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Anti-Protozoal and Anti-Fungal Evaluation of 3,5-Disubstituted 1,2-Dioxolanes

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ABSTRACT

Endoperoxides are a class of compounds, which is well-known for their antimalarial properties, notably, but few reports exist about 3,5-disubstituted 1,2-dioxolanes. After having designed a new synthetic route for the preparation of these substances, they were evaluated against 4 different agents of infectious diseases, protozoa (*Plasmodium*, *Leishmania*) and Fungi (*Candida* and *Aspergillus*). Whereas moderate antifungal activity was found for our products, potent antimalarial and antileishmanial activities were observed for a few compounds. The nature of the substituents linked to the endoperoxide ring seems to play an important role in the bioactivities.

Endoperoxides are a source of potent bioactive compounds, such as important anti-malarial drugs artemisinin, artemether and arterolane.^{1–4} 1,2-Dioxane is the predominant endoperoxide structure among all peroxy natural products, however plakinic acids A-P illustrate a major family of endoperoxides exhibiting a 1,2-dioxolane scaffold with antiprotozoal, anti-HIV, antibacterial or cytotoxic activities.^{5,6} These products are in particular 3,3,5,5-tetrasubstituted on the 1,2-dioxolane ring and the interest of chemists for these compounds culminated in studies towards their total synthesis.^{7–9} Because plakinic acids, and endoperoxides in general, exhibit potent bioactivities, medicinal chemistry studies carried out by Woerpel & Clardy reported moderate antimalarial activity of many synthetic 3,3,5,5-tetrasubstituted 1,2-dioxolanes obtained from formal [3+2] cycloadditions.¹⁰ Nevertheless, Woerpel & Stockwell found also that one of the described endoperoxide, called FINO2, initiates ferroptosis through GPX4 inactivation.^{11,12} Vennerstrom *et al.* also prepared a 1,2-dioxolane analogue of arterolane using the same technique, but they found that it was about 100-fold less active than the original compound (Figure 1).^{13–15}

At the same time, mycangimycin, a new polyene endoperoxide, was identified in 2009 from some *Streptomyces* strains living in symbiosis with the southern pine beetle *Dendroctonus frontalis*.^{16,17} This compound bears a 3,5-disubstituted 1,2-dioxolane ring and was reported to possess excellent antimalarial and antifungal activities [EC₅₀ = 0.052 μ M against *Plasmodium falciparum*, MIC = 0.61 μ M against *C.*

albicans WT]. The nature of the substitution is relatively uncommon among natural products containing a 1,2-dioxolane, and by consequence we wanted to know how we could access to such a structure.¹⁸ For this purpose, we developed new methodologies to synthesize specifically 3,5-disubstituted 1,2-dioxolane rings.^{19–21} Having in hand a small chemical library resulting from our synthetic methodology studies, we decided to assess their anti-fungal, antimalarial and anti-leishmanial properties (Table 1).

Figure 1. Selected examples of reported bioactive 1,2-dioxolanes.

Chemical synthesis

The preparation of 3,5 disubstituted 1,2-dioxolanes was achieved by using two different approaches, both of them employing an oxidative ring expansion of cyclopropanols as a key step. In the first method, cyclopropanols **3b-e** were obtained by applying a Kulinkovich reaction with ethyl formate from bromides **1b-e**. Alternatively, **3a** was prepared by methylenation of aldehyde **2a**, followed by a reductive cyclization with CrCl_2 and water, because of the hindrance of *tert*-butyl group which did not permit the use of the original pathway. Cyclopropanols **3a-e** in hand, oxidative ring expansion was achieved with $\text{Mn}(\text{acac})_2$, providing 1,2-dioxolanols **4a-e** in good yields. Then, Lewis acid catalyzed acetylation furnished the corresponding acetate derivatives **5a-e**. Acetate derivatives **5a-e** were then substituted with various neutral nucleophiles by using conditions reported by

us; namely a stoichiometric protocol with a strong Lewis acid such as TiCl_4 at low temperature (-40°C) corresponding to "Conditions A",¹⁹ and a catalytic procedure using mild Lewis acid such as $\text{Sc}(\text{OTf})_3$ at room temperature, corresponding to "Conditions B".²⁰ Sakurai reaction with different allylsilanes was performed to build 1,2-dioxolanes **6c-e** and **7c-9c**; Reaction with TMSCN or TMSN_3 afforded compounds **10c** and **11c**, while Et_3SiH gave **12c**; Mukaiyama aldol reactions furnished ketones **13c-14c** from the corresponding silylenol ether and thioester **15c-16c** were obtained from the corresponding silylenol ether and thioester **15c-16c** were obtained from the corresponding silyl ketene(thio)acetals. It is noteworthy that hydroxyl compounds **4a-e** exist in equilibrium with the open hydroperoxy aldehyde form, while the structure of **5a-e**, **10c** and **11c** is blocked. Their hydrolysis in a biological medium might be possible, however 1,2-dioxanyl derivatives are much more resistant to acid hydrolysis than their tetrahydrofuranyl analogues. (Figure 2)

Figure 2. Synthesis of dioxolanes **4a-e**, **5a-e**, **6c-e**, **8c-17c**. "dr" represents *trans:cis* diastereomeric ratios. (A) or (B) refers to the conditions A or B used for the nucleophilic substitution.

Figure 3. Synthesis of dioxolanes **18f** and **19f**

A second approach is based on our pioneering work towards synthesis of analogues of mycangimycin.¹⁸ Kulinkovich reaction between olefin **20** and methyl palmitate **21** using Cha's protocol²² afforded cyclopropanol **22** and subsequent oxidative ring

expansion with molecular oxygen gave dioxolanol **23**. Reduction of the ketal with Et_3SiH and triflic acid at -78°C , followed by the silyl ether cleavage provided alcohol **18f** with the *cis* configuration predominating. Oxidation of the hydroxygroup

with periodic acid and RuCl₃ furnished **19f**, which corresponds to

the saturated side chain analogue of mycangimycin. (Figure 3)

Antifungal Evaluation

All twenty-three 1,2-dioxolanes were evaluated for antifungal activity against *Candida albicans* (CAAL93) and *Aspergillus fumigatus* ASFU76 clinical isolates; for antimalarial activity against *Plasmodium falciparum* 3D7 strain; for antileishmanial activity against *Leishmania donovani* LV9 axenic amastigote

stage. Some selected 1,2-dioxolanes were also tested against *Leishmania donovani* LV9 intramacrophage form and a cytotoxicity test was performed with those compounds on HUVEC cells to calculate the selectivity index for treatment against malaria and leishmaniasis. Results are reported in Table 1.

Table 1. Inhibitory concentration (IC₅₀), expressed in µM, of 3,5-disubstituted 1,2-dioxolanes against *Candida albicans* CAAL93 & *Aspergillus fumigatus* ASFU76 strains (controls: fluconazole & amphotericin B); against *Plasmodium falciparum* 3D7 (control: chloroquine); against *Leishmania donovani* LV9 under axenic amastigote form and intramacrophage form (control: miltefosine). Evaluation of toxicity (CC₅₀, µM) towards HUVEC cells line (control: chloroquine and miltefosine) and selectivity indexes (SI) to evaluate beneficial effect of antimalarial and antileishmanial activities (on axenic amastigote form) versus human cell death.

Compounds	<i>Candida albicans</i> (CAAL93) (IC ₅₀ , µM)	<i>Aspergillus fumigatus</i> (ASFU76) (IC ₅₀ , µM)	<i>Plasmodium falciparum</i> (3D7) (IC ₅₀ , µM)	<i>L. donovani</i> LV9 axenic amastigote (IC ₅₀ , µM)	<i>L. donovani</i> LV9 Intramacrophage (IC ₅₀ , µM)	Toxicity HUVEC (CC ₅₀ , µM)	SI <i>P. falciparum</i>	SI <i>L. donovani</i>
4a	>100	>100	0.34 ±0.03	>100	>100	12.23 ±1.63	35	<0.12
5a	>100	>100	0.02 ±0.009	>100	44.26 ±16.09	12.56 ±1.16	628	<0.13
4b	>100	>100	0.12 ±0.02	>100	>100	28.19 ±4.03	234	<0.28
5b	>100	>100	0.1 ±0.02	>100	32.86 ±13.38	59.32 ±6.07	293	<0.59
4c	>100	>100	>1	>100	>100	71.68 ±6.08	<71	<0.72
5c	>100	>100	>1	70.65 ±10.52	>100	57.83 ±1.49	<57	0.82
6c	25.24 ±3.40	>100	0.1 ±0.03	>100	ND	0.7 ±0.16	7	<0.01
7c	35.73 ±2.00	35.73 ±5.12	0.17 ±0.05	6.82 ±1.04	>100	11.69 ±1.72	68	1.71
8c	33.39 ±2.63	>100	0.15 ±0.02	1.50 ±0.09	28.35 ±15.44	6.13 ±0.55	40	4.09
9c	>100	>100	>1	1.55 ±0.21	33.56 ±8.76	2.29 ±0.14	<2	1.48
10c	>100	>100	0.03 ±0.01	19.15 ±1.77	37.61 ±11.08	12.17 ±1.48	405	0.64
11c	>100	>100	>1	42.87 ±6.16	>100	10.94 ±3.92	<10	0.26
12c	>100	>100	>1	49.77 ±1.16	ND	10.64 ±2.35	<10.6	0.2
13c	>100	>100	>1	4.42 ±1.34	>100	6.09 ±0.93	<6	1.38
14c	>100	>100	0.37 ±0.10	>100	ND	9.02 ±0.43	24	<0.09
15c	>100	>100	0.25 ±0.05	1.88 ±0.31	38.93 ±13.59	1.89 ±0.55	7	1.01
16c	>100	>100	>1	2.20 ±0.49	>100	1.82 ±0.44	<1.8	0.83
5d	>100	>100	>1	>100	ND	ND	ND	ND
6d	>100	>100	>1	>100	ND	ND	ND	ND
5e	>100	>100	>1	56.60 ±3.32	ND	ND	ND	ND
6e	>100	>100	>1	6.78 ±1.23	ND	ND	ND	ND
18f	>100	>100	>1	5.57 ±0.18	ND	ND	ND	ND
19f	>100	>100	>1	6.16 ±0.21	ND	ND	ND	ND
Fluconazole	0.30 ±0.04	-	-	-	-	-	-	-
Ampho. B	-	0.33 ±0.00	-	-	-	-	-	-
Chloroquine	-	-	0.0166 ±0.0055	-	-	31.50 ±1.40	1897	ND
Miltefosine	-	-	-	1.7 ±0.573	0.61 ±0.14	54.3 ±2.14	ND	32

ND: not determined

Antifungal evaluation

The results reported in Table 1 showed that most of compounds were inactive towards the tested fungi clinical isolates. Only compounds **6c**, **7c** and **8c** showed moderate antifungal activity, **7c** being only active against *Aspergillus*, but with IC₅₀ values approximately one hundred times higher than

references. It is also disappointing to see that the saturated analog of mycangimycin **19f** showed absolutely no inhibitory properties against tested fungi. By consequence, the role of the unsaturation on the side chain of mycangimycin is probably crucial for the antifungal activity.

Antimalarial evaluation

Evaluation of the antimalarial activity concluded that eleven compounds out of twenty-three exhibited a potential antimalarial activity below micromolar range (**4a**, **4b**, **5a**, **5b**, **6c-8c**, **10c**, **14c**, **15c**). Among them, **5a** and **10c** displayed powerful properties in this area with activities in the same range as chloroquine. Their strong antimalarial activity seems associated with the nature of the alkyl substituent, because α -branched substituents showed good antimalarial properties overall (**4a-b**, **5a-b**). The substituent put at position 3 in the last nucleophilic substitution (See Figure 2) plays also an important role, and azide obviously enhances significantly antiplasmodial properties of compound **10c**. Antimalarial activity is significantly higher for these compounds, with selectivity indexes of 628 and 405 for **5a** and **10c**, respectively. In contrast, saturated analogue of mycangimycin **19f** showed no activity below the micromolar range, which means that the potent antimalarial property of the natural product is probably not only associated with the endoperoxide scaffold, but also with the unsaturated side chain. Some complementary studies were also performed by using a mass spectrometry technique, which can detect hemin-binding of chemicals with potential antimalarial molecules such as artemisin.²³⁻²⁵ Although a certain number of our 1,2-dioxolanes showed covalent hemin-binding, no correlation with the observed antimalarial activity could be deduced (e.g. the most active compounds **5a** and **10c** did not show any hemin-binding). This suggests that the mechanism might not be the same as other known antimalarial endoperoxides,^{14,26,27} with possibly off target interactions. Moreover, azide **10c** might undergo reduction with glutathione-hemin and hemin,²⁸ to provide an unstable amino-dioxolane,²⁹ which might interfere directly on the biomineralisation of hemin by the parasite.

Antileishmanial evaluation

Among twenty-three compounds, nine 1,2-dioxolanes displayed an antileishmanial activity below 10 μ M on axenic amastigotes (**7c-9c**, **13c**, **15c**, **16c**, **6e**, **18f**, **19f**). Among them, compounds **8c**, **9c**, **15c** and **16c** exhibited a potent antileishmanial activity less than or equal to 2 μ M; the excellent bioactivities in this series seem linked to the nature of the substituents such as haloalkyl (**8c&9c**) and ethanethioates (**15c&16c**). However, the selectivity index is significantly favorable only in the case of compound **8c** (SI = 4.1). The antileishmanial activity was also evaluated with selected compounds on the intramacrophage form of *Leishmania donovani* LV9. Among 14 compounds, six exhibited a moderated effect in this series (**4a-b**, **8c**, **9c**, **10c**, **15c**). **4a-b** and **10c** showed some activity under these conditions whereas **13c** and **16c** lost their antileishmanial properties. Antileishmanial activity of **7c**, **13c**, and **16c** extinguished entirely on intramacrophage amastigotes, suggesting they could not cross the macrophages and parasitophorous vacuole properly. Nevertheless, compound **8c** is so far the best compound in the two systems and screening of further analogues of this structure would need to be undertaken.

In conclusion, this is the first time that a small library of 3,5-disubstituted dioxolanes (twenty-three compounds) was intensively tested for these infectious diseases, i.e. candidiasis, aspergillosis, malaria and leishmaniasis. Whereas no major antifungal activity was found for these compounds, we discovered that eleven compounds showed a significant antimalarial activity and nine, an interesting antimalarial activity. Among them, **5a** and **10c** demonstrated excellent antimalarial properties with a high selectivity towards parasite compared to

human cells, which should be further investigated as drug candidates. Compounds **8c-9c** and **15c-16c** displayed excellent antileishmanial activities. However, the selectivity is overall poor compared to human cells, and compound **9c** being the sole substance in the series which gives an acceptable index selectivity. Further evaluation of new series of compounds would be needed to optimize these preliminary promising results. Particularly azides and α -branched alkyl chain as substituents proved to give excellent antimalarial properties, and preparation of products combining these two substituents will be interesting to test. Haloalkyl substituents and thioesters proved to give the best results against *L. donovani*, therefore the synthesis of new endoperoxides bearing these two groups will be a next objective in the synthesis of antileishmanial compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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