Design of Anti-infectious Agents from Lawsone in a Three-Component Reaction with Aldehydes and Isocyanides

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ACCESS

ABSTRACT: The first effective synthetic approach to naphthofuroquinones via a reaction involving lawsone, various aldehydes, and three isocyanides under microwave irradiation afforded derivatives in moderate to good yields. In addition, for less-reactive aldehydes, two naphtho-enaminodione quinones were obtained for the first time, as result of condensation between lawsone and isocyanides. X-ray structure determination for 9 and 2D-NMR spectra of 28 confirmed the obtained structures. All compounds were evaluated for their anti-infectious activities against Plasmodium falciparum, Leishmania donovani, and Mycobacterium tuberculosis. Among the naphthoquinone series, 17 exhibited comparatively the best activity against P. falciparum (IC_{50} = 2.5 μM) and M. tuberculosis (MIC = 9 μM) with better (P. falciparum) or equivalent (M. tuberculosis) values to already-known naphthoquinone compounds. Among the two naphtho-enaminodione quinones, 28 exhibited a moderate activity against P. falciparum (IC_{50} = 9 μM) and M. tuberculosis (MIC = 3.5 μM and SI > 28), rendering it very competitive to the reference drug miltefosine. All compounds were studied through molecular modeling on their potential targets for P. falciparum, Pfbc1, and PfDHODH, where 17 showed the most favorable interactions.

INTRODUCTION

Known as dyes in the chemical industry, quinones are also an important family of major impact as biologically active compounds.

The quinone fragment is present in many natural products with important biological roles in humans, animals, and plants, rendering it one of the most attractive structures in medicinal chemistry. In this respect, various non-natural molecules bearing the quinone scaffold have been prepared and evaluated for anticancer, antifungal, antimalarial, and other biological activities.

Among various classes of quinones, naphthoquinones and especially naphthoquinone derivatives have attracted increasing interest for their pharmacological activities. Naphthoquinone derivatives can be obtained from natural sources but also via synthetic approaches, giving rise to compounds with various structures and biological properties, for example, cytotoxicity against tumor cells, inhibition of tyrosine kinases receptors, and antimalarial or antidiabetic activity.

Due to their pharmacological activities, many synthetic efforts have been dedicated to their preparation. Two main synthetic routes have been adopted: (i) reaction of 2,3-dichloro-1,4-naphthoquinone with 1,3-dicarbonyl reagents and (ii) strategies based on the [3 + 2] annulation of 2-hydroxy-1,4-naphthoquinone with various reagents. We can also refer to two other synthetic approaches using either 3-iodolawsone or thio-substituted 1,4-naphthoquinone.

Lawsone seems to be an important fragment while searching for antimalarial agents. Atovaquone (7) (Figure 2) is reported to be the leading drug interacting with the cytochrome bc1 complex, a specific mitochondrial target of P. falciparum. Cytochrome bc1 and dihydroorotate dehydrogenase (DHODH) are the two main biological targets of Plasmodium mitochondrion, and they are also biochemically related. Atovaquone acts as a competitive inhibitor at the Q_{b} site of the cytochrome bc1 complex. Unfortunately, strong P. falciparum resistance against atovaquone arises rapidly in the field.
In this respect, efforts to design and prepare naphthoquinones and naphthofuroquinones based on lawsone scaffold have been pursued worldwide.\textsuperscript{26} Borgati et al.\textsuperscript{12} reported the synthesis of a series of naphthoquinones and naphthofuroquinones through functionalization of lawsone either on the 2-hydroxy group affording alkoxy derivatives or on the C-3 alkene carbon atom, followed by cyclization, thus leading to naphthofuroquinones. Among all the synthesized compounds, naphthofuroquinone 6 exhibited the lowest IC\textsubscript{50} value against the chloroquine-resistant \textit{P. falciparum} W2 strain (Figure 2). Based on docking studies, the authors proposed that compounds of this series, especially 6, could act as inhibitors of either cytochrome \textit{bc1} or DHODH.

Oramas-Royo et al.\textsuperscript{27} reported the synthesis and antiplasmodial activities of a series of 1,2,3-triazole-naphthoquinone conjugates. Compound 8 (Figure 2) exhibited the second lowest IC\textsubscript{50} value against the chloroquine-resistant \textit{P. falciparum} W2 strain, while molecular docking on the potential target PfDHODH showed very favorable interactions.

In this context, we report our efforts in constructing naphtho(furo)quinone derivatives obtained via a three (or two)-component reaction of lawsone with aldehydes and isocyanides. First, their syntheses will be described. Then, their biological activities will be reported against three important pathogens, \textit{P. falciparum}, \textit{L. donovani}, and \textit{M. tuberculosis}, for which the problem of multiresistance to the current therapy is pressing. In addition, we report docking studies of several drugs on cytochrome \textit{bc1} and DHODH molecular targets.

## RESULTS AND DISCUSSION

### Chemistry

Multicomponent reactions are an important synthetic tool in medicinal chemistry.\textsuperscript{28} They are one-pot reactions employing more than two starting compounds, where the majority of atoms are incorporated into the final compound.\textsuperscript{29} Nowadays, many basic multicomponent reactions are named ones (Ugi, Passerini, Strecker, Biginelli, etc.), while many others have been developed for the synthesis of heterocyclic compounds such as 2-styryl quinolines, quinazolines,\textsuperscript{30,31} and imidazoles.\textsuperscript{32} Concerning the naphthofuran system, synthesis of 2-amino-naphtho[2,3-\textit{b}]furan-4,9-diones has been reported by Teimouri et al.\textsuperscript{33} as one-pot three-component condensation among lawsone (1), an aldehyde, and an alkyl-isocyanide under reflux in toluene. Later on, Jiménez-Alonso et al.\textsuperscript{34} reported a methodology using ethylenediamine-diacetic acid (EDDA) as a catalyst, thus reducing the reflux time in toluene.

The mechanistic aspect of the synthesis of these adducts is considered as follows: first, a Knoevenagel condensation between lawsone (1) and an aldehyde leads to the formation of a conjugated enone; this step is followed by a [4 + 1] cycloaddition reaction of the heterodiene moiety of the Knoevenagel adduct with an isocyanide. This reaction affords
Scheme 1. Possible Mechanism of the Multicomponent Domino Reaction of Lawsone

Scheme 2. Model Domino Reaction Used for the Optimization of Reaction Conditions

Table 1. Reaction Conditions and Yields for the Model Domino Reaction

| methodology   | reaction time | equivalents (lawsone/aldehyde: isocyanide) | catalyst | solvent | lawsone conversion<sup>a</sup> | isolated yield<sup>b</sup> |
|---------------|---------------|---------------------------------------------|----------|---------|-------------------------------|---------------------------|
| reflux        | 120 min       | 1:1.2:1.2                                  | EDDA     | PhMe    | 90%                          | 30%                       |
| irradiation (μw) | 2 × 15 min    | 1:1:1                                       | Et₃N     | DCE     | 12%                          | 11%                       |
| irradiation (μw) | 60 min        | 1:1:1                                       | EDDA     | DCE     | 60%                          | 59%                       |
| irradiation (μw) | 2 × 60 min    | 1:1.5:1                                    | EDDA     | DCE     | 100%                         | 66%                       |
| thermal activation | 60 min       | 1:1.5:1                                    | EDDA     | DCE     | 100%                         | 59%                       |
| thermal activation | 60 min       | 1:1:1                                       | Et₃N     | DCE     | 70%                          | 57%                       |

<sup>a</sup>Calculated based on the <sup>1</sup>H NMR spectrum of the crude reaction mixture at the end of the reaction. <sup>b</sup>Calculated based on the starting amount of lawsone. μw stands for microwave.

The reaction was first conducted in the presence of solvents (toluene and 1,2-dichloroethane DCE) and catalysts (Et₃N and EDDA), favoring the Knoevenagel condensation. All reactions were conducted under reflux for 2 h and monitored by thin-layer chromatography (TLC) every 30 min. Our best result under these conventional conditions was obtained when using EDDA in toluene: the obtained yield of the final compound 9 was 30%. A monowave 50 thermic reactor was also used. The reaction in DCE in the presence of 10% of EDDA (1 h of heating at 182 °C, 15 bar) provided the target naphthofuroquinone derivative 9 in 59% yield after PuriFlash purification.

Then, we performed the reaction under microwave irradiation for 1 h (160 °C, 6 bars, 300 W). When operating in the presence of 1:1:1 mole equiv of reactants, after work up and purification, we obtained the deep violet-colored naphthofuroquinone derivative 9 in 59% yield. Some traces of a yellow compound an intermediate iminolactone that leads to the final naphthofuroquinone derivative after rearrangement (Scheme 1).

To optimize the reaction conditions (catalyst, solvent, reaction time, and activation method), the reaction between lawsone (1), 4-chlorobenzaldehyde, and tert-butyl isocyanide (Scheme 2 and Table 1) was first used as a model reaction. In fact, embelin has been recently reported<sup>35</sup> to undergo this three-component reaction under microwave irradiation. However, until now, there was no equivalent study for lawsone (1). Microwave-assisted organic synthesis has attracted great attention in the last decades and has proven to be particularly useful in the construction of important heterocyclic systems<sup>36</sup> such as benzothiazoles<sup>37</sup> and benzoxazoles. Microwave-assisted multicomponent reactions are also part of this progress, having been emerged as useful tools for the elaboration of relevant heterocycles.<sup>38</sup>
were also isolated, without further identification until now. By increasing the ratio of aldehyde to 1.5 equiv, the yield of 9 was slightly increased up to 66%. The use of more equivalents of aldehyde did not lead to better results. Conversely, the purification and isolation of the naphthofuroquinone became problematic in the presence of excess of aldehyde since the aldehyde was eluted in very near proximity to the desired naphthofuroquinone.

The microwave irradiation using 1:1:1 equiv of reactants and 10% of EDDA as a catalyst was therefore considered as the

### Table 2. Structures and Isolated Yields of Products

| Entry | Product | R₁ | R₂ | Aldehyde conversion | Isolated Yield |
|-------|---------|----|----|---------------------|----------------|
| 1     | 9       |    |    | 70%                 | 59%            |
| 2     | 10      |    |    | 30%                 | 30%            |
| 3     | 11      |    |    | 60%                 | 55%            |
| 4     | 12      |    |    | 45%                 | 25%            |
| 5     | 13      |    |    | 30%                 | 30%            |
| 6     | 14      |    |    | 80%                 | 35%            |
| 7     | 15      |    |    | 20%                 | 19%            |
| 8     | 16      |    |    | 95%                 | 52%            |
| 9     | 17      |    |    | 91%                 | 53%            |
| 10    | 18      |    |    | 50%                 | 46%            |
| 11    | 19      |    |    | 45%                 | 42%            |
| 12    | 20      |    |    | 40%                 | 40%            |
| 13    | 21      |    |    | 54%                 | 12%            |
| 14    | 22      |    |    | 60%                 | 45%            |
| 15    | 23      |    |    | 55%                 | 55%            |
| 16    | 24      |    |    | 45%                 | 40%            |
| 17    | 25      |    |    | 30%                 | nd             |
| 18    | 26      |    |    | 10%                 | 8%             |
| 19    | 27      |    |    | 35%                 | 30%            |
| 20    | 28      |    |    | -                   | 40%            |
| 21    | 29      |    |    | -                   | 50%            |

*Calculated based on the ¹H NMR spectrum of the crude reaction mixture at the end of the reaction. Calculated based on the starting amount of lawsone.*

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optimal conditions. Then, we performed this reaction with a series of aldehydes and three different isocyanides, namely, tert-butyl, n-butyl, and cyclohexyl isocyanides. Table 2 summarizes the obtained results.

When using p-chlorobenzaldehyde (entry 1), the domino adduct 9 was obtained in 59% yield with respect to the starting amount of aldehyde (the conversion of aldehyde was 70%). A similar result was evidenced when using 3,4,5-trimethoxybenzaldehyde (entry 2): the domino reaction afforded adduct 10 in 30% yield. Despite the fact that the aldehyde conversion was rather poor (30%), we managed to isolate a second product from the reaction mixture. It was identified as compound 28 (yield 20%), where the tert-butyl isocyanide has reacted directly with lawsone (entry 20).

Noteworthily, this compound is a mixture of cis- and trans- isomers in a 1:1 molar ratio. This point will be further discussed below. Concerning the reaction with 3,4-dimethoxybenzaldehyde (entry 2) gave a more complex mixture, leading after purification to adduct 10 in 30% yield. Despite the fact that the aldehyde conversion was rather poor (30%), we managed to isolate a second product from the reaction mixture. It was identified as compound 28 (yield 20%), where the tert-butyl isocyanide has reacted directly with lawsone (entry 20). Noteworthily, this compound is a mixture of cis- and trans-isomers in a 1:1 molar ratio. This point will be further discussed below. Concerning the reaction with 3,4-dimethoxybenzaldehyde, the aldehyde conversion was only 45% and a complex mixture was obtained in which compound 28 (entry 20) was not detected. Purification led to compound 12 in 25% yield (entry 4).

Two heteroaromatic bicyclic aldehydes were then chosen to carry out the domino reaction. The reaction of N-methyl indazole aldehyde in the presence of lawsone and tert-butyl isocyanide (ratio 1:1:1) in the presence of EDDA (10%) under microwave irradiation for 2 h provided the domino compound 13 with 30% yield after purification (the aldehyde conversion was 30%), indicating a high selectivity for this domino reaction (entry 5). In addition, the same adduct 28 (cis- and trans-isomers) as above was isolated in 40% yield. On the other hand, reaction of indole aldehyde (entry 6) afforded a complex mixture. A poor yield of the domino adduct 14 (35%) with an 80% aldehyde conversion was obtained, while compound 28 (entry 20) was not detected. Finally, starting from 5-nitrofuraldehyde, the domino compound 15 was obtained in poor yield (19%) with 20% aldehyde conversion (entry 7).

Piperidine-4-carbaldehydes possessing either a Boc or a benzyl protecting group on the nitrogen atom provided very similar results (entries 8 and 9). In both cases, the corresponding naphtho-furoquinones 16 and 17 were obtained in 52 and 53% yield, respectively, after purification. Compound 28 (entry 20) was not detected. The conversion of the aldehyde was higher than 90%.

The three-component reaction was also studied with commercially available cyclohexyl and n-butyl isocyanides. All reactions were conducted with five aldehydes representative of those studied above: N-benzylpiperidine carbaldehyde, p-chlorobenzaldehyde, 3,4,5-trimethoxybenzaldehyde, N-methyl-indazole carbaldehyde, and 5-nitrofuraldehyde.

In the reactions of cyclohexyl isocyanide (entries 10–14) and using p-chlorobenzaldehyde, 3,4,5-trimethoxybenzaldehyde, N-methyl-indazole, or N-benzyl piperidine carbaldehyde, the corresponding naphtho-furoquinones 18–21 were obtained in 46, 42, 40, and 42% yields, respectively (entries 10–12 and 14).

Finally, the reactions of cyclohexyl isocyanide with 5-nitrofuraldehyde provided a complex mixture from which 22 was isolated in 12% yield (entry 13).

Concerning reactions with n-butyl isocyanide (entries 15–19), the best results in terms of yield and aldehyde conversion were obtained with aromatic aldehydes (entries 15 and 16). Naphtho-furoquinones 23 and 24 were obtained in 55 and 40% yield, respectively, after purification. N-Benzyl piperidine carbaldehyde led to a 30% yield of naphtho-furoquinone 27 with 35% aldehyde conversion (entry 19), while 5-nitrofuraldehyde resulted in only 10% of aldehyde conversion and afforded compound 26 (entry 18) in very poor yield in a complex mixture. Reaction of n-butyl isocyanide with N-methyl-indazole carbaldehyde afforded compound 29 with 50% yield (entry 21), while naphtho-furoquinone 25 could be identified in a complex mixture. Compound 25 was unstable in different purification conditions.

To summarize, a library of 19 novel naphtho-furoquinones was synthesized under microwave irradiation in moderate yields (three-component reaction, Scheme 3). In some cases, where probably aldehydes are less reactive, a two-component condensation between lawsone and tert-butyl or n-butyl isocyanide occurred, affording novel naphtho-enaminodione quinones 28 and 29 (two-component reaction, Scheme 3).

X-ray and 2D-NMR Analysis of Compound 9. Compound 9 was recrystallized in methylene chloride. The obtained single crystals, which appeared as purple platelets, were analyzed by X-ray diffraction (Figure 3). Mo-Kα radiation was used, and the compound showed a P21/c space group (crystallographic Data in the Supporting Information). Inspection of the structure...
shows that the aromatic halogen ring exhibited a dihedral angle of −44.2° with respect to the furan ring (C₆b/C₇/C₈/C₉), while the carbon atom of the tert-butyl group (C₁₀) was almost coplanar with the furan ring. The observed dihedral angle (O₁/C₂/N₁/C₁₀) had a small value of 16.3°, thus indicating a potential useful space for coordination between the oxygen atom of the carbonyl group (C₉ = O₁) and the oxygen atom of the furan ring (O₁).

Compound 9 was also analyzed by 2D-NMR at 298 K. All the ¹H and ¹³C signals were assigned based on the chemical shifts, spin−spin coupling constants, splitting patterns, and signal intensities and by using ¹H−¹H COSY45, ¹H−¹³C HSQC, and ¹H−¹³C HMBC experiments (see the Experimental Section).

In addition, the attribution of C4 and C9 of compound 9 was based on the previous study of Borgati et al., where the authors performed a complete, comparative ¹H and ¹³C signal assignment of ρ-naphthoquinones and ortho- and para-naphthofuroquinones. In their case, the presence of a proton at C3 allowed the differentiation/identification of C4 and C9 by HMBC correlation with a ∆δ between C4 and C9 as high as 8 ppm. Then, we assigned the chemical shifts of C9 and C4 carbon atoms at 169.6 and 182.0 ppm, respectively, which is consistent with the reliable assignment of Borgati et al.

The ¹H and ¹³C chemical shifts of 9 are given in Table 3. The ¹³C and HSQC spectra show 20 different carbon signals for 9. From the ¹³C data, it was possible to identify one cyclohexanedione (δC 169.58 and 181.95), one phenyl chloride group (δC 129.2, 130.8, and 133.8), six sp²-hybridized carbons (δC 99.0 from 99.0 to 159.3), and four sp³-hybridized carbons (δC 108.6 to 157.9).

Table 3. ¹H and ¹³C NMR Data Assignments of Compound 9 in CDCl₃ at 298 K.

| ¹H and/or ¹³C numbering | ¹H chemical shift, ppm | ¹³C chemical shift, ppm |
|------------------------|------------------------|------------------------|
| C-2                    | 159.3                  |                        |
| C-9a                   | 144.2                  |                        |
| C-9                    | 169.6                  |                        |
| C-8a                   | 133.2                  |                        |
| CH-8                   | 8.19                   | 126.1                  |
| CH-7                   | 7.72                   | 133.8                  |
| CH-6                   | 7.63                   | 132.4                  |
| CH-5                   | 8.04                   | 126.5                  |
| C-4a                   |                        | 133.2                  |
| C-4                    |                        | 182.0                  |
| C-3a                   |                        | 130.3                  |
| C-3                    |                        | 99.0                   |
| C-1                    |                        | 133.6                  |
| CH-2b                  | 7.44                   | 130.8                  |
| CH-3b                  | 7.47                   | 129.2                  |
| C-4b                   |                        | 133.8                  |
| CH-5b                  | 7.47                   | 129.2                  |
| CH-6b                  | 7.44                   | 130.8                  |
| NH                     | 4.86                   |                        |
| CH-3-11,12,13          | 1.52                   | 30.1                   |

For the sake of clarity, the chemical shifts are reported starting from the furan (C2), then the naphthoquinone moiety (C9a to C3a), followed by the p-chlorophenyl ring (C1 to C6b), and finally the N-tert-butyl group (HN1, C10 to C13).

2D-NMR Analysis of Compound 28. A complete 2D-NMR analysis was also conducted for compounds 28 (28a/28a’) and 29 (29a/29a’), especially using HMBC correlations. Both compounds are present in two stereoisomers in a 1:1 ratio.

Compound 28a/28a’ is analyzed here (for compound 29a/29a’, see the Supporting Information) at a temperature of 298 K. All the ¹H and ¹³C signals were assigned on the basis of chemical shifts, spin−spin coupling constants, splitting patterns, and signal intensities and by using ¹H−¹H COSY45, ¹H−¹³C HSQC, and ¹H−¹³C HMBC experiments (see the Experimental Section).
evaluated activities of simple synthons (lawsone and iodolawsone (Figure 4), which are supposed to target the cytochrome sone) along with the reference drugs atovaquone for H37Rv. In addition, we have also included in Table 5 the two other pathogens, namely, M. tuberculosis, miltefosine for L. donovani, and streptomycin for P. falciparum. We have also included two antimalarial compounds were first evaluated in vitro on the axenic form of L. donovani and Streptomyces for M. tuberculosis. We have also included two antimalarial compounds from the literature: MMV00757143-45 and XCV46 (Figure 4), which are supposed to target the cytochrome bc1 complex and/or the dihydroorotate dehydrogenase (DHODH). The docking scores of all compounds regarding these two mitochondrial targets were also evaluated, and the obtained values are summarized in Table 6.

Activities against P. falciparum. Depending on the isocyanide used for the synthesis, three series can be distinguished bearing the tert-butyl, N-butyl, and N-cyclohexyl scaffolds. Concerning the first series of compounds (9–17), all but one exhibited IC50 values above 10 μM against P. falciparum. Conversely, compound 17 had an IC50 value of 2.5 μM, which, owing to its low cytotoxicity on Vero cells (86 μM), confers to the molecule promising pharmacological characteristics. Compound 17 possesses a (1-benzyl-piperidin-4-yl) substituent at position 3 of the furan ring, while an analogous functionality (4-chlorophenyl cyclohexyl) is present in atovaquone. Interestingly, the N-Boc derivative 16 of compound 17 exhibits an IC50 value higher than 10 μM, indicating that this protecting group results in loss of antiplasmodial activity of the compound.

Concerning the second naphthofuroquinones series (18–22), bearing an N-cyclohexyl ring, four compounds out of five evaluated, exhibited IC50 values in the range of 3.6–11 μM. Compound 21, bearing a nitro-furan moiety at C3 of the furan ring, exhibited an IC50 value higher than 10 μM, similar to compounds 15 and 26 of the other two families bearing the same substituent. Compound 22 exhibits the relatively better activity with an IC50 value of 3.6 μM, compared to compounds 18, 19, and 20 (11, 6, and 10 μM, respectively). Nevertheless, possessing a 3,4,5-trimethoxy phenyl group at C3 of the furan ring has a higher SI value (>16 vs 34 for 17), rendering this compound potentially the most interesting of this series, from a pharmacological point of view.

In the third series of compounds (23–27) bearing the n-butyl group, compound 27 having the same scaffold as compound 17 of the previous series at C3 exhibits the best activity with an IC50 value of 4 μM. Nevertheless, its higher cytotoxicity on Vero cells (24 μM) and consequently its weak SI (6) renders compound 27 less interesting in comparison to 17. In this series, compound 24 having a 3,4,5-trimethoxy phenyl group attached to C3 has an IC50 in the same range as 27 but exhibits a much lower cytotoxicity value (>100 μM), rendering 25 potentially more interesting.

Finally, concerning these series of naphthofuroquinones, it is worth pointing out that many of our compounds are 2- to 5-fold more active against P. falciparum than derivative 6 reported by Borgati et al.12

Among the other evaluated compounds, while lawsone and iodolawsone are weak to very weak inhibitors of P. falciparum in vitro, compounds 28 and 29 seem more interesting. For both couples of compounds bearing either a N-tert-butyl or N-butyl group, the IC50 values are 1.4 and 1.9 μM, respectively, associated with a weak cytotoxicity (CC50 > 50 μM) resulting in promising SIs (>36 and >26, respectively). Noteworthily, it is the first time that enamidino systems possessing also an α,β-dione functionality were evaluated against P. falciparum. These compounds keep the 1,4-quinoine scaffold where the 2-hydroxy group of the atovaquone is modulated to a ketone group. The 2-hydroxy function of atovaquone plays a crucial role in the interaction of the drug with the bc1 complex, and this interaction is believed to be disturbed in atovaquone-resistant Plasmodium. Thus, modification at this position may be an interesting starting point for further development of drugs able to circumvent atovaquone resistance.

Activities against L. donovani. Naphthoquinones are well known for their reported antileishmanial activities. All our new compounds were first evaluated in vitro on the axenic form of L. donovani, the parasite responsible for visceral leishmaniasis in humans. Six of them, namely, compounds 8, 16, 18, 28, 29, and iodolawsone, exhibited IC50 values lower than 10 μM, thus justifying an evaluation on the L. donovani intramacrophage amastigote model, which is closer to the pathological conditions. Before carrying out this experiment, it was necessary to evaluate the cytotoxicity of the compounds on the RAW 264.7 macrophage as the host cell of the in vitro Leishmania model.

| 1H and/or 13C numbering | 1H chemical shift, ppm | 13C chemical shift, ppm |
|------------------------|------------------------|------------------------|
|                        | 28a | 28a’ | 28a | 28a’ |
| C-7                    | 108.6 | 109.3 |
| C-8                    | 176.8 | 177.5 |
| C-9                    | 181.7 | 182.0 |
| C-12                   | 132.3 | 132.3 |
| CH-13                  | 8.18 | 8.19 |
| CH-14                  | 7.72 | 7.72 |
| CH-15                  | 7.84 | 7.82 |
| CH-16                  | 8.16 | 8.27 |
| C-11                   | 134.5 | 136.3 |
| C-10                   | 184.5 | 181.9 |
| CH-1                   | 8.55 | 8.67 |
| NH                     | 12.07 | 12.22 |
| C-3                    | 56.5 | 56.5 |
| CH3-4,5,6              | 1.52 | 1.52 |

*The chemical shifts are reported starting by the ring (C7 to C10), followed by the alkene side chain.
All the six compounds exhibited a reasonable cytotoxicity, with CC\textsubscript{50} values higher than 40 μM (Table 5). Compound 28 was less cytotoxic with a CC\textsubscript{50} value higher than 100 μM and consequently a SI higher than 28. Then, the six compounds were eligible to be evaluated on the intramacrophage amastigote model. Except iodo-lawsone (IC\textsubscript{50} = 13.2 μM and SI = 4.3), the compounds exhibited IC\textsubscript{50} values lower than 10 μM, and compounds 28 and 29 showed potent activities in close range of the reference drug miltefosine. Notably, compound 28 showed the best activity (IC\textsubscript{50} = 3.5 μM) and the best SI (> 28) that is better than that of miltefosine. Compared to lawsone, the substitutions leading to 28 increased the antileishmanial activity, while they significantly lowered the cytotoxicity. It should be noted that 28 was also the most active and selective compound against \textit{P. falciparum} (IC\textsubscript{50} = 1.4 μM and SI > 36).

**Activities against \textit{M. tuberculosis} H37Rv.** Among all the compounds evaluated, naphthofuroquinones 17, 22, and 27 exhibit interesting activities against \textit{M. tuberculosis} H37Rv strain as well as against \textit{P. falciparum}.

Compound 17 (MIC = 9 μM) contains a benzyl-N-piperidine scaffold at position C3 of the furan ring and a tert-butyl group attached to the nitrogen atom at position C2. Compound 22 (MIC = 17−34 μM) has the same benzyl-N-piperidine scaffold at C3, while a N-cyclohexyl fragment is attached to C2. Compound 27 (MIC = 18 μM) contains the same benzyl-N-piperidine scaffold at C3 and a N-butyl function at C2. Naphthofuroquinone derivatives have been reported by Ho-Yeon Song to possess antituberculous activities. In a recent article, the authors reported a series of benzonaphthofuran- andiones obtained by direct intramolecular annulation of 2-chloro-3-phenoxynaphthoquinone derivatives. The compound

### Table 5. Biological Evaluation of Naphthoquinones Derivatives Reported Herein

| compound | \textit{P. falciparum} F32ARTIC<sub>50</sub> (μM) | CC<sub>50</sub><sup>a</sup> (μM) against Vero cells | SI for Plasmodium parasites | L. \textit{donovani}<sup>b</sup> IC<sub>50</sub> (μM) | CC<sub>50</sub><sup>c</sub> (μM) against macrophages | SI for \textit{Leishmania} parasites | \textit{M. tuberculosis} H37Rv MIC (μM) |
|----------|---------------------------------|-------------------------------|---------------------------|-----------------|-----------------------------|-----------------------------|-------------------------------|
| 9        | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 10       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 11       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 12       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 13       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 14       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 15       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 16       | >10                             | >10                           |                          |                 |                             |                             | >147                          |
| 17       | 2.5                             | 86                            | 34                       |                 |                             |                             |                 |
| 18       | 11                             | 50                            | 4.5                      | 9.41            | 54.21                       | 5.76                        | 39                           |
| 19       | 6                               | >100                          | >16                      |                 |                             |                             | >147                          |
| 20       | 10                              |                               |                          |                 |                             |                             | 150                          |
| 21       | >10                             |                               |                          |                 |                             |                             | 20                           |
| 22       | 3.6                             | 36                            | 10                       |                 |                             |                             | 17−34                        |
| 23       | >10                             |                               |                          |                 |                             |                             | 84                           |
| 24       | 9                               | >100                          | >10                      |                 |                             |                             | 147                          |
| 25       | >10                             |                               |                          |                 |                             |                             | 84                           |
| 26       | >10                             |                               |                          |                 |                             |                             | 18                           |
| 27       | 4                               | 24                            | 6                        |                 |                             |                             | 18                           |
| 28       | 1.4                             | >50                           | >36                      | 3.50            | >100                        | >28.5                       | 62−124                       |
| 29       | 1.9                             | >50                           | >26                      | 6.33            | 46.48                       | 7.34                        | >147                          |

lawsone (1)  
6<sup>c</sup>  
iodo-lawsone  
avatoquine (7)  
miltefosine  

**<sup>a</sup>Cytotoxicity was evaluated against Vero cells and expressed as CC\textsubscript{50}, and the corresponding SI was relative to the ratio CC\textsubscript{50}/IC\textsubscript{50} for \textit{P. falciparum}.**<sup>b</sup> The molecules were tested against both LV9 \textit{Leishmania donovani} axenic amastigote forms and intramacrophage amastigote forms. The values written on this table correspond to the activities on the second form. Cytotoxicity was evaluated against macrophage RAW 264.7 cells and expressed as CC\textsubscript{50}. The SI calculated corresponds to the ratio CC\textsubscript{50}/IC\textsubscript{50} (intramacrophage amastigote forms).<sup>d</sup> SI evaluated on Hep GAA16 cells. IC\textsubscript{50}: inhibitory concentration 50%. CC\textsubscript{50}: cytotoxic concentration 50%.

**Figure 4.** Structures of compounds also analyzed for comparison purposes.
Table 6. Computational Evaluation of Naphthoquinone Derivatives as Mitochondrial Antimalarial Agents

| compound | P. falciparum F32-ARTIC<sub>a</sub> (μM) | P. falciparum bc1 docking score (kcal/mol) | P. falciparum DHODH docking score (kcal/mol) |
|----------|------------------------------------------|---------------------------------------------|---------------------------------------------|
| 9        | >10                                      | -9.7                                       | -6.8                                        |
| 10       | >10                                      | -7.1                                       | -7.7                                        |
| 11       | >10                                      | 0.0                                        | -7.0                                        |
| 12       | >10                                      | -9.2                                       | -8.3                                        |
| 13       | >10                                      | 0.0                                        | -6.8                                        |
| 14       | >10                                      | -3.5                                       | -6.9                                        |
| 15       | >10                                      | -3.5                                       | -7.3                                        |
| 16       | >10                                      | -8.9                                       | -9.4                                        |
| 17       | 2.5                                      | -12.2                                      | -11.0                                       |
| 18       | 11                                       | -9.5                                       | -8.2                                        |
| 19       | 6                                        | -8.8                                       | -9.0                                        |
| 20       | 10                                       | -2.6                                       | -6.3                                        |
| 21       | >10                                      | -10.0                                      | -7.3                                        |
| 22       | 3.6                                      | -6.4                                       | -9.3                                        |
| 23       | >10                                      | -11.2                                      | -11.1                                       |
| 24       | 9                                        | -10.4                                      | -9.2                                        |
| 26       | >10                                      | -10.5                                      | -9.0                                        |
| 27       | 4                                        | -10.8                                      | -9.3                                        |
| 28       | 1.4                                      | a = -7.2                                   | a' = -7.0                                   |
|          |                                          | a' = -7.0                                  | a' = -7.0                                   |
| 29       | 1.9                                      | a = -7.5                                   | a = -7.1                                    |
|          |                                          | a' = -7.3                                  | a' = -7.5                                   |
| lawsone  (1) | >10                                      | -6.5                                       | -6.6                                        |
| 6        | 11.65                                    | -8.7                                       | -8.7                                        |
| iodo-lawsone  (2) | >50                                      | -6.9                                       | -7.1                                        |
| atovaquone (7) | 0.001                                   | -12.1                                      | -10.7                                       |
| MMV007571 | 1                                       | -8.6                                       | -7.3                                        |
| XCV      | 0.073                                    | -11.2                                      |                                              |

Figure 5. Compound BNF15, active against drug-resistant Mycobacterium tuberculosis.

N═(CH<sub>3</sub>)<sub>2</sub> substituent on the benzofuran fragment. The authors reported that BNF15 was effective against all drug-sensitive and drug-resistant *M. tuberculosis* isolates tested and effectively killed intracellular *M. tuberculosis* and nontuberculous mycobacteria. The authors point out that (i) the naphthofuran system could be a valuable fragment for compounds targeting *M. tuberculosis* and (ii) a fine tuning of the substitution pattern of the benzofuran frame is crucial in order to enhance the activities by more than two log units.

**Computational Studies on Mitochondrial Targets of *P. falciparum***. In order to rationalize the activities against *P. falciparum*, we carried out molecular docking on the two targets expected to interact with our compounds, that is, cytochrome bc1 (bc1) and dihydorotate dehydrogenase (DHODH). In fact, naphthoquinone derivatives based on lawsone scaffold are known to interfere with these two mitochondrial targets of *P. falciparum*. 26

The AlphaFold2-modeled structure of the cytochrome enzyme (Q7HP03) was used to characterize the predicted interactions between the compounds described and the *P. falciparum* target bc1.69 All local quality measures of the predicted protein structure were assessed as very good (Figure 6), that is, per-residue confidence scores (pLDDT) for the model were all very high near the binding site region (central groove inside the α-helices, near Tyr 263) and had low expected position error (dark green in Figure 7, in angstrom) for the predicted alignment errors (PAE), that is, the values for expected position error at residue x when the predicted and true structures are aligned on residue y.

For the DHODH target, the X-ray crystal structure of *PfDHODH* (Protein Data Bank (PDB) 7l)30 was retrieved from the PDB having a resolution of 1.6 Å, adequate for docking studies.31 Hence, we docked all naphthoquinone conjugates into the putative quinone binding tunnel formed by the N-terminal domain to check if our compounds are susceptible to interact with this target.

The naphthofuroquinone derivatives were docked into the binding site of *P. falciparum* bc1 and also *PfDHODH*, and their predicted interactions showed similar binding modes and interaction partners as compared to the known inhibitors atovaquone, MMV007571, and XCV. Good docking scores and binding poses were thus obtained (Table 6 and Figure 8). Compounds 17, 19, 22, 24, and 27 were predicted to exhibit the best binding.

The naphthoquinone ring was predicted to be able to bind in two different binding modes: flipped and unflipped with respect to atovaquone, and their interactions were also similar to those of another known inhibitor, MMV007571. There is good enrichment of the top-ranked compounds in Table 6 with known strong inhibitors appearing in the top-ranked compounds (in bold).

Several compounds that were experimentally active had a stronger predicted interaction with both *Pf bc1* and DHODH (Table 5), such as 17, 27, and atovaquone.

Among the enaminone compounds 28 and 29, compound 29α (−7.45 kcal/mol) was predicted to make good use of bridging water molecules in the binding site with better predicted docking scores in the hydrated binding site, the latter which may be advantageous for the binding energy and/or specificity for some compounds (Figure 9).52,53 This is also the case for compound XCV (−10.79 kcal/mol). Compound 11 binds mostly through van der Waals contacts, both in bc1 and in DHODH. Compound 22 probably acts through inhibition of *P. falciparum* DHODH as it has a stronger predicted interaction with this enzyme than with *P. falciparum* bc1, as compared to controls. XCV in DHODH makes strong use of hydrogen bonds and π–π stacking with His185, as well as hydrogen bonds to Arg285. Atovaquone makes π–π stacking interactions with Phe171 in DHODH and with Phe264 in bc1. MMV007571 also interacts mainly through van der Waals contacts in bc1. Binding to several *P. falciparum* targets at the same time may lead to a compound possessing stronger inhibition properties, as well as the potential to avoid resistance.

The ADME properties for all compounds were predicted with Swiss-ADME.54 All compounds passed Lipinski’s rule of five for bioavailability, most of them also passing Ghose, Veber, Egan, and
and Muegge filters or lead-likeness.\textsuperscript{55} PAINS warnings\textsuperscript{56} were present only for the well-known quinone group.

The good properties of predicted binding, experimental inhibition, interactions inside the hydrated and nonhydrated binding sites, multitarget binding,\textsuperscript{57} and favorable molecular properties for further optimization of the compounds make them valuable features to continue their development as possible leads for malaria therapeutics.

\section*{CONCLUSIONS}

Based on lawson, the first synthetic approach of a three (or two)-component reaction involving various aldehydes and three commercially available isocyanides is reported. Under microwave irradiation, this approach afforded a series of naphthofuroquinones in moderate to good yields. In addition, with the less-reactive aldehydes, we obtained for the first time two naphtho-enaminodione quinones derived from the direct condensation of lawson with the corresponding isocyanide derivative.

Among the naphthofuroquinone series, compound 17 exhibited the best activity against \textit{P. falciparum}, which is more than 4 times better than the previous optimal reported in the naphthofuroquinone series.\textsuperscript{12} In addition, compound 17 exhibited the best SI = 34 among the synthesized naphthofuroquinones. Considering the molecular docking of 17 on the potential targets Pfbc1 and PfDHODH, this compound showed very favorable interactions, thus making it a valuable starting point for further development.

Noteworthily, the two naphtho-enaminodione quinone compounds 28 and 29 also exhibited antimalarial activities in the micromolar range. Compound 28 exhibited the best activity against \textit{P. falciparum} (IC\textsubscript{50} = 1.4 \textmu M) and the highest SI (> 36), while molecular docking predicted favorable interactions with both targets. Then, naphtho-enaminodiones quinones should be thus considered as interesting compounds in terms of novelty in synthesis but also due to their biological activities.

Against \textit{M. tuberculosis}, the same compound 17 exhibited the best activity (MIC = 9 \textmu M). It is about 7-fold less active than the known naphthofuroquinone derivative BNF15. A fine tuning of the substitution pattern should be important in order to increase the activity of compound 17 against \textit{M. tuberculosis}.

Finally, compounds 28 and 29 have potent activities against \textit{L. donovani} that are close to that of the reference drug miltefosine. More importantly, compound 28 showed the best activity (IC\textsubscript{50} = 3.5 \textmu M) and the best SI (> 28) that is higher than that of miltefosine. Compound 28 is one of the rare compounds which are active against both \textit{P. falciparum} and \textit{L. donovani}.

In addition, compound 28 is worth to be evaluated in vivo against the \textit{L. donovani}/BALB/c mouse model.

\section*{METHODS}

\subsection*{Experimental Section.} Our reagents and solvents were purchased from Sigma-Aldrich, TCI, Alfa Aesar, and Fluorochem and used as received without any further purification. Microwave irradiation reactions were performed in a CEM Discover SP Microwave model 909150/SN: DC 9208.
apparatus. A monowave 50 thermic reactor was obtained from Anton Paar. TLC was performed on silica gel 60 F$_{254}$ plates (Merck). The compounds and the reaction mixtures were visualized on the TLC plates by irradiation with UV light. For

Figure 8. Binding modes and interactions for compounds in the binding site of Pf bc1.
flash column chromatography, a PuriFlash XS520Plus system was used in combination with PF-30SIHP-JP-F0040 columns. When PE/A* is mentioned in the Experimental Section, A* stands for the DCM/AcOEt (8:2) system of solvents. $^1$H and $^{13}$C NMR spectra for the reported compounds were recorded on a Bruker Avance I 300 MHz (300 MHz for $^1$H and 75 MHz for

Figure 9. Binding modes and interactions for compounds in the binding site of *P. falciparum* DHODH. X-ray crystal structure co-crystallized with inhibitor XCV in white, self-docked in magenta (RMSD = 0.18 Å), and water molecules as spheres.
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3-(3-Bromophenyl)-2-(tert-butylamino)naphtho[2,3-b]-furan-4,9-dione (10). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time four cycles × 60 min. Aldehyde conversion 30%. The crude product was purified by FCC with cyclohex/AcOEt (9:1) to yield 50 mg (30%) of the product as a violet solid (mp 193−194 °C). Rf (cyclohex/AcOEt 9:1) = 0.26. 1H NMR (400 MHz, CDCl3): δ 8.14 (dd, J = 7.6, 0.9 Hz, 1H), 8.01 (dd, J = 7.6, 0.9 Hz, 1H), 7.68 (dt, J = 7.5, 1.4 Hz, 1H), 7.60 (dt, J = 7.5, 1.4 Hz, 2H), 7.46 (ddd, J = 7.9, 1.9, 1.1 Hz, 1H), 7.42−7.36 (m, 1H), 7.30 (t, J = 7.9 Hz, 1H), 4.86 (s, 1H), 1.50 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 181.7 (C==O), 169.6 (C==O), 159.4 (C), 144.1 (C), 133.8 (CH), 133.2 (CH), 133.2 (C), 133.2 (C), 133.2 (CH), 133.2 (CH), 130.4 (CH), 130.3 (C), 126.1 (CH), 126.2 (CH), 123.2 (C), 98.7 (C), 54.2 (C), 30.1 (3 × CH3), HRMS calcd for C22H18BrNO3+: [M + H]+ = 424.0548; found, 424.0548.

2-(tert-Butylamino)-3-(3,4,5-trimethoxyphenyl)naphtho[2,3-b]-furan-4,9-dione (11). The compound was synthesized by following the above-mentioned general procedure (0.3 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 60%. The crude product was purified by FCC with Hex/AcOEt (7:3) to yield 66 mg (55%) of the product as a violet solid (mp 218−220 °C). Rf (Hex/AcOEt 7:3) = 0.23. 1H NMR (400 MHz, CDCl3): δ 8.81 (dd, J = 7.6, 1.0 Hz, 1H), 8.02 (dd, J = 7.6, 1.0 Hz, 1H), 7.67 (td, J = 7.5, 1.3 Hz, 1H), 7.59 (td, J = 7.5, 1.3 Hz, 1H), 6.72 (s, 2H), 4.98 (s, 1H), 3.90 (s, 3H), 3.89 (s, 6H), 1.50 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 181.9 (C==O), 169.4 (C==O), 159.6 (C), 153.6(C), 144.0 (C), 137.7 (C), 137.7 (C), 133.8 (CH), 133.4 (2C), 132.3 (CH), 130.4 (CH), 130.3 (C), 126.1 (CH), 125.6 (C), 106.7 (CH), 100.5 (C), 61.0 (CH3), 56.4 (2 × CH3), 54.1 (C), 30.2 (3 × CH3), HRMS calcd for C24H19NO6+: [M + H]+ = 436.1760; found, 436.1761.

2-(tert-Butylamino)-3-(4-chlorophenyl)naphtho[2,3-b]-furan-4,9-dione (9). The compound was synthesized by following the above-mentioned general procedure (0.23 mmol lawsone). Reaction time three cycles × 60 min. Aldehyde conversion 70%. The crude product was purified by FCC with Hex/AcOEt (9:1) to yield 50 mg (59%) of the product as a violet solid (mp 237−239 °C). Rf (Hex/AcOEt 9:1) = 0.18. 1H NMR (400 MHz, CDCl3): δ 8.17 (dd, J = 7.6, 1.0 Hz, 1H), 8.02 (dd, J = 7.6, 1.0 Hz, 1H), 7.69 (td, J = 7.5, 1.3 Hz, 1H), 7.61 (td, J = 7.5, 1.3 Hz, 1H), 7.49−7.34 (m, 4H), 4.82 (s, 1H), 1.50 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.0 (C==O), 169.6 (C==O), 159.4 (C), 144.2 (C), 133.9 (CH), 133.7 (C), 133.4 (C), 133.3 (C), 132.5 (CH), 130.9 (2 × CH), 130.4 (C), 129.3 (2 × CH), 128.8 (C), 126.6 (CH), 126.3 (CH), 99.1 (C), 54.3 (C), 30.2 (3 × CH3). HRMS calcd for C22H17ClNO3+: [M + H]+ = 380.1053; found, 380.1058.

2-(tert-Butylamino)-3-(3,4-dimethoxyphenyl)naphtho[2,3-b]-furan-4,9-dione (12). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion...
conversion 45%. The crude product was purified by FCC with petroleum ether (PE)/AcOEt (8:2) to yield 35 mg (25%) of the product as a violet solid (mp 226−228 °C). Rf (Hex/AcOEt 8:2) = 0.17. 1H NMR (400 MHz, CDCl3): δ 8.16 (dd, J = 7.6, 0.9 Hz, 1H), 8.03 (dd, J = 7.6, 0.9 Hz, 1H), 7.68 (td, J = 7.5, 1.4 Hz, 1H), 7.59 (td, J = 7.5, 1.4 Hz, 1H), 7.06 (d, J = 1.9 Hz, 1H), 7.03−6.92 (m, 2H), 4.93 (s, 1H), 3.93 (s, 6H), 1.49 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.0 (C=O), 169.3 (C=O), 159.7 (C), 149.3 (C), 148.8 (C), 143.9 (C), 133.8 (CH), 133.5 (C), 133.4 (C), 132.3 (CH), 130.6 (C), 126.5 (CH), 126.2 (CH), 122.7 (C), 121.5 (C), 113.3 (CH), 111.6 (CH), 100.6 (C), 56.2 (CH3), 56.1 (CH3), 54.1 (C), 30.2 (3 × CH3). HRMS calcld for C24H24N2O8 [M + H]+ = 406.1654; found, 406.1655.

2-(tert-Butylamino)-3-(1-methyl-1H-indazol-5-yl)-naphtho[2,3-b]furan-4,9-dione (13). The compound was synthesized by following the above-mentioned alternative procedure (0.5 mmol lawsone). Reaction time 48 h. Aldehyde conversion 30%. The crude product was purified by FCC with PE/AcOEt (4:6) to yield 60 mg (30%) of the product as a blue-violet solid (mp 219−221 °C). Rf (PE/AcOEt 4:6) = 0.32. 1H NMR (400 MHz, CDCl3): δ 8.13 (dd, J = 7.6, 1.1 Hz, 1H), 7.99−7.90 (m, 2H), 7.75 (dd, J = 0.8 Hz, 1H), 7.66 (td, J = 7.5, 1.3 Hz, 1H), 7.56 (td, J = 7.5, 1.3 Hz, 1H), 7.49−7.40 (m, 2H), 5.11 (s, 1H), 4.04 (s, 3H), 1.50 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.0 (C=O), 169.0 (C=O), 159.8 (C), 143.8 (C), 139.3 (C), 133.7 (CH), 133.5 (C), 133.3 (C), 132.9 (CH), 132.2 (CH), 130.8 (C), 128.6 (CH), 126.4 (CH), 126.1 (CH), 124.3 (C), 122.3 (C), 121.6 (CH), 109.4 (CH), 100.7 (C), 54.1 (C), 35.6 (CH3), 30.1 (3 × CH3). HRMS calcld for C26H26N2O8 [M + H]+ = 406.1661; found, 406.1659.

2-(tert-Butylamino)-3-(1H-indol-2-yl)naphtho[2,3-b]furan-4,9-dione (14). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 80%. The crude product was purified by FCC with cyclohexene/AcOEt (9:1) to yield 50 mg (35%) of the product as a blue solid (mp 219−220 °C). Rf (cyclohexene/AcOEt 9:1) = 0.24. 1H NMR (400 MHz, CDCl3): δ 11.37 (s, 1H), 8.14 (dd, J = 7.6, 1.3 Hz, 2H), 7.70 (td, J = 7.5, 1.4 Hz, 1H), 7.63 (td, J = 7.5, 1.4 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.50 (dd, J = 8.1, 0.6 Hz, 1H), 7.24−7.18 (m, 1H), 7.13−7.06 (m, 1H), 6.40 (d, J = 1.4 Hz, 1H), 5.46 (s, 1H), 1.63 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 183.6 (C=O), 168.9 (C=O), 159.4 (C), 143.7 (C), 135.4 (C), 134.4 (C), 133.2 (C), 132.9 (C), 123.4 (CH), 129.5 (C), 128.8 (C), 128.6 (C), 127.1 (CH), 126.2 (CH), 122.5 (CH), 120.3 (CH), 119.8 (CH), 111.4 (CH), 97.5 (CH), 93.5 (C), 54.7 (C), 30.1 (3 × CH3). HRMS calcld for C26H26N2O8 [M + H]+ = 385.1552; found, 385.1553.

2-(tert-Butylamino)-3-(5-nitrofuran-2-yl)naphtho[2,3-b]furan-4,9-dione (15). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 20%. The crude product was purified by FCC with PE/DCM (4:6) to yield 45 mg (19%) of the product as a purple solid (mp 274−276 °C). Rf (PE/DCM 3:7) = 0.21. 1H NMR (400 MHz, CDCl3): δ 8.16 (dd, J = 14.5, 7.2 Hz, 2H), 7.77−7.64 (m, 3H), 7.48 (d, J = 3.9 Hz, 1H), 6.97 (s, 1H), 1.63 (s, 9H). 13C NMR (151 MHz, DMSO-d6): δ 180.0 (C=O), 168.7 (C=O), 159.6 (C), 149.8 (C), 143.4 (C), 133.7 (CH), 132.5 (CH), 132.1 (C), 131.8 (C), 127.6 (C), 125.9 (CH), 125.1 (CH), 123.5 (C), 114.7 (CH), 110.8 (CH), 87.4 (C), 54.0 (C), 28.8 (3 × CH3). HRMS calcld for C20H13N2O6 [M + H]+ = 381.1087; found, 381.1090.

terButyl 4-(2-(tert-Butylamino)-4,9-dioxo-4,9-dihydronaphtho[2,3-b]furan-3-yl)piperidine-1-carboxylate (16). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 95%. The crude product was purified by FCC with PE/AcOEt (8:2) to yield 95 mg (52%) of the product as a violet oil. Rf (PE/AcOEt 8:2) = 0.22. 1H NMR (400 MHz, CDCl3): δ 8.15−8.09 (m, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.67 (td, J = 7.5, 1.5 Hz, 1H), 7.61 (td, J = 7.5, 1.5 Hz, 1H), 4.24 (s, 2H), 2.99 (tt, J = 12.4, 3.6 Hz, 1H), 2.80 (t, J = 11.9 Hz, 2H), 1.95 (qd, J = 12.7, 4.3 Hz, 2H), 1.68−1.61 (m, 2H), 1.50 (s, 9H), 1.46 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 182.6 (C=O), 169.7 (C=O), 158.5 (C), 155.1 (C), 144.5 (C), 133.7 (CH), 133.3 (C), 133.2 (C), 132.4 (CH), 131.3 (C), 126.5 (CH), 126.1 (CH), 105.2 (C), 79.7 (C), 60.5 (C), 54.3 (C), 32.5 (2 × CH2), 30.4 (3 × CH3), 30.2 (2 × CH2), 28.6 (3 × CH3). HRMS calcld for C29H23N2O12 [M + H]+ = 453.2389; found, 453.2383.
conversion 91%. The crude product was purified by FCC with DCM/MeOH/Et2N (98:2:1) to yield 70 mg (53%) of the product as a violet solid (mp 168–170 °C). Rf (DCM/MeOH/ Et2N 98:2:1) = 0.35. 1H NMR (400 MHz, CDCl3): δ 8.15 (dd, J = 7.6, 1.0 Hz, 1H), 8.07 (dd, J = 7.6, 1.0 Hz, 1H), 7.68 (td, J = 7.5, 1.4 Hz, 1H), 7.62 (td, J = 7.5, 1.4 Hz, 1H), 7.43–7.29 (m, 5H), 4.64 (s, 1H), 3.62 (s, 2H), 3.05 (d, J = 10.2 Hz, 3H), 2.18–2.07 (m, 4H), 1.72 (d, J = 12.6 Hz, 2H), 1.47 (s, 9H). 13C NMR (101 MHz, CDCl3): δ 184.11 (C=O), 183.01 (C=O), 169.20 (C), 158.90 (C), 133.70 (CH), 133.60 (C), 133.30 (C), 132.20 (CH), 131.50 (CH), 129.50 (CH), 128.40 (2 × CH), 127.30 (CH), 126.40 (2 × CH), 126.10 (CH), 63.40 (CH₂), 54.30 (C), 54.20 (2 × CH₂), 30.20 (2 × CH₂), 30.40 (3 × CH₂), 29.80 (CH). IR (cm⁻¹): 1183 (C=O), 1201 (C–N), 1396 and 1460 (CH₂), 1532 (ArH), 1558 (ArH), 1560 (ArH), 1631 (C=O), 1634 (ArH), 1672 (C=O), 3316 (N–H). HRMS calcld for C28H33N2O6₉ [M + H]⁺ = 443.2335; found, 443.2333.

3-(4-Chlorophenyl)-2-(cyclohexylamino)naphtho[2,3-b]furan-4,9-dione (18). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawson). Reaction time two cycles × 60 min. Aldehyde conversion 50%. The crude product was purified by FCC with PE/AcOEt (9:1) to yield 74 mg (46%) of the product as a violet solid (mp 187–189 °C). Rf (PE/AcOEt 9:1) = 0.33. 1H NMR (400 MHz, CDCl3): δ 8.16 (dd, J = 7.6, 1.0 Hz, 1H), 8.01 (dd, J = 7.6, 1.0 Hz, 1H), 7.68 (td, J = 7.5, 1.3 Hz, 1H), 7.60 (td, J = 7.5, 1.3 Hz, 1H), 7.47–7.33 (m, 4H), 4.82 (d, J = 8.3 Hz, 1H), 3.82 (ddd, J = 10.3, 8.2, 4.0 Hz, 1H), 2.13–2.01 (m, 2H), 1.82–1.70 (m, 2H), 1.70–1.59 (m, 2H), 1.42 (t, J = 18.3, 4.7 Hz, 2H), 1.32–1.17 (m, 2H). 13C NMR (101 MHz, CDCl3): δ 182.00 (C=O), 169.50 (C=O), 159.10 (C), 143.50 (C), 133.90 (CH), 133.60 (C), 133.40 (C), 133.20 (C), 132.40 (C), 131.10 (C), 130.80 (2 × CH), 129.20 (2 × CH), 128.70 (C), 126.60 (CH), 126.30 (CH), 97.60 (C), 52.50 (CH), 34.00 (2 × CH₂), 25.40 (CH₃), 24.80 (2 × CH₂). HRMS calcld for C28H21ClNO₉ [M + H]⁺ = 426.1210; found, 406.1206.

2-(Cyclohexylamino)-3-(3,4,5-trimethoxyphenyl)naphtho[2,3-b]furan-4,9-dione (19). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawson). Reaction time two cycles × 60 min. Aldehyde conversion 45%. The crude product was purified by FCC with cyclohex/AcOEt (8:2) to yield 77 mg (42%) of the product as a blue solid (mp 163–165 °C). Rf (cyclohex/AcOEt 8:2) = 0.26. 1H NMR (400 MHz, CDCl3): δ 6.15 (dd, J = 7.6, 1.0 Hz, 1H), 8.02 (dd, J = 7.6, 1.0 Hz, 1H), 7.68 (td, J = 7.5, 1.3 Hz, 1H), 7.59 (td, J = 7.5, 1.3 Hz, 1H), 6.73 (s, 2H), 4.94 (d, J = 8.5 Hz, 1H), 3.90 (s, 3H), 3.89 (s, 6H), 2.08 (dd, J = 12.1, 3.0 Hz, 2H), 1.80–1.70 (m, 2H), 1.69–1.59 (m, 1H), 1.50–1.36 (m, 2H), 1.33–1.12 (m, 4H). 13C NMR (101 MHz, CDCl3): δ 182.00 (C=O), 169.30 (C=O), 159.30 (2C), 153.60 (C), 143.30 (C), 137.70 (C), 133.80 (CH), 133.40 (C), 133.30 (C), 132.30 (CH), 131.10 (C), 126.50 (CH), 126.20 (CH), 125.50 (C), 106.80 (2 × CH), 99.00 (C), 61.00 (CH₂), 56.40 (2 × CH₂), 52.40 (CH), 34.00 (2 × CH₂), 25.40 (CH₂), 24.80 (2 × CH₂). HRMS calcld for C28H23NO6⁺ [M + H]⁺ = 407.1243; found, 407.1243.
2-(Butylamino)-3-(3,4,5-trimethoxyphenyl)naphtho[2,3-b]furan-4,9-dione (24). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time four cycles × 60 min. Aldehyde conversion 45%. The crude product was purified by FCC with Hex/AcOEt (7:3) to yield 69 mg (40%) of the product as a dark blue oil. R\text{f} (Hex/AcOEt 7:3) = 0.21. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.16 (dd, \(J = 7.6, 0.9\) Hz, 1H), 8.03 (dd, \(J = 7.6, 0.9\) Hz, 1H), 7.68 (td, \(J = 7.5, 1.4\) Hz, 1H), 7.60 (td, \(J = 7.5, 1.4\) Hz, 1H), 6.73 (s, 2H), 5.05 (t, \(J = 5.9\) Hz, 1H), 3.91 (s, 3H), 3.90 (s, 6H), 3.52 (dd, \(J = 13.2, 7.0\) Hz, 2H), 1.64 (dt, \(J = 19.8, 7.5\) Hz, 2H), 1.41 (dq, \(J = 14.6, 4.7\) Hz, 2H). 0.96 (t, \(J = 7.4\) Hz, 3H). \textsuperscript{13}C NMR (101 MHz, CDCl\textsubscript{3}): \(\delta\) 182.0 (C=O), 169.4 (C=O), 160.0 (C), 153.6 (2 × C), 143.3 (C), 137.7 (C), 133.8 (CH), 133.4 (C), 133.3 (C), 132.3 (CH), 131.2 (C), 126.6 (CH), 126.2 (CH), 125.5 (C), 106.8 (2 × CH\textsubscript{3}), 98.8 (C), 61.0 (CH\textsubscript{3}), 56.4 (2 × CH\textsubscript{3}), 43.2 (CH\textsubscript{3}), 32.3 (CH\textsubscript{3}), 20.1 (CH\textsubscript{3}), 13.8 (CH\textsubscript{3}). IR (cm\textsuperscript{-1}): 1124 (C=O), 1412 (CH\textsubscript{3}), 1355 and 1454 (CH\textsubscript{3}), 1539 (ArH), 1584 (C=O), 1600 (C=O), 3323 (N=H). HRMS calced for C\textsubscript{25}H\textsubscript{25}N\textsubscript{2}O\textsubscript{6} \textsuperscript{+} [M + H]\textsuperscript{+} = 436.1760; found, 436.1759.

2-(Butylamino)-3-(5-nitrofuran-2-yl)naphtho[2,3-b]furan-4,9-dione (26). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 10%. The crude product was purified by FCC with PE/DCM (3:7) to yield 16 mg (8%) of the product as a purple oil. R\text{f} (PE/DCM 3:7) = 0.12. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 8.20–8.09 (m, 2H), 7.72 (dq, \(J = 14.5, 7.5\) Hz, 1H), 7.48 (d, \(J = 4.0\) Hz, 1H), 6.76 (t, \(J = 6.6\) Hz, 1H), 3.72 (dd, \(J = 12.9, 6.9\) Hz, 2H), 1.85–1.72 (m, 2H), 1.42–1.29 (m, 2H), 1.03 (t, \(J = 7.3\) Hz, 3H). \textsuperscript{13}C NMR (151 MHz, DMSO-d\textsubscript{6}, 100 °C): \(\delta\) 180.1 (C=O), 168.5 (C=O), 160.2 (C), 149.6 (C), 142.5 (C), 133.7 (CH), 132.4 (CH), 132.1 (C), 131.9 (C), 128.6 (C), 125.8 (CH), 125.1 (CH), 124.0 (C), 114.8 (CH), 111.1 (CH), 85.6 (C), 42.2 (CH\textsubscript{3}), 30.7 (CH\textsubscript{3}), 18.8 (CH\textsubscript{3}), 12.9 (CH\textsubscript{3}). HRMS calculated for C\textsubscript{26}H\textsubscript{30}N\textsubscript{2}O\textsubscript{6} \textsuperscript{+} [M + H]\textsuperscript{+} = 379.0930; found, 379.0923.

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3-((Butylamino)methylene)naphthalene-1,2,4(3H)-trione (28). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time two cycles × 60 min. Aldehyde conversion 35%. The crude product was purified by FCC with DCM/MeOH (99:1) and then with DCM/MeOH (97:3) to yield 45 mg (30%) of the product as a blue-violet oil. \( \delta \) (CDCl\( _3 \)) = 0.42. \(^{1}H\) NMR (300 MHz, CDCl\( _3 \)): 8.15–8.09 (m, 1H), 8.03 (dd, \( J = 7.5, 1.2 \) Hz, 1H), 7.66 (td, \( J = 7.5, 1.5 \) Hz, 1H), 7.58 (td, \( J = 7.5, 1.5 \) Hz, 1H), 7.37–7.27 (m, 5H), 5.08 (s, 1H), 5.37 (2H), 3.49 (dd, \( J = 13.1, 7.0 \) Hz, 2H), 2.14 (t, \( J = 10.9 \) Hz, 2H), 1.97 (qd, \( J = 12.4, 3.2 \) Hz, 2H), 1.67–1.57 (m, 2H), 1.41 (dq, \( J = 14.4, 7.3 \) Hz, 2H), 0.96 (t, \( J = 7.3 \) Hz, 3H). \(^{13}C\) NMR (101 MHz, CDCl\( _3 \)): \( \delta \) 183.4 (C=O), 168.4 (C=O), 159.7 (C), 142.5 (C), 137.8 (C), 133.8 (C), 133.8 (CH), 133.2 (C), 132.3 (C), 132.0 (CH), 129.6 (2 × CH), 128.4 (2 × CH), 127.4 (CH), 126.3 (CH), 126.1 (CH), 120.2 (C), 63.5 (CH\(_3\)), 54.1 (2 × CH\(_3\)), 43.6 (2 × CH\(_3\)), 32.4 (CH\(_3\)), 30.9 (CH\(_3\)), 30.0 (CH), 20.1 (CH\(_3\)), 13.9 (CH\(_3\)). HRMs calcd for \( C_{29}H_{36}NO_{3}^{+} [M + H]^{+} \) = 443.2335; found, 443.2334.

3-((Butylamino)methylene)naphthalene-1,2,4(3H)-trione (29). The compound was synthesized by following the above-mentioned general procedure (0.4 mmol lawsone). Reaction time 24 h. Aldehyde conversion 22%. The crude product was purified by FCC with PE/AcOEt (5:5) and then with PE/AcOEt (4:6) to yield 129 mg (50%) of the product as a yellow solid (mp 159 °C decomposition). \( R_f \) (Hex/AcOEt 1:1) = 0.42. \(^{1}H\) NMR (300 MHz, CDCl\( _3 \)): \( \delta \) 12.17 (s, 1H), 12.04 (s, 1H), 8.65 (d, \( J = 14.9 \) Hz, 1H), 8.54 (d, \( J = 15.0 \) Hz, 1H), 8.29–8.21 (m, 1H), 8.16 (dd, \( J = 7.2, 2.8, 1.4 \) Hz, 1H), 7.77 (td, \( J = 7.6, 1.5 \) Hz, 1H), 7.68 (td, \( J = 6.6, 1.5, 1.5 \) Hz, 1H), 1.49 (s, 9H). \(^{13}C\) NMR (75 MHz, CDCl\( _3 \)): \( \delta \) 181.7 (C=O), 181.6 (C=O), 177.7 (C=O), 157.7 (CH), 157.2 (CH), 135.1 (CH), 134.9 (CH), 134.2 (C), 133.1 (CH), 133.0 (CH), 132.9 (C), 128.1 (CH), 127.6 (CH), 127.1 (CH), 126.6 (CH), 109.6 (C), 109.0 (C), 56.1 (C), 56.0 (C), 29.6 (3 × CH\(_3\)). IR (cm\(^{-1}\)): 1348 and 1428 (CH\(_3\)), 1561 (ArH), 1562 (ArH), 1594 (C=O), 1611 (C=O), 1659 (C=O), 1668 (ArH). HRMs calcd for \( C_{32}H_{36}NO_{3}^{+} [M + H]^{+} \) = 258.1130; found, 258.1132.

X-ray Analysis of Compound 9. Data were collected at low temperature (100 K) on a Bruker APEX II diffractometer using a micro-focus-sealed X-ray tube, Mo-K\( _\alpha \) radiation (\( \lambda = 0.71073 \) Å), and equipped with an Oxford Cryosystems Cryostream Cooler Device. The structures were solved by Direct Methods using a SHELXLS-97\(^{29}\) and refined by means of least-squares procedures on \( F^{2} \) with the aid of the program SHELXL2016\(^{58}\) included in the software package WinGX version 1.63.\(^{59}\) The Atomic Scattering Factors were taken from International tables for X-ray crystallography.\(^{60}\) All hydrogen atoms were placed geometrically, except for H1 carried by the N1 atom which was located by Fourier difference maps. They were refined using an overlap model.

All nonhydrogen atoms were anisotropically refined, and in the last cycles of refinement, a weighting scheme was used, where weights are calculated from the following formula: \( w = 1/\sigma(Fo)^{2} + (ap)^{2} + bP \), where \( P = (Fo^{2} + 2Fc^{2})/3 \).

Drawing of molecules were performed with the program ORTEP3\(^{61}\) with 30% probability displacement ellipsoids for nonhydrogen atoms.

2D NMR Analysis of Compounds 9, 28, and 29. In the present study, \(^{1}H\) and \(^{13}C\) NMR spectroscopies were used for the characterization of compounds 9, 28, and 29. NMR samples were prepared by dissolving 10–20 mg of each compound in 600 \( \muL \) of CDCl\( _3 \). All spectra were recorded on a Bruker Avance 600 spectrometer equipped with a 5 mm triple-resonance inverse Z-gradient probe (TBI \(^{1}H\), \(^{13}P\), and BB). All chemical shifts for \(^{1}H\) and \(^{13}C\) are relative to TMS using \(^{1}H\) (residual) or \(^{13}C\) chemical shifts of the solvent as a secondary standard. Compound 9 was also analyzed by 2D-NMR experiment at a temperature of 298 K. All the \(^{1}H\) and \(^{13}C\) signals were assigned on the basis of chemical shifts, spin–spin coupling constants, splitting patterns, and signal intensities and by using \(^{1}H\)-H COSY, \(^{1}H\)-\(^{13}C\) HSQC, and \(^{1}H\)-\(^{13}C\) HMBC experiments. Gradient-enhanced \(^{1}H\) COSY was performed by including eight scans for per increment. \(^{1}H\)-\(^{13}C\) correlation spectra using a gradient-enhanced HSQC sequence (delay was optimized for \( T_{1}{\text{CH}} \) of 145 Hz) was obtained with 16 scans per increment. Gradient-enhanced HMBC experiment was performed, allowing 62.5 ms for long-range coupling evolution (64 scans were accumulated). Typically, 2048 t2 data points were collected for 256 t1 increments.

Computational and Docking Experiments. The AlphaFold2-predicted structure of the protein Pfbc1 was downloaded from the European Bioinformatics Institute website\(^{62}\) and preprocessed with PrepWizard [Schrodinger LLC. 2021] to add hydrogen, minimize the structure, and resolve the ionization states and clashes. The binding site was specified in analogy to the structure 4pd4 from the PDB.\(^{63}\)

The X-ray crystal protein structure (7T01, resolution = 1.60 Å) for Pf DHODH was downloaded from the PDB\(^{62}\) and preprocessed with PrepWiz [Schrodinger LLC. 2021] both with and without water molecules. Co-factors (FNM—flavin mononucleotide and ORO—orotic acid) were kept in the structure. The co-crystallized ligand DSM782 (XCV, N-(1-([5-cyano-1H-pyrazol-3-yl]ethyl)-3-methyl-4-[[1-(6-trifluoromethyl)pyridin-3-yl)cyclopropyl]-1H-pyrrrole-2-car-
boxamide) was also extracted and re-docked (self-dock) for comparison and as a control.

Chemical compounds were imported as SMILES or drawn, energy-minimized with Maestro [Schrödinger LLC, 2021], and then processed with LigPrep [Schrödinger LLC. 2021] for assigning tautomers and ionization states around pH 7 ± 2. The synthesized compounds and known inhibitors were used as controls.

Molecules were docked into the binding site of bc1 using Glide XP [Schrödinger LLC, 2021], including aromatic hydrogens as donors, halogen acceptors, and other settings.54,55

The input file is provided below:

```
FORCEFIELD OPLS_2005
GRID_CENTER − 1.9900714545454548, 9.326240250000001
GRIDFILE glide-grid_AF-Q7_2.zip
HBOND_ACCEP_HALO True
HBOND_DONOR_AROMH True
INCLUDE_INPUT_RINGS True
LIGANDFILE todock.sdf
POSTDOCK_XP_DELE 0.5
PRECISION XP
WRITE_XP_DESC False
```

Antiplasmodial Activity and Cytotoxicity. The different compounds were evaluated in vitro for their antiplasmodial activity against the *P. falciparum* resistant strain F32-ART, selected after 144 intermittent and increasing doses of artemisinin, according to our published procedures.64,65 Briefly, the antimalarial effect was first determined by Sybr Green at two doses (1 and 10 μM), in triplicate, and on two independent experiments. For compounds inducing around or more than 50% parasite growth inhibition at 10 μM, a new chemosensitivity assay using four doses, each one tested in triplicate, was then performed to determine their exact IC₅₀ values. For the best antimalarial compounds, their cytotoxicity was obtained using Vero cells, leading to the calculation of their SI as the ratio cytotoxicity/activity.

Antituberculous Activity: MIC Determination by Resazurin Reduction Microplate Assay. To determine the in vitro activity of the compounds (MIC90) in *M. tuberculosis* H37Rv, the resazurin reduction microplate assay was performed as previously described.66 Briefly, serial 2-fold dilutions (starting from 64 μg/mL (for H37Rv)) of each drug were prepared in 96-well black plates (Fluororunc, Thermo Fisher, Waltham, MA, USA) in 100 μL of Middlebrook 7H9 medium, without the addition of Tween 80. Then, log-phase cultures were diluted (OD₆00 = 0.0005) and added in a 96-well black plate. Growth controls containing no compound and sterile controls without inoculum were also included. After 7 days of incubation at 37 °C, 10 μL of resazurin (0.025% w/v) was added to each well, and bacterial viability was assessed after a further 24 h of incubation using a Fluoroskan Microplate Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA; excitation = 544 nm and emission = 590 nm). Bacterial viability was calculated as a percentage of resazurin turnover in the absence of compound. Streptomycin was used as a positive control. Results were expressed as the average of at least three independent replicates.

Antileishmanial Activity and Cytotoxicity on RAW 264.7 Macrophages. Cell Lines. The mouse monocyte/macrophage cell line RAW 264.7 and *L. donovani* (MHOM/ET/67/HU3, also called LV9) promastigotes and axenic amastigotes were maintained according to the protocols described in Pomel et al., 2021.69

Evaluation of Compound Cytotoxicity on RAW 264.7 Macrophages. Cytotoxicity was evaluated on RAW 264.7 macrophages using the resazurin method as detailed in Pomel et al., 2021.69

In Vitro Antileishmanial Evaluation on *L. donovani* Axenic Amastigotes. This evaluation was performed using the SYBR Green method as previously described.69 IC₅₀ values were calculated using the ICEstimator version 1.2 software (http://www.antimalarial-icestimator.net/runregression1.2.htm). Miltefosine was used as the reference drug.

In Vitro Antileishmanial Evaluation on Intramacrophage Amastigotes. Determination of cytotoxicity, as presented above, was used to select the highest drug concentrations that could be studied on the *L. donovani* intramacrophage amastigote model using RAW 264.7 cells. Macrophages were infected with *L. donovani* axenic amastigotes according to a ratio of 10 parasites per macrophage. In these conditions, the percentage of infected macrophages was around 80%, and the mean number of amastigotes per infected macrophage was 4 to 5 in the untreated controls. The in vitro treatment was applied 24 h post-infection, and the treatment duration was 48 h. The results of the effect of the compounds are given as percentage reduction of parasite growth, measured using the SYBR Green incorporation method. The activity of the compounds is expressed as IC₅₀ calculated using the ICEstimator version 1.2 software (Pomel et al., 2021).

Miltefosine was used as the reference drug.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03421. Crystallographic data of compound 9 (CIF)

1H and 13C NMR spectra, HRMS, and 2D NMR for compounds 9, 28, and 29 and UV–vis spectra for compounds 17, 22, 24, and 28 (PDF)

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C.L.K. carried out the synthetic work, wrote part of the manuscript, and prepared the Supporting Information; M.N. directed and wrote the computational studies; A.R. followed up the discussion and Experimental Section of this topic; A.T.G.S, C.L.K. carried out the synthetic work, wrote part of the manuscript, and prepared the Supporting Information; M.N. directed and wrote the computational studies; A.R. followed up the discussion and Experimental Section of this topic; A.T.G.S.

Notes
The authors declare no competing financial interest.

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