS: antimalarial, terpenoid, isonitrile, structure-activity relationship, natural product synthesis

ABSTRACT: Several kalihinol natural products, members of the broader isocyanoterpene family of antimalarial agents, are potent inhibitors of *Plasmodium falciparum*, the agent of the most severe form of human malaria. Our previous total synthesis of kalihinol B provided a blueprint to generate many analogues within this family, some as complex as the natural product and some much simplified and easier to access. Each analogue was tested for blood-stage antimalarial activity using both drug-sensitive and -resistant *P. falciparum*. Many considerably simpler analogues of the kalihinols retained potent activity, as did a compound with a different decalin scaffold made in only three steps from sclareolide. Finally, one representative compound showed reasonable stability toward microsomal metabolism, suggesting that the isonitrile functional group that is critical for activity is not an inherent liability in these compounds.

The impact of natural products on the development of modern antimalarial therapy cannot be overstated.[1] The discovery of the alkaloid quinine—one of the WHO Model List of Essential Medicines—inspired the development of several clinically used drugs for malaria prophylaxis and therapy, including mefloquine, the 4-aminoquinolines chloroquine and amodiaquine, and the 8-aminoquinolines primaquine and tafenoquine. More recently, another natural product, artemisinin, and its semi-synthetic analogues have been used in combination with other antimalarials as a first line treatment for *P. falciparum* malaria worldwide. The chemical diversity and potentially novel modes of action of natural products make them ideal targets for the development of new classes of potent antimalarials.

The isocyanoterpenes (ICTs) are a group of sponge-derived polycyclic terpenoids bearing the unusual isonitrile functional group.[2] Among the ICTs that have been evaluated for antimalarial activity,[3–5] kalihinol A (**1**, Figure 1) shows the most potent activity against chloroquine-resistant FCR-3 *Plasmodium falciparum*.[5,6] In an important recent development, Shenvi and co-workers demonstrated that representative ICTs have potent liver-stage antimalarial activity in addition to well-established blood-stage potency.[7] Interestingly, many structurally diverse ICTs are potent antiplasmodic agents *in vitro*; however, most of the potent compounds display a common structural feature—the isonitrile-bearing cyclohexane highlighted in red in **1**. Comparison of the structural features of each of the ICT subgroups revealed the kalihinols as superior starting points for potential antimalarial lead compound identification; by contrast, the adociane- and amphilectane-type compounds (e.g., **4**–**6**) are particularly hydrophobic. The discovery of simplified kalihinol analogues that retain antimalarial potency, that are easier to make, and that have improved physicochemical characteristics is one of the goals of our research program.

Our recent synthesis of kalihinol B (**2**),[8] the THF-containing congener of kalihinol A, provided a blueprint for quick access to simplified analogues and the impetus to make them: kalihinol B was nearly as active as kalihinol A in spite of the significant structural change. The dramatic simplification of both structure and synthesis that would accrue from removal of the complex pendant heterocycles could conceivably lead to attractive candidates for further antimalarial development.[9–12] In this letter, we describe the synthesis and antimalarial activity of several simplified kalihinol analogues, and show that the motif highlighted in structure **1** is not an absolute determinant of potency, a conclusion also recently reached by Shenvi and coworkers.[7] We further demonstrate that the key isonitrile functional group might not be as serious a metabolic liability as one might expect.

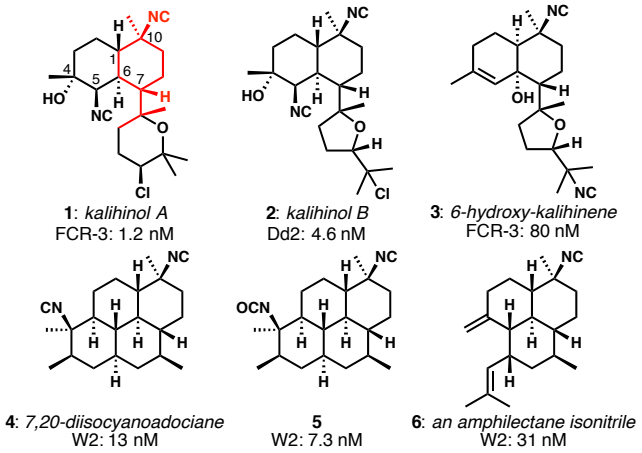
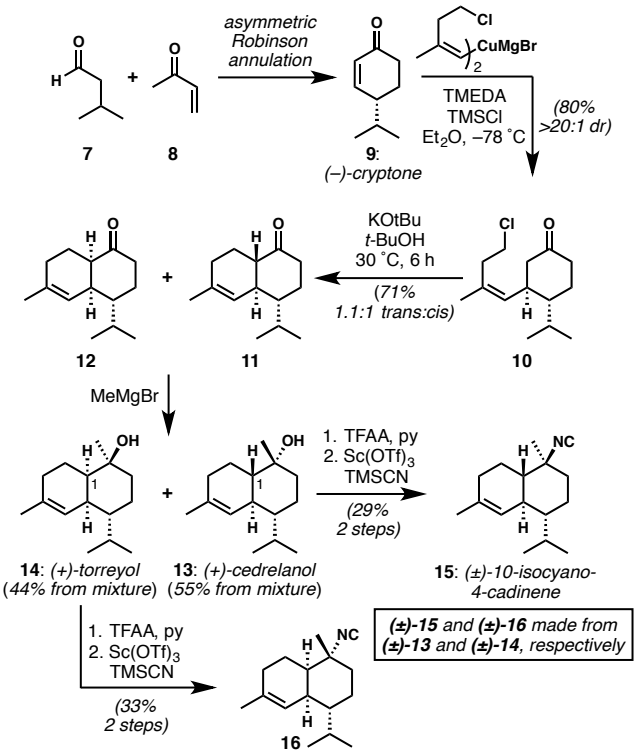


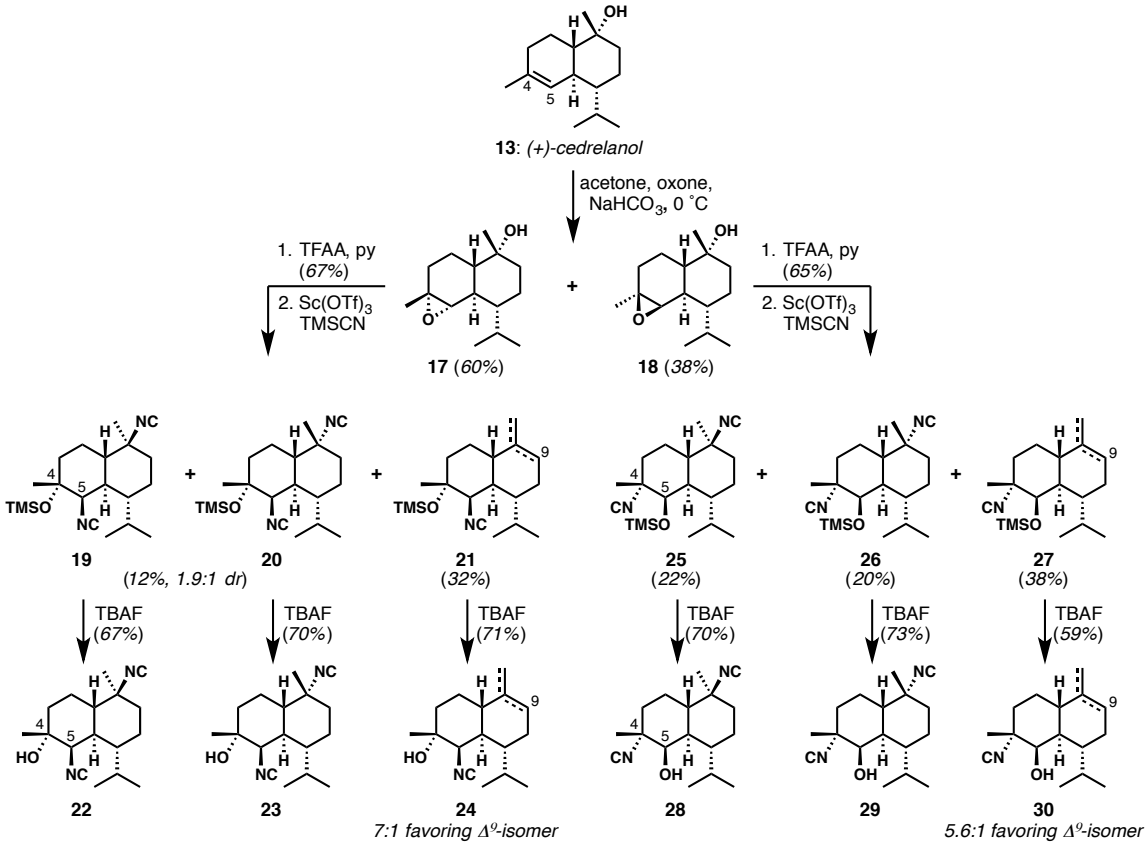
Figure 1. Representative isocyanoterpenes and their activities against drug-resistant *P. falciparum* (FCR-3, Dd2, and W2; numbers shown are IC_{50} values)

The synthesis of all simplified kalihinol analogues, in which the heterocyclic ring was replaced with an isopropyl appendage, started with the synthesis of enantioenriched (+)-cedrelanol (**13**, Scheme 1). (−)-Cryptone (**9**) was obtained in enantioenriched form via a Robinson annulation featuring the catalytic asymmetric Michael addition of isovaleraldehyde to methyl vinyl ketone, using the procedure and catalyst of Gellman,[13] as used by Baran.[14] The remainder of the cedrelanol synthesis exactly paralleled the sequence of steps from our kalihinol B synthesis.[8] Two-stage Piers annulation onto cryptone provided the decalones (**11** and **12**) as a mixture of *cis* and *trans* ring fusions. Nucleophilic methylation of the isomeric mixture provided cedrelanol (**13**) and its stereoisomer torreyol (**14**), which were readily separable. Although lacking stereochemical control at C1, this synthesis of these sesquiterpenoids is particularly direct, delivering each in enantioenriched form in only five steps. Application of Shenvi's isonitrile installation[15] to *racemic samples* of **13**[15] and **14** provided the well-known mono-isonitrile (±)-10-isocyano-4-cadinene (**15**),[16] which has been made several times before, but via lengthier sequences,[10,15,17,18] and **16**, respectively. The latter is the C10 epimer of 10-isocyano-4-amorphene.[16,19]

Synthetic (+)-cedrelanol was used to generate six different bis-isonitrile analogues (Scheme 2). Epoxidation of the C4–C5 alkene was not as highly stereocontrolled as in our kalihinol B synthesis, and **17** and **18** were formed in about a 1.5:1 ratio. Activation of the tertiary alcohol of each diastereomer was uneventful. However, attempted isocyanation at C10 with concomitant isocyanolysis of the epoxide yielded multiple products in each case, which were fully characterized after



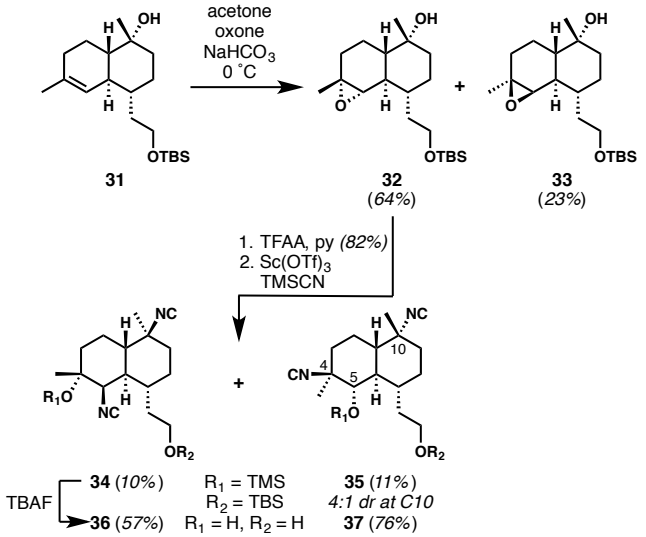
Scheme 1. Synthesis of (+)-cedrelanol, (+)-torreyol, (±)-10-isocyano-4-cadinene, and stereoisomer **16** via Piers-type annulation onto enantioenriched cryptone



Scheme 2. Conversion of (+)-cedrelanol into six different simplified kalihinol analogues

fluoride-mediated removal of the silyl ether (from silylation of the opened epoxide by TMSCN). From α-epoxide **17**, all products contained the C5-isonitrile, as expected on the basis of the Fürst–Plattner ring-opening. In this case, invertive displacement of the tertiary trifluoroacetate ester was far from smooth; both stereoisomeric products **19** and **20** were isolated, along with **21**, which derived from the competing elimination of the axial tertiary trifluoroacetate ester. Similar problems were observed in our kalihinol B synthesis.[8] With β-epoxide **18**, the same general trend was observed, with a somewhat improved production of bis-isonitrile diastereomers **25** and **26**, which were formed as C4-isonitrile regioisomers on account of Fürst–Plattner ring-opening. Elimination products (**27**) were also observed.

We believed that significant changes to the C7-appendage (*cf.* kalihinol A vs. B) would have little effect on antimalarial potency. We therefore aimed to access kalihinol-based chemical probe molecules featuring functional handles attached at C7 for mechanism of action studies. The latter stages of a synthesis of analogues with a free primary hydroxyl group are shown in Scheme 3. Appropriate groups can easily be appended by esterification or by conversion of the hydroxyl group to an azide and the use of alkyne/azide cycloaddition methods. The synthesis proved straightforward starting from 4-(*tert*-butyldimethylsilyloxy)butanal.[20] Robinson annulation (in the racemic manifold), Piers annulation, and nucleophilic methylation afforded decalin **31** (steps are not shown, but are similar to those in Scheme 1[20]). Epoxidation afforded a *ca.* 2.5:1 mixture of diastereomers **32** and **33**, and application of the two-fold isonitrile introduction to the major diastereomer provided β-hydroxyisonitrile isomers **36** and **37**, in low yields over the three-step sequences. In accord with the Fürst–Plattner principle, diisocyanide **34** resulted from nucleophilic epoxide opening at C5 to give the C4/C5 diaxial product and invertive displacement of the C10 trifluoroacetate to afford the equatorial isonitrile. However, competitive nucleophilic epoxide opening at C4, possibly a result of chelation of the Lewis acid to the epoxide and the pendant silyl ether, afforded the C4/C5 diequatorial product **35**. While some kalihinane natural products with equatorial C4-tertiary hydroxyl group and C5-isonitrile are known (the "isokalihinol" series[21]), we were



Scheme 3. Synthesis of kalihinol-based chemical probe precursors **36** and **37**

surprised to observe this product because it is likely to arise from the anti-Fürst–Plattner ring-opening of the epoxide. Interestingly, this C4/C5 diequatorial product was isolated as a mixture of C10 epimers, of which the C10 axial isonitrile was predominant (*ca.* 4.4:1 dr). This result was also unexpected, because the C10 equatorial isonitrile, resulting from invertive displacement, is normally the major diastereomer under the reaction conditions. Both of these unusual results speak to the subtle and unpredictable reactivities in complex multifunctional settings.

In addition to these planned, simplified analogues, two congeners of kalihinol B were made (Figure 2)[20]. Monoisonitrile **38** arose (as a mixture of inseparable alkene isomers) from unwanted elimination during attempted C10 isonitrile introduction. C14-nitrile **39** resulted from an attempt to convert a C15-trifluoroacetoxy group into a C15-isonitrile related to 6-hydroxykalihinene (**3**); hydride shift and cation trapping on the carbon atom of cyanide explains its formation.

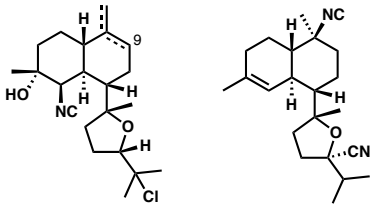
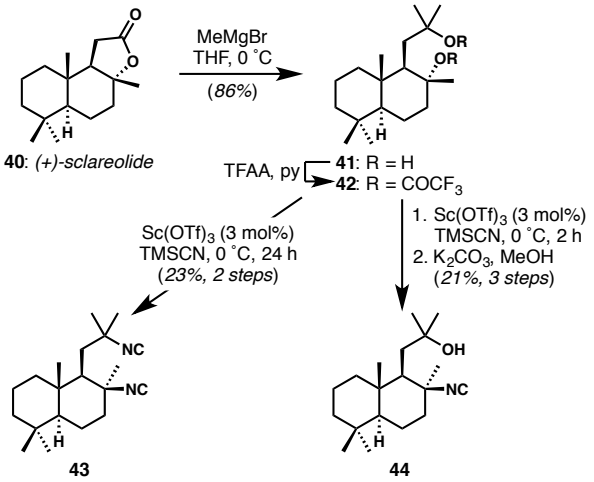


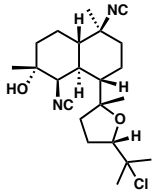
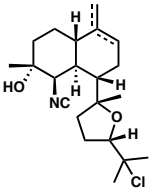
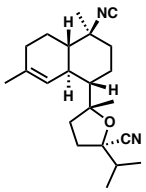
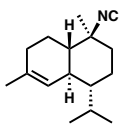
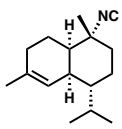
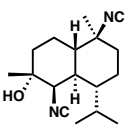
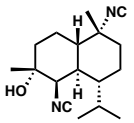
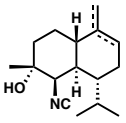
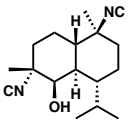
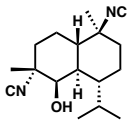
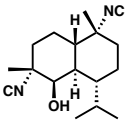
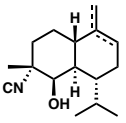
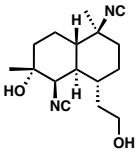
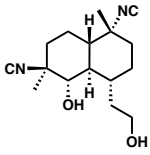
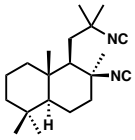
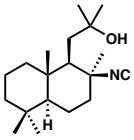
Figure 2. Two analogues from our kalihinol B synthesis efforts

Finally, with the goal of accessing ICT-like structures via simple sequences from readily available materials, we produced two isonitrile-bearing compounds from the inexpensive sesquiterpenoid sclareolide (Scheme 4). Superficially, these compounds were meant to reproduce the complex cyclohexane highlighted in **1** (Figure 1); however, we note that the different decalin ring fusion forces the isonitrile in sclareolide-derived compounds **43** and **44** to be oriented axially, whereas those in structures **1** through **6** have their isonitriles in an equatorial disposition. This procedure gave rise to two complex ICT analogues in only three steps.



Scheme 4. Synthesis of two sclareolide-derived ICT-like compounds

Table 1. Results of antiplasmodial assays of synthetic ICTs and analogues against drug-sensitive (3D7) and drug-resistant (Dd2) strains of *P. falciparum* (numbers shown are IC_{50} values in nM)

	 **2**: kalihinol B	 **38** (3:1)	 **39**	 (±)-**15**: 10-isocyano-4-cadinene	 (±)-**16**	 **22**
3D7	8.4	139	175	705	180	12
Dd2	4.6	144	123	247	45	16
	 **23**	 **24** (7:1)	 **28**	 (±)-**28**	 **29**	 **30** (5.6:1)
3D7	2.9	138	15	27	1150	312
Dd2	31	200	17	46	958	529
	 (±)-**36**	 (±)-**37**	 **43**	 **44**		
3D7	302	27	1.9	244		
Dd2	205	24	1.6	416		

All of the compounds synthesized as described above were evaluated for activity against drug-sensitive 3D7 and chloroquine-resistant Dd2 *P. falciparum* strains (Table 1). Interestingly, all compounds showed strong antimalarial activity, with IC_{50} values ranging from 1.6 nM to *ca.* 1 μM. Mono-isonitrile kalihinol B analogues **38** and **39** retained reasonable potency despite having isonitriles in different positions. 10-Isocyano-4-cadinene (**15**), which has been previously tested by Wood,[11,22] was not as potent as its *cis*-decalin stereoisomer (**16**, also epimeric at C10). Most importantly, a variety of kalihinol analogues lacking the complex THP or THF rings of kalihinols A and B, respectively, retained great potency. Analogue **22**[10,22] retains all other structural features of **1** and **2**, and is potent. Individually, the change in configuration at the isonitrile-bearing C10 (see **23**) or the interchange of C4/C5 substituents (see **28**, with the C4-isonitrile and C5-secondary alcohol) do not significantly affect potency. The activity of the compounds arising from undesired trifluoroacetate elimination is similar across the board (compare **38**, simplified version **24**, and **30** with its swapped C4/C5 substituents). The least active compound in the series of C7-isopropyl compounds is **29**, with unnatural C10 configuration *and* C4/C5 substituent interchange. The two chemical probe precursors provided confounding results, wherein analogue (±)-**36**, with its full set of natural features, proved an order of magnitude less active than (±)-**37**, in which both the C10 center is inverted, C4 bears an *equatorial* isonitrile, and C5 bears an *equatorial* hydroxyl group (note this compound differs in the C4 and C5 configurations relative to **29**). Finally, and provocatively, sclareolide-derived bis-isonitrile **43** was the most potent compound synthesized (IC_{50} of 1.6 nM against Dd2 strain), with essentially equal potency to kalihinol A, the most potent ICT ever reported (IC_{50} of 1.2 nM against FCR-3 strain). Mono-isonitrile **44** was significantly less active. Shenvi and colleagues have made closely related compounds to **43** and **44** from dihydrosclareol, and reported potent activities.[7]

Our findings include: (1) the apparent insignificance of the complex heterocyclic rings in the natural kalihinols; (2) the potential significance of the *cis*-decalin that is poorly represented among kalihinol natural products previously evaluated for antimalarial activity; (3) the flexibility with respect to axially or equatorially disposed C10 isonitriles;[23] and finally (4) the flexibility with respect to the C4/C5 β-hydroxy isonitrile regiochemistry and configurations in some cases. We note that the motif highlighted in Figure 1 does not seem to be the definitive pharmacophore; unfortunately, it remains difficult to make any clear structure-activity conclusions with the data currently available from natural products and from synthesis work from our labs and those carried out previously by others.[5,7–9]

Our concerns for the viability of kalihinol compounds as potential preclinical leads hinged in part on the expected *in vivo* instability of the isonitrile function. Compound (±)-**37**, one of the chemical probe precursors, retained potency in spite of the

serious structural changes relative to the natural kalihinols and in spite of its racemic nature. This compound was subjected to microsomal stability assays, using both human and murine liver microsomes. Interestingly, we found that this potent ICT analogue showed significant stability in the presence of liver microsomes, with half-lives of 142 min (human) and 87 min (murine).[24] While more studies are warranted, this preliminary result suggests that there is nothing intrinsically poor about the salient isonitrile functional groups that are so intimately tied to potency in the ICT family of antimalarials.

The kalihinol scaffold appears to be a promising starting point for the development of interesting antimalarial lead compounds. Many of the simplified analogues synthesized in this study retain high potency and are much easier to access than the natural products; however, the results do not show clear SAR trends. The ICTs have been shown previously to show good selectivity indices[3,5] with respect to mammalian cytotoxicity and to have multi-stage activity;[7] the latter point conflicts previous notions that their activity is due to inhibition of pathways for heme detoxification.[25] We have now shown that the salient isonitrile functional groups are not a metabolic liability. Future studies aimed to engineer the kalihinane scaffold to identify simpler congeners with improved physicochemical properties and high *in vitro* and *in vivo* efficacy are warranted, as are investigations into the incompletely understood mechanism(s) of action of these antimalarial agents.

ASSOCIATED CONTENT

Supporting Information

In the associated PDF, we include general experimental details, experimental procedures for the synthesis of and characterization data for all new compounds. Experimental details and raw data for the antimalarial assays and information about the metabolic stability experiments for compound (±)-**37** are also provided.

The Supporting Information is available free of charge on the ACS Publications website.

AUTHOR INFORMATION

Corresponding Author

*choukri.benmamoun@yale.edu, karinel@ucr.edu, cdv@uci.edu

Author Contributions

The manuscript was written through contributions of all authors.

Funding Sources

CBM's research is supported by NIH (AI123321; AI097218; GM110506; AI09486; AI119691) and The Bill and Melinda Gates Foundation (1021571) grants.
This study was financially supported by the University of California, Riverside (NIFA-Hatch-225935 to KGLR). The funding body had no role in the design of the study, in collection, analysis, and interpretation of data, or in writing the manuscript.
MED was supported in part by an Allergan Graduate Fellowship.

ACKNOWLEDGMENT

The following reagents were obtained through the MR4 as part of the BEI Resources Repository, NIAID, NIH: *Plasmodium falciparum* strains 3D7 (MRA-102) deposited by D.J. Carucci and Dd2 (MRA-156) deposited by Thomas Wellems.

ABBREVIATIONS

ICT, isocyanoterpene; TMEDA, tetramethylethylenediamine; TFAA, trifluoroacetic anhydride; TMS, trimethylsilyl; TBS, *tert*-butyldimethylsilyl; TBAF, tetra-*n*-butylammonium fluoride.

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4. Only a select few of the over 50 kalihinane natural products have been evaluated in antimalarial assays, reflecting the relatively late discovery of this activity relative to the isolation of many of these compounds.

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9. In their investigations on the kalihinols, the Wood group made a large number of analogues based on the cadinane, or "truncated kalihinane" framework. They synthesized five isonitrile-containing compounds, including (±)-**15** (10-isocyano-4-cadinene) and (±)-**22**. Others were made that bore isothiocyanates and formamides in the usual positions of the isonitriles; these functional groups have also been seen in natural products co-isolated with ICTs. Further analogues were made with nitriles and azides. None of the compounds devoid of isonitriles demonstrated appreciable antimalarial activity, See refs 10, 11, and 12.

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20. Please see the Supporting Information for details.

21. See, for example: Fusetani, N.; Yasumuro, K.; Kawai, H.; Natori, T.; Brinen, L.; Clardy, J. Kalihinene and Isokalihinol B, Cytotoxic Diterpene Isonitriles from the Marine Sponge *Acanthella klethra Tetrahedron Lett.* **1990**, *31*, 3599–3602.

22. The Wood group found that (±)-**15** had IC_{50} values of 2.2 μM against HB3 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) strains of *P. falciparum* (ref 11). Compound (±)-**22** had IC_{50} values of 80 nM against both HB3 and Dd2 strains (ref 10).

23. The Shenvi group recently noted that the potential pharmacophore corresponding to a conformationally constrained cyclohexane bearing a tertiary equatorial isonitrile (as shown in **1**) did not adequately account for antimalarial activities of compounds from their studies, including analogues related to **43**. See ref 7.

24. Assay performed under contract by Shanghai ChemPartner Co. Ltd.

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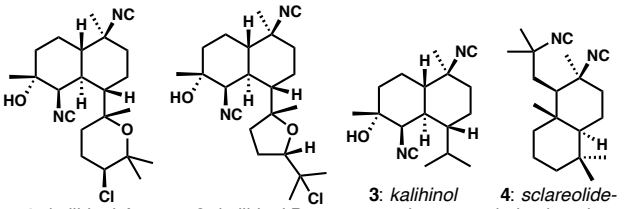
For Table of Contents Use Only:

Antimalarial Properties of Simplified Kalihinol Analogues

Mary Elisabeth Daub, Jacques Prudhomme, Choukri Ben Mamoun, Karine G. Le Roch, Christopher D. Vanderwal



1: *kalihinol A* **FCR-3: 1.2 nM***

2: *kalihinol B* **Dd2: 4.6 nM**

3: *kalihinol analogue* **Dd2: 16 nM**

4: *sclareolide-derived analogue* **Dd2: 1.6 nM**

*IC_{50} values against drug-resistant strains of *P. falciparum*