A Divergent Synthesis of Numerous Pyrroloiminoquinone Alkaloids Identifies Promising Antiprotozoal Agents

Griffin L. Barnes[1‡], Nicholas L. Magann[1‡], Daniele Perrotta[1], Fabian M. Hörmann[1], Sebastian Fernandez[1], Pratap Vydyam[3], Jae-Yeon Choi,[3] Jacques Prudhomme[4], Armund Neal[4], Karine G. Le Roch[4], Choukri Ben Mamoun[3], and Christopher D. Vanderwal*[1,2]

[1]Department of Chemistry, 1102 Natural Sciences II, University of California, Irvine, CA 92697-2025, USA.

[2]Department of Pharmaceutical Sciences, 856 Health Sciences Road, Suite 5400, University of California, Irvine, CA 92697, USA.

[3]Department of Internal Medicine, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT, USA.

[4]Department of Molecular, Cell and Systems Biology, University of California Riverside, CA, USA.

ABSTRACT: On the basis of a streamlined route to the pyrroloiminoquinone (PIQ) core, we made 16 natural products spread across four classes of biosynthetically related alkaloid natural products, and multiple structural analogues, all in ≤8 steps longest linear sequence (LLS). The strategy features a Larock indole synthesis as the key operation in a five-step synthesis of a key methoxy-PIQ intermediate. Critically, this compound was readily diverged via selective methylation of either (or both) of the imine-like or the pyrrole nitrogens, which then permitted further divergence by either *O*-demethylation to *o*-quinone natural products or displacement of the methoxy group with a range of amine nucleophiles. Based on a single, early report of their potential utility against the malaria parasite, we assayed these compounds against several strains of *Plasmodium falciparum*, as well as two species of the related protozoan parasite *Babesia*. In combination with evaluations of their human cytotoxicity, we identified several compounds with potent (low-nM IC_{50}) antimalarial and antibabesial activities that are much less toxic toward mammalian cells and therefore are promising lead compounds for antiprotozoal drug discovery.

Introduction

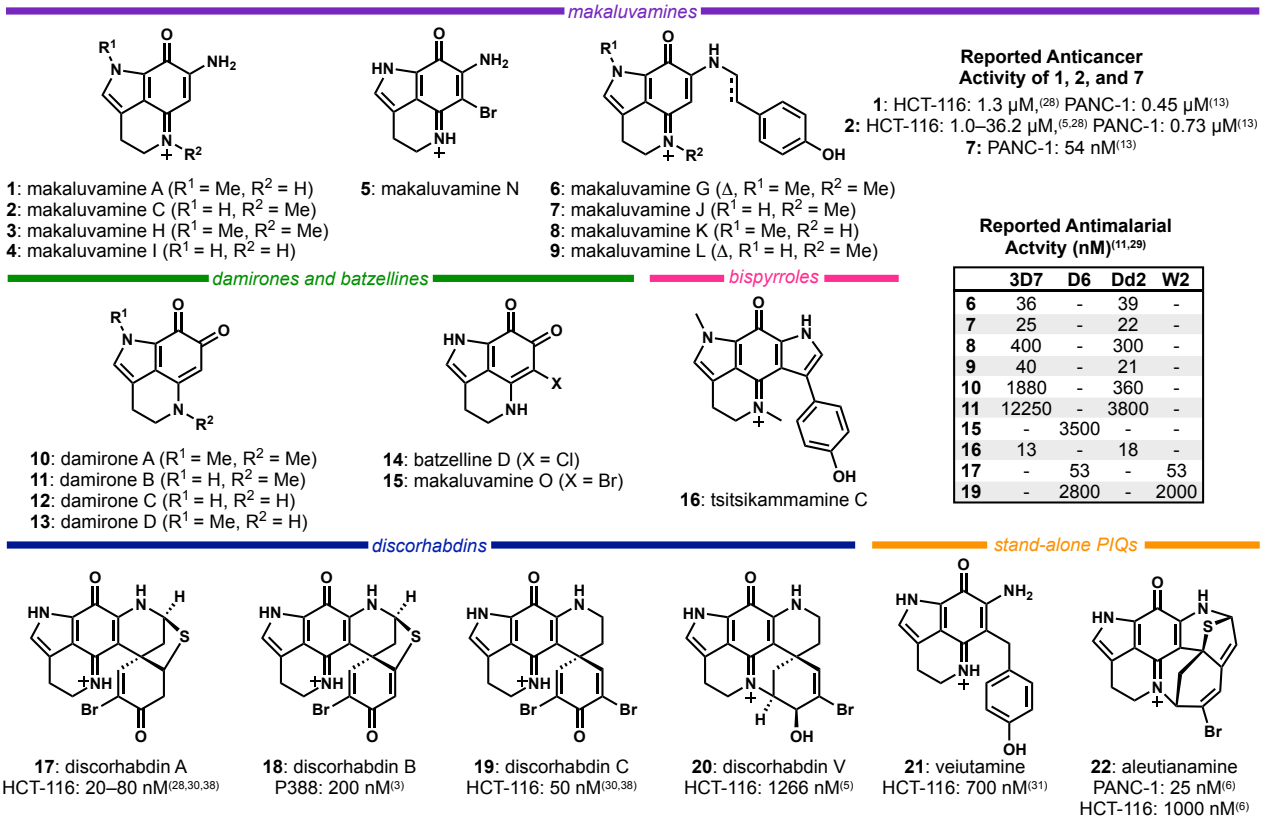
The diversity of alkaloids generated by the oxidation of tryptophan or tryptamine is astounding, and their biological activities show remarkable breadth. Among this rather loose collection of secondary metabolites, the pyrroloiminoquinone (PIQ) alkaloids (Figure 1) vary in complexity from the relatively simple makaluvamines A, C, H, I,[1] and N (**1**–**5**),[2] which are barely decorated variants of the basic PIQ ring system, to the polycyclic, polyfunctional discorhabdins A, B,[3] C,[4] and V,[5] (**17**–**20**) and aleutianamine (**22**)[6] (Figure 1).

Beyond the proposed biosynthetic construction of the core ring system by oxidation of tryptamine that gives rise to the damirones[7] and batzellines,[8,9] further complexity arises via incorporation of an ammonia equivalent to give the simple makaluvamines and by inclusion of arylethylamine groups on the PIQ scaffold to give alkaloids such as makaluvamine G (**6**)[10] and its congeners. Fused or spirocyclic PIQs such as tsitsikammamine C (**16**)[11] and discorhabdins, respectively then arise via oxidative C–C bond formations of electron-rich aromatics or benzylic-type C–H bonds. Clearly, nature makes opportunistic use of oxidizable functionality in the biosynthesis of this family, and doesn't stop there, as demonstrated by its most complex members. It is likely that chemists have only scratched the surface of the PIQ family's structural diversity.

The most prevalent biological activity reported for the PIQs is anticancer.[12] Some representative data are shown in Figure 1. For example, several of the makaluvamines demonstrate anticancer activity against colon (HCT-116) and pancreatic (PANC-1) cell lines, with makaluvamine J showing particularly potent PANC-1 activity (IC_{50}: 54 nM), with a reasonable selectivity index that was not found with many of its congeners.[13] Several of the discorhabdins are also quite potent anticancer agents. Natural products are at the mercy of the assays available to the isolation chemists, and once a family of natural products is associated with a particular type of activity, that can define future biological investigations of previously known or newly discovered members.

In a single publication by Quinn and co-workers, a small subset of PIQs isolated from a sponge of the genus *Zyzzya* was shown to have promising activity against both chloroquine-sensitive and multi-drug-resistant strains (3D7 and Dd2, respectively) of the human-malaria-causing parasite *Plasmodium falciparum*.[11] Makaluvamines G, J, and L (**6**, **7**, and **9**), as well as tsitsikammamine C (**16**) each demonstrated IC_{50} values below 50 nM against each strain. Importantly, these compounds were significantly less toxic toward human embryonic kidney cells (HEK-293); therapeutic indices (TI) with respect to this sample human cell line were thus ~50 or greater. Tsitsikammamine C (**16**) was both the most potent antimalarial and least cytotoxic toward HEK-293 cells, with an SI of ≥200. Despite this report from over a decade ago, it appears that there has been little follow-up on the antimalarial potential of the PIQs. Notably, a few related discorhabdins were potent inhibitors of D6 and W2 clones of *P. falciparum in vitro*; however, they proved toxic in *P. bergheii*-infected mice.[14] In general, tricyclic PIQs are less potent cytotoxins than the more complex discorhabdins.

Our group has a strong track record in the synthesis of antimalarial natural products and designed analogues, and collaborative investigations into their structure/activity relationships (SAR) and mechanisms of action.[15–18] Babesiosis is a related, tick-borne disease caused by protozoan parasites of the genus *Babesia*.[19] With its prevalence dramatically increasing worldwide, and with no dedicated therapeutic agents against it, babesiosis is often treated with antimalarial agents, with varying levels of success.[20–27] As such, there is considerable pressure to develop targeted antibabesial chemotherapeutics.[19]



	3D7	D6	Dd2	W2
6	36	-	39	-
7	25	-	22	-
8	400	-	300	-
9	40	-	21	-
10	1880	-	360	-
11	12250	-	3800	-
15	-	3500	-	-
16	13	-	18	-
17	-	53	-	53
19	-	2800	-	2000

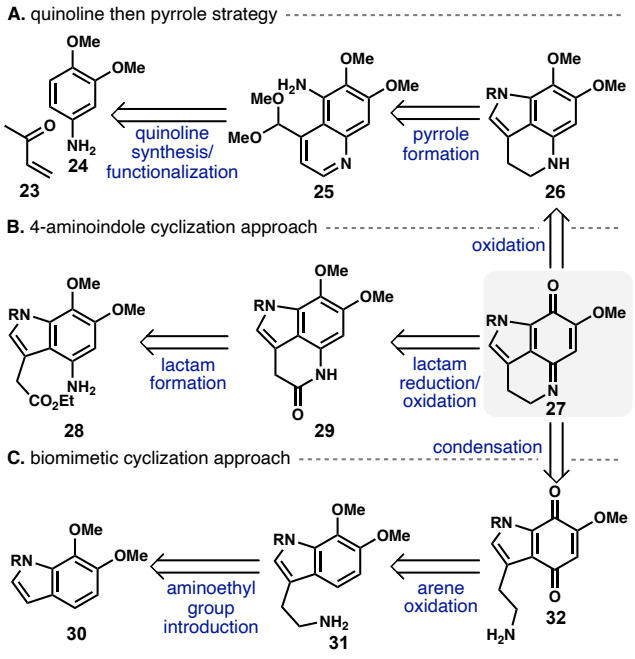
Figure 1. Representative examples highlighting structural diversity and biological activity within the PIQ class of alkaloids. All data given are IC_{50} values.

Here, we report how we have leveraged an optimized synthesis of the core heterocyclic scaffold of the PIQ alkaloids, along with a critical selective *N*-methylation strategy, to access numerous members of the class. We provide significant new data on the potent antimalarial and antibabesial activity of these promising compounds.

Background

The first synthesis of a marine alkaloid of the PIQ class was accomplished over 30 years ago by Yamamura and co-workers, who demonstrated the utility of an electrophilic methoxy iminoquinone of type **27** (Scheme 1).[32–36] This landmark achievement demonstrated the clean reactivity of **27** with primary amine nucleophiles, which is well-suited for convergent synthesis applications. This approach eventually enabled access to a handful of makaluvamines, batzellines and discorhabdins. Thus, many groups have targeted similar intermediates en route to PIQ targets. This is a rich area of heterocycle synthesis that has been frequently, but not comprehensively, reviewed.[12,13,37–42] In general, this electrophilic PIQ tricycle is formed via one of three overarching approaches (Scheme 1) **A.** pyrrole annulation onto a functionalized quinoline intermediate pioneered by Joule, inspiring a handful of similar approaches;[43–51] **B.** cyclization of a 4-aminoindole, as was first executed by Yamamura and later used by several other groups;[32,36,43,52,53] and **C.** a biomimetic strategy in which an oxidized tryptamine equivalent enables cyclization of a pendent amine onto an aromatic ring, followed by oxidation to the iminoquinone.[54–58]

Of these approaches, the biomimetic cyclization strategy has inspired the most innovation because of its value in greatly simplifying the construction of the PIQ core, prompting the application of state-of-the-art C–N bond-forming methodologies. Major advancements to this end include the use of aryne chemistry,[59–64] transition metal-catalyzed cross-coupling[65,66] and oxidative coupling reactions.[67–70,64] The latter two strategies were employed successfully in very recent total syntheses of PIQ alkaloids by the groups of Tokuyama, Wood, Stoltz and Burns.[65,71–73]



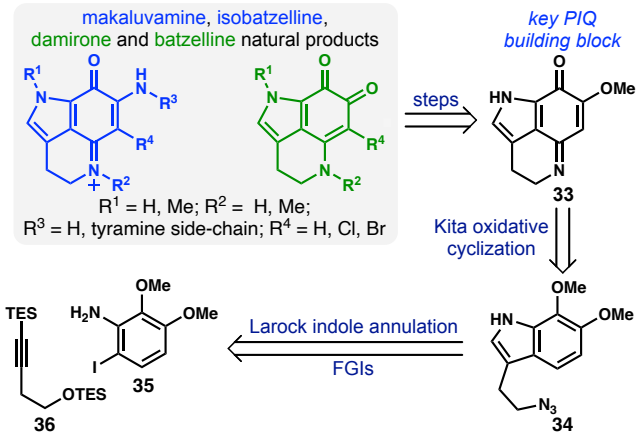
Scheme 1. Strategies for the Synthesis of Versatile PIQ Core Intermediates of Type 27

Despite the many advancements in PIQ total synthesis since Yamamura's first success, only a handful of groups elaborated their tricyclic intermediates to access a broad range of PIQ alkaloids. The lengthy syntheses required to elaborate commercially available materials to appropriately functionalized tryptamine precursors as a prelude to assembly of the PIQ core apparently limited their practical applications to PIQ alkaloid synthesis. And, while the Burns group made *N*-Ts damirone C via a short sequence of 6–7 steps, they did not advance this intermediate to the synthesis of any natural products; the *o*-quinone motif salient to the damirones has proven considerably less useful for amine condensation compared to the vinylogous imidate found in compounds like **27**.[70] *One of the key missing elements in an approach able to afford large numbers of PIQ alkaloids from a common core is the selective access to the four different N-methylation patterns found in these alkaloids.* Given the apparent impact of the methylation pattern on both antimalarial activity and cytotoxicity (see below), the ability to control methylation is critical to advancing our understanding of the biological potential of the PIQ alkaloids.

Results and Discussion

Synthesis of PIQ Alkaloids and Analogues

The strategy we developed to interrogate antiparasitic PIQ structural space is depicted in Scheme 2. Central to this work was our aim to access members of the structurally related makaluvamine, damirone, batzelline, isobatzelline and tsitsikammamine natural products to establish structure/activity relationships (SAR) among and within these natural product families. While a handful of other groups have used synthetic PIQs to interrogate SAR, these studies were confined to anticancer activity. [30,74–82]

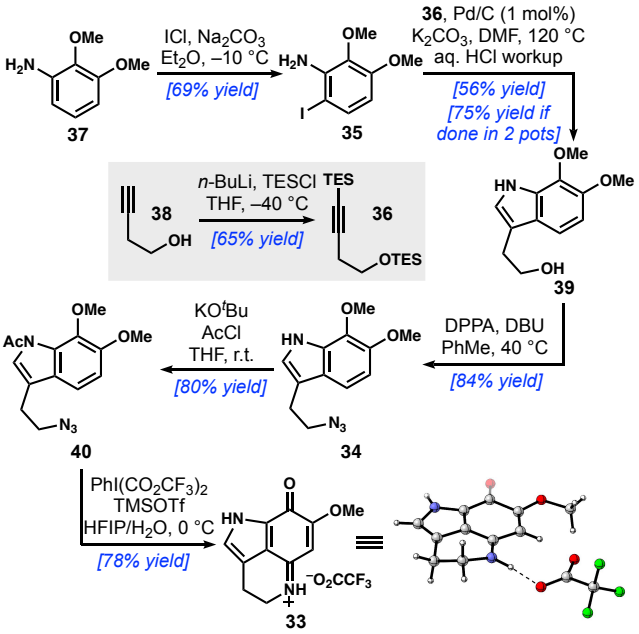


Scheme 2. A Plan to Prosecute Divergent Syntheses of PIQ Alkaloids

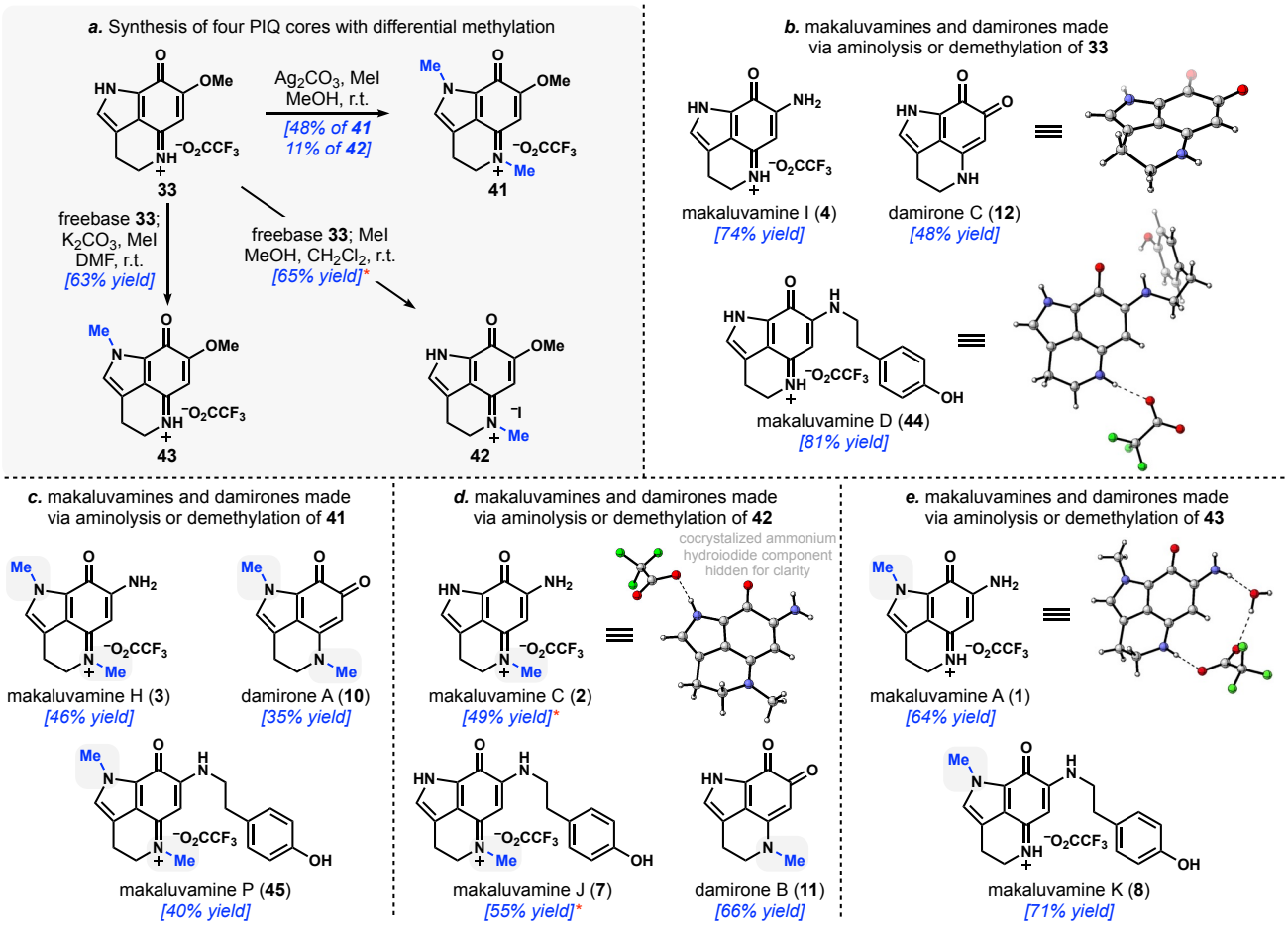
Despite the significant body of synthetic work focusing on PIQs that hints at the possibility of a divergent synthesis, no single approach has been able to efficiently link many related PIQ natural product families to a common synthetic intermediate and produce a large number of congeners.[35,66] This problem stems largely from the early installation of key functional groups, especially the *N*-methyl groups. These limitations are also likely related in many cases to long synthetic sequences and perceived instability of the PIQ core ring system.[54,58] Nonetheless, it was clear that developing a late-stage divergent synthesis would vastly improve our ability to access a library of PIQs with the goal of learning more about their antiprotozoal activity; thus, we embarked upon a concise scalable synthesis of the known electrophilic PIQ core **33**, from which we would design and implement maximally divergent chemistry.

We were attracted to the hypervalent-iodine-mediated oxidative cyclization chemistry developed by Kita and co-workers to forge the piperidine ring of **27**.[67,68] However, preparation of the methoxy indole precursor bearing the critical azide requires a lengthy synthesis (see Supporting Information for details) using existing technology.[30,83] We thus designed a concise synthesis of PIQ **33** (Scheme 2) that centered on a strategic Larock disconnection, which permits rapid access to substituted tryptophols or tryptamine derivatives, thus rapidly setting up for Kita's oxidative cyclization. At the outset of our work, the Larock disconnection had yet to be employed in the preparation of pyrroloiminoquinone alkaloids, despite its identification as an important strategy for the synthesis of tryptophan-based natural products.[84–86] Very recently, Wood and co-workers documented the utility of a Larock indole synthesis coupled with a Kita-type oxidative cyclization en route to makaluvamines A and K; however, the overall sequence to intermediates of type **27** was >10 steps, LLS, although the indole synthesis was shown to be quite scalable.[72] Additionally, the Stoltz group reported an attractive tandem Larock/Buchwald–Hartwig process to assemble a reduced variant of the PIQ core, which was then used to make five alkaloids.[66] As we show here, our approach compares favorably to these recent achievements, as we are able to procure 16 alkaloids via our highly optimized approach via sequences that are in most cases multiple steps shorter.

The synthesis of tryptophol **39** (Scheme 3) began with the construction of the iodoaniline building block **35** via a regioselective iodination (4:1 *rr*, 69% yield of **35**) with ICl under biphasic conditions.[87] Known alkyne building block **36** (prepared in one step)[88] underwent Larock annulation with **35** in the presence of K_2CO_3 and Pd/C (1 mol%) to generate indole **39** in 56% yield following acidic workup or in 75% yield over two steps by employing a discrete hydrolysis of the crude bis-silylated indole intermediate.[88] Regardless, the bis-silylated immediate annulation product was sensitive to partial desilylation during purification and we found it most convenient to



Scheme 3. Multigram Scale Synthesis of Building Block 33



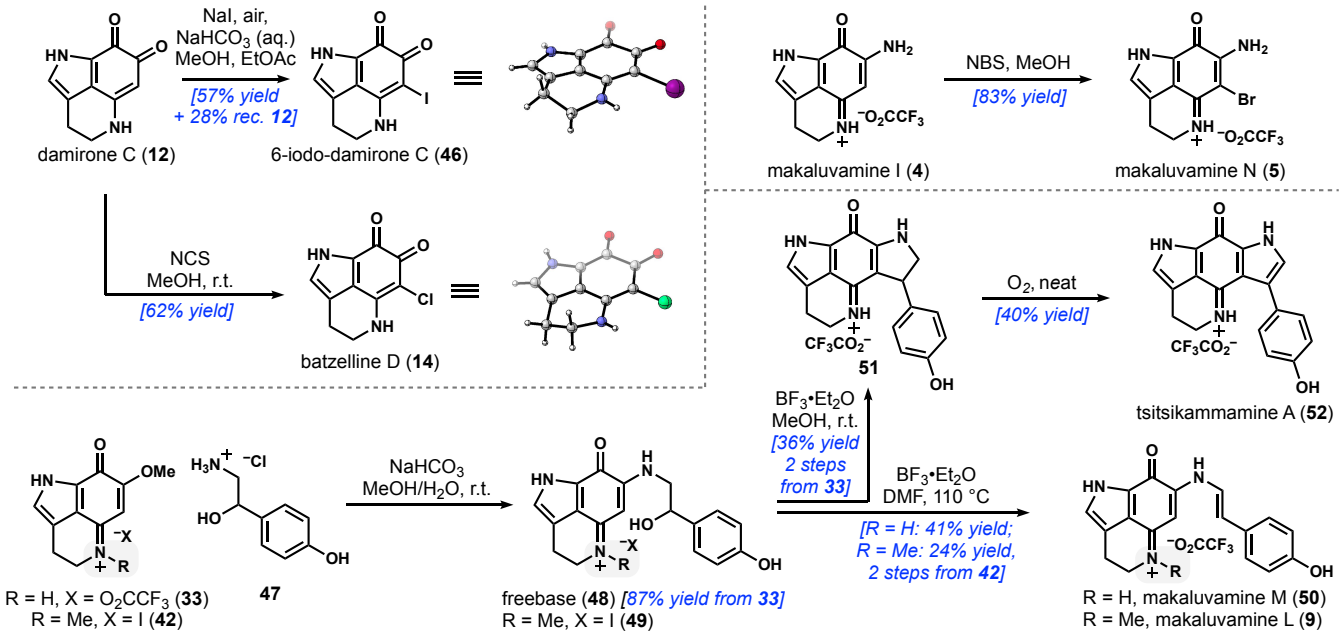
Scheme 4. Synthesis of 11 PIQ Alkaloids. (a) Selective methylation of PIQ core 33. (b–e) Conversion of each differentially methylated core to PIQ alkaloids. For details, see text and Supporting Information. *Yield calculated on the basis of purity determined by Q-NMR.

remove both TES groups prior to chromatographic purification. Deoxyazidation of known tryptophol **39**[89] using diphenylphosphoryl azide and DBU delivered azide **34** in high yield.[90] Acetylation of the indole nitrogen of **34** provided the requisite oxidative cyclization precursor **40**. The optimized yield for the PIFA-mediated oxidative cyclization of **40** reported by Kita and coworkers is 51%.[68] Because we were unwilling to concede such a loss of material, we further optimized this key transformation. While conducting a screen of reaction solvents commonly used for this type of transformation, we uncovered the importance of order of reagent addition. Specifically, we observed significantly improved reaction profiles and higher isolated yields when H_2O was added following the addition of the other reagents, thus increasing the yield for PIQ **33** to 78%, thereby easing purification and improving material throughput.

Using the optimized synthetic sequence (Scheme 3), we generated >11 g of the key PIQ intermediate **33** in a week of lab time, starting from aniline **37**. Importantly we observed that both the freebase and trifluoroacetic acid (TFA) salt of this compound are significantly more stable than previously reported.[54] We observed that while **33** and other PIQs (see below) are highly reactive in solution,[91] only minor degradation of a solid sample stored at –20 °C was detected over a period of greater than 6 months. The remarkable stability of the TFA salt of PIQ **33** is highlighted by the ability to generate crystals of sufficient quality for single crystal X-ray analysis through the slow evaporation of a MeOH solution on the benchtop.

With ample quantities of tricyclic electrophile **33** in hand, we aimed to develop efficient routes to multiple makaluvamine and damirone natural products that differ primarily by *N*-methylation pattern. Quinn and co-workers' study suggested that the *N*-methylation significantly affects the antimalarial activity PIQ alkaloids, with those bearing *N*-methylated iminium functions displaying the greatest potency.[11] To investigate the generality of this observation, we required a means to selectively methylate both the imine and the pyrrole. In almost all prior work methylation at each of these sites is carried out before the formation of the iminoquinone.[61,62,92] Instead, we looked to develop conditions for the direct methylation of **33** (Scheme 4), thus allowing for strategic diversification first to different *N*-methylated variants of **33** and from those intermediates to makaluvamines by amine condensation and damirones by *O*-demethylation.

Initial attempts at exhaustive methylation of the TFA salt of **33** using the best literature precedent (K_2CO_3 and MeI in DMF[61,62]) were met with non-specific decomposition. Fortunately, switching to Ag_2CO_3 and MeI reproducibly yielded the desired bis-methylated PIQ **41** (48%) along with the imine-methylated PIQ **42** (11%) after careful optimization. Switching to the freebase of **33** enabled selective methylation of the pyrrole and imine *N*-atoms. The higher nucleophilicity of the imine relative to the pyrrole allowed the imine methylated product **42** to be obtained selectively with MeI in CH_2Cl_2/MeOH. The *N*-methylated pyrrole **39** was accessed by revisiting the original K_2CO_2, MeI, DMF conditions with the freebase of **33**. Careful



Scheme 5. Synthesis of halogenated PIQs, tsitsikammamine A, makaluvamine M and makaluvamine L.

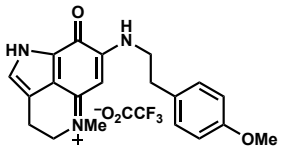
optimization of the conditions was required to ensure complete conversion while minimizing decomposition, ultimately affording pyrrole *N*-methylated product **43** in 61% yield. In this way, we gained access to four electrophilic, tricyclic PIQ cores with all possible methylation patterns of the two nitrogen atoms; these could be used in either *O*-demethylation reactions to deliver the *o*-quinone damirone and batzelline alkaloids, or by amine condensation to afford makaluvamines.

We accessed makaluvamine I (**4**) and D (**44**) from the TFA salt of **33** via aminolysis with NH_4Cl and tyramine as amine sources respectively.[35,49,63] The inclusion of sat. aq. $NaHCO_3$ ensured the complete conversion of the TFA salt of **33** to aminolysis products, especially when the reactants were also ammonium salts; inconsistent levels of conversion resulted when it was omitted. The synthesis of the *ortho*-quinone alkaloid damirone C (**12**) from **33** proceeded via NaI-mediated demethylation of the vinylogous imidate. This reaction, inspired by the conditions employed by Tokuyama in their synthesis of damirone B (**11**),[61] provided damirone C (**12**).[1] If aqueous workup procedures were used, a significant quantity of 6-iodo-damirone C (**46,** Scheme 5) was isolated in conjunction with **12**. Based on control experiments with electrophilic halogenating agents (see below), this reaction likely occurred via the air oxidation of iodide anion. Interestingly, this product was not observed if aqueous workup was omitted. The optimized conditions for demethylation provided damirone C in 48% yield. The chemistry described for the synthesis of makaluvamines I and D, and damirone C, proved general for the synthesis of their differentially methylated congeners as shown in Scheme 4c–e, with the exception of damirone A, which was generated under different conditions (see Supporting Information). This synthesis of eleven PIQ alkaloids served as proof-of-principle that divergent methylation of the PIQ core would be a powerful strategy in this area of natural product space.

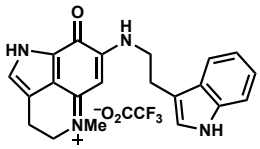
The halogenated congeners of the makaluvamines and damirones, known as the isobatzellines and batzellines respectively, have only scarcely been evaluated for antimalarial activity.[29] Though the electrophilic introduction of halogen atoms via the enaminone of the damirones is well-precedented,[64,69,73] the direct introduction of halogens onto the *para*-iminoquinone framework had not been accomplished. We document that both reactivities are efficient (Scheme 5), by showing chlorination of damirone C (**12**) to batzelline D (**14**) under modified literature conditions,[69] and the conversion of makaluvamine I (**4**) to makaluvamine N (**47**) upon treatment with NBS in methanol.

We made three final PIQ natural products. Condensation of **33** with aminoalcohol **47** led to **48**, which could be directly dehydrated with $BF_3{\cdot}OEt_2$ in protic solvent to give the conjugated side chain of makaluvamine M (**50**). Interestingly, treatment of this same intermediate with the same Lewis acid in polar aprotic solvent at elevated temperatures effected cyclization to pyrroline (**51**), which was easily oxidized to give the fused tetracyclic core of the tsitsikammamines (in this case tsitsikammamine A, **52**). At this point, we had only made these two compounds from non-methylated electrophilic PIQ core **33**; of course, our access to the differentially methylated cores provides a blueprint to make the congeners of makaluvamine M and tsitsikammamine A with different methylation patterns. As a first step to corroborate this assertion we synthesized makaluvamine L (**9**) from imine-methylated PIQ **42** via the same two-step sequence applied to access makaluvamine M (**50**).

Overall, our doubly divergent approach led to the first syntheses of makaluvamines H, L, and M. With respect to alkaloids that had been previously made, we accomplished the shortest syntheses of batzelline D; damirones A, B, and C; makaluvamines I, J, K, N, and P; as well as tsitsikammamine A. Further, with the advent of our divergent methylation protocol that permits ready access to all four electrophilic vinylogous imidate PIQ cores, we have a simple blueprint to easily make many analogues by amination. For example, on the basis of the strong activity (see below) of the imine *N*-methylated makaluvamines bearing phenethylamine-type side chains (makaluvamines J and P), we aminated **42** with *O*-methoxytyramine and tryptamine, arriving at compounds **53** and **54**, the latter of which was very recently reported as a potent inhibitor of PANC-1 cells.[92] These compounds, as described below, are particularly potent antiprotozoal agents.



PIQ-1 (**53**)



PIQ-2 (**54**)

Figure 2. Two unnatural analogues made by amination of 42

Critical Application of Q-NMR for Many Intermediates

Despite the vast literature surrounding PIQ syntheses, few groups have interrogated the purity of their PIQ intermediates or final products beyond NMR spectroscopic homogeneity. In their syntheses of discorhabdin natural products, Heathcock and coworkers claimed that "inorganic salts often seemed to contaminate the product, so extra care was necessary to ensure that the product was pure".[58] However, no further description was given as to the nature of these impurities. Additionally, hygroscopicity of charged PIQs is precedented. In the report of its isolation, discorhabdin C was characterized by X-ray diffraction as the HCl salt, which was hydrated by no less than six water molecules per unit cell.[4] In our studies, we found that some positively charged PIQ intermediates had unusually high affinity for water and organic solvents. In most cases, bulk water could be removed by interconversion to the corresponding neutral, freebase form, followed by routine desiccation methods. However, when the freebase PIQ was inaccessible (such as the N-Me iminium ions), or when hydroscopic PIQs were obtained, our best efforts were made to assign their purity using quantitative NMR spectroscopy in the presence of an internal standard. Additionally, we seized every opportunity to characterize our products by X-ray diffraction to better understand their solid-state composition. In doing so, we hoped to bolster the validity of our isolated yields as well as the biological assays discussed below.

Biological Assessment of 25 PIQ Alkaloids and Analogues

Our goal for the biology of these PIQ alkaloids was to delve more deeply into the antiprotozoal activity of these compounds, to recapitulate the potent antimalarial activity and high therapeutic index reported by Quinn for makaluvamine J (**7**) and related compounds, and to gain a better sense of SAR and selectivity with respect to toxicity to a broader range of pathogens and human cell lines. For this purpose, we assayed 25 compounds—a combination of 12 natural products and 13 synthetic analogues—for antimalarial activity (*Plasmodium falciparum* drug-resistant 3D7, Dd2, and W2 strains), antibabesial activity (*Babesia duncani, and B. divergens* Rouen strains), several strains of three different species of fungus, and four human cell lines. A summary of the data is presented in Figure 3 with heat mapping/color-coding; IC_{50} values for the antimalarial and antibabesial activity are shown along with the therapeutic index (TI) calculated against the average cytotoxicity of the compounds against the four human cell lines (complete data sets can be found in the Supporting Information).

The two representative methoxy-PIQ tricycles **33** and **41** are micromolar antiprotozoals, with moderate TIs. *o*-Quinones damirone C, batzelline D, and analogue PIQ-5 are poorly active antiprotozoals and show virtually no TI. In the cases of the PIQs derived from ammonolysis of the methoxy core, sub-micromolar activities are observed for makaluvamines I and C, but only the latter shows any modest TI. Here, we can begin to observe the importance of methylation patterns on SAR: pyrrole *N*-methylation trends with poor activity and imine *N*-methylation seems to bolster activity and attenuate toxicity against mammalian cell lines.

In those PIQs with tyramine-derived side chains, there is a general increase in potency with respect to the simpler compounds. The standouts in all cases are those with the imine *N*-methylated, as in makaluvamines J, P, PIQ-1, PIQ-2, and makaluvamine L. Again, pyrrole *N*-methylation (compare makaluvamines J and P) slightly decreases activity and slightly increases cytotoxicity for an overall decrease in TI. Pyrrole bromination as in PIQ-12 abrogates activity compared to the parent makaluvamine D, as does tyramine phenol bromination (see PIQ-11 and PIQ-13). *O*-Methylation (PIQ-1), however, increases activity, with a concomitant increase in cytotoxicity; however, this compound shows 3.5 nM activity against *B. divergens* Rouen and maintains a promising TI. The other compound that is noteworthy is PIQ-2, bearing a tryptamine motif; it shows potencies ≤31 nM in all assays (9 nM against *P. falciparum* 3D7 and <2 nM against *B. divergens* Rouen). Tsitsikammamine A, the only PIQ with a tetracyclic core that we tested, was significantly less potent than the analogous uncyclized compound makaluvamine D.

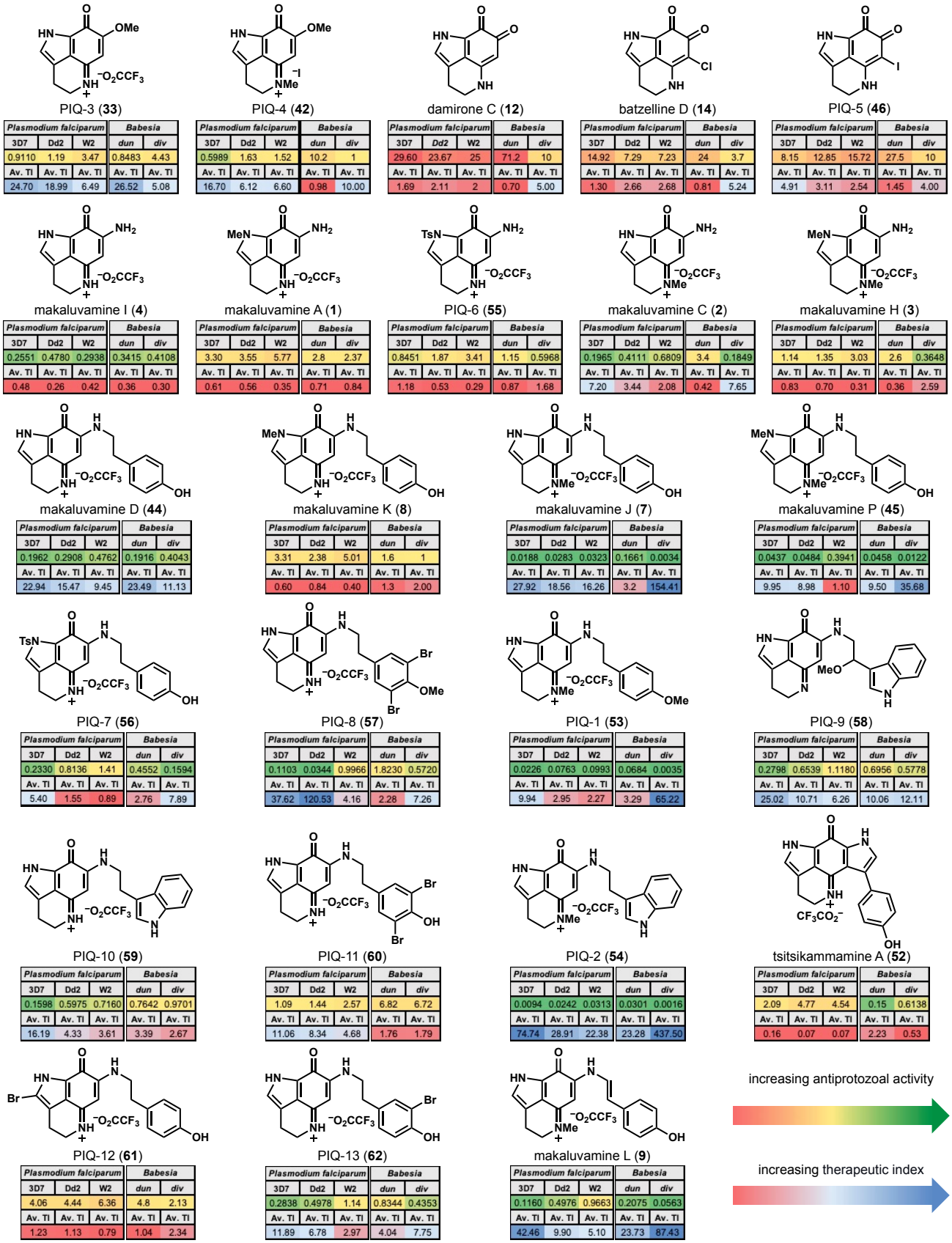
It is important to recognize that TI values naturally increase as the antiprotozoal activity decreases; the raw data in the Supporting Information will indicate, for example, that makaluvamine K, which is roughly an order of magnitude less active than makaluvamine D, is not that much more cytotoxic.

The combination of an *N*-methylated imine core with a lipophilic side chain in many cases leads to potent antiprotozoal activity. In some cases, the increase in potency is accompanied by an increase in cytotoxicity; however, we find that the whole class of compounds are very poorly active in antifungal models (little activity up to 100 μM concentrations, see the Supporting Information), indicating that they are not just broadly toxic. Clearly, there are levers that can be pulled to try to maximize antiprotozoal potency while minimizing mammalian cell toxicity; these will include the identity of the greasy side chain and the choices of groups with which to alkylate the imine nitrogen atom.

As mentioned above, significant emphasis has previously been placed on the discorhabdin family of PIQs as a result of their potent cytotoxicity. Most—but not all—of the simpler PIQs are significantly less cytotoxic than the discorhabdins, and many have other potentially important activities, are far easier to access, and appear to have promising selectivity. It is still worthy of note that some of the simpler alkaloids—such as makaluvamine J (54 nM PANC-1[1,13,91]) do have potentially valuable anticancer activities. Clearly, an understanding of the mechanisms of action for both their antiprotozoal activity and human cytotoxicity will be of great value in determining the future potential of this class of compounds.

Conclusions

We have developed an efficient synthesis of the PIQ alkaloids that has permitted extensive biological evaluation of numerous natural products and structural analogues. The effectiveness of this route hinged on a scalable Larock indole synthesis that enabled access to >10 g batches of the known, key electrophilic PIQ core structure **33**, which was then diverged in two stages. A critical and enabling advance is found in the selective *N*-methylation protocols that delivered four different electrophiles that could then be further diverged either via *O*-demethylation to afford damirones or substitution with amines to yield



Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.9110	1.19	3.47	0.8483	4.43
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
24.70	18.99	6.49	26.52	5.08

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.5989	1.63	1.52	10.2	1
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
16.70	6.12	6.60	0.98	10.00

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
29.60	23.67	25	71.2	10
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
1.69	2.11	2	0.70	5.00

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
14.92	7.29	7.23	24	3.7
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
1.30	2.66	2.68	0.81	5.24

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
8.15	12.85	15.72	27.5	10
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
4.91	3.11	2.54	1.45	4.00

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.2551	0.4780	0.2938	0.3415	0.4108
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
0.48	0.26	0.42	0.36	0.30

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
3.30	3.55	5.77	2.8	2.37
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
0.61	0.56	0.35	0.71	0.84

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.8451	1.87	3.41	1.15	0.5968
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
1.18	0.53	0.29	0.87	1.68

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.1965	0.4111	0.6809	3.4	0.1849
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
7.20	3.44	2.08	0.42	7.65

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
1.14	1.35	3.03	2.6	0.3648
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
0.83	0.70	0.31	0.36	2.59

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.1962	0.2908	0.4762	0.1916	0.4043
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
22.94	15.47	9.45	23.49	11.13

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
3.31	2.38	5.01	1.6	1
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
0.60	0.84	0.40	1.3	2.00

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.0188	0.0283	0.0323	0.1661	0.0034
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
27.92	18.56	16.26	3.2	154.41

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.0437	0.0484	0.3941	0.0458	0.0122
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
9.95	8.98	1.10	9.50	35.68

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.2330	0.8136	1.41	0.4552	0.1594
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
5.40	1.55	0.89	2.76	7.89

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.1103	0.0344	0.9966	1.8230	0.5720
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
37.62	120.53	4.16	2.28	7.26

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.0226	0.0763	0.0993	0.0684	0.0035
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
9.94	2.95	2.27	3.29	65.22

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.2798	0.6539	1.1180	0.6956	0.5778
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
25.02	10.71	6.26	10.06	12.11

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.1598	0.5975	0.7160	0.7642	0.9701
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
16.19	4.33	3.61	3.39	2.67

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
1.09	1.44	2.57	6.82	6.72
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
11.06	8.34	4.68	1.76	1.79

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.0094	0.0242	0.0313	0.0301	0.0016
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
74.74	28.91	22.38	23.28	437.50

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
2.09	4.77	4.54	0.15	0.6138
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
0.16	0.07	0.07	2.23	0.53

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
4.06	4.44	6.36	4.8	2.13
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
1.23	1.13	0.79	1.04	2.34

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.2838	0.4978	1.14	0.8344	0.4353
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
11.89	6.78	2.97	4.04	7.75

Plasmodium falciparum			Babesia	
3D7	Dd2	W2	*dun*	*div*
0.1160	0.4976	0.9663	0.2075	0.0563
Av. TI	Av. TI	Av. TI	Av. TI	Av. TI
42.46	9.90	5.10	23.73	87.43

Figure 3. Biological data for 25 PIQs assayed against malaria- and babesiosis-causing protozoan parasites, as well as their therapeutic indices. 3D7, Dd2, and W2 are representative drug resistant strains of *Plasmodium falciparum*; dun represents *Babesia duncani*, div represents *Babesia divergens* Rouen strain, and Av. TI is the therapeutic index arrived by comparing the activity against the protozoan parasites with the average value of the cytotoxicities against the human cell lines HCT-116, HEK, HeLa, and HepG2.

makaluvamines. Several other PIQs were made from key intermediate **33**, leading to a total of 16 natural alkaloids; multiple unnatural analogues were also obtained. Three of the natural products were synthesized for the first time, and 11 of the remaining 13 were made via the shortest sequence to date, despite the significant quantity of prior art in this area.[93] Notably, our syntheses of tsitsikammamine A (**51**) (8 steps LLS) and makaluvamine N (**47**) (7 steps LLS) access these natural products in just over half the number of steps of previously reported approaches (15 and 12 steps LLS respectively).[66,78]

Inspired by a seminal report on their potential as antimalarial agents, we evaluated these compounds against drug-resistant strains of *Plasmodium falciparum*, the related protozoan parasites *Babesia duncani* and *Babesia divergens*,[94,95] several species and strains of yeast, and four human cell lines. These efforts revealed, in particular, that imine *N*-methylated makaluvamines and analogues are extremely potent antiprotozoal agents. We recapitulated the earlier results of Quinn by confirming that these compounds are indeed potent (sub-50 nM IC_{50}) antimalarials, but we also showed for the first time that they are even more potent (IC_{50} = 2–4 nM for **53** and **54**) against *B. divergens* Rouen, which causes babesiosis, a tick-borne illness of ever-increasing concern that lacks effective front-line therapeutics.[94] We also learned that these potent PIQ antiprotozoal agents are not simply broad toxins, as they show little antifungal activity and many have significant therapeutic indices with respect to human cell lines.

This work represents only the beginning of our SAR on PIQ alkaloids with respect to antiprotozoal activity. On the basis of the results described below, we aim to evaluate different pyrrole and imine *N*-capping groups on the makaluvamine scaffold, and we will interrogate the impact of the largely hydrophobic substituents on the N9 position.[92] The refined SAR obtained from these studies will set the stage for the design and application of natural-product-derived chemical probe compounds for detailed mechanism of action studies, following collaborative workflows that we have recently used successfully in the area of malaria chemotherapeutics.[96]

AUTHOR INFORMATION

Corresponding Author

*cdv@uci.edu

Author Contributions

‡These authors contributed equally.

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SUPPORTING INFORMATION

Supplemental discussion of reactivity studies, experimental procedures for the synthesis of new compounds, tabulated spectral data supporting the structural assignment of these compounds, NMR spectra for these compounds, X-ray diffraction information for compounds **2**, **12**, **13**, **14**, **33**, and **46** and biological assay descriptions and results. Also, a summary of previous synthetic efforts in this area.

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Graphical Abstract