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# Semisynthesis and antimicrobial activity of novel guttiferone-A derivatives

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ABSTRACT

Six derivatives of guttiferone-A (LFQM-79, 80, 81, 82, 113 and 114) were synthesized and evaluated for their antimicrobial activity against the opportunistic or pathogenic fungi *Candida albicans* (ATCC 09548), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 69548), *Candida tropicalis* (ATCC 750), *Cryptococcus neoformans* (ATCC 90012), *Trichophyton tonsurans, Microsporum gypseum* and also against the opportunistic and pathogenic Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 11778) and Gram-negative *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella typhimurium* (ATCC 14028), *Proteus mirabilis* (ATCC 25933). The antimicrobial activities of derivatives were compared with guttiferone-A and they presented to be more potent than the original molecule and sometimes greater than standard drugs established in therapeutics. The current study showed that derivatives of guttiferone-A possess potent antimicrobial activity and are relatively non-cytotoxic, which reveal these new molecules as promising new drug prototype candidates, with innovative structural pattern.



## 1. Introduction

Infectious diseases represent a critical issue for health and are the major cause of morbidity and mortality worldwide. Despite significant progress in human medicine, infectious diseases caused by microorganisms are still a serious threat to public health. The impact is even greater in developing countries due to unavailability of medicine in all locations, the practice of self-medication and the emergence of microorganism drug resistance.[1]

The polyisoprenylated benzophenone derivatives compounds are a very restricted class of plant natural products, occurring mainly in the family Guttiferae that excels due to benzophenones usually replaced by isoprenyl or geranyl subunits, which can be cyclized or not. For several polyisoprenylated benzophenones, antimicrobial, cytotoxic, antiviral, antioxidant and antiinflammatory activities have been reported.[2]

The species *Garcinia brasiliensis* is native to the Amazon region and is cultivated throughout the Brazilian territory and is known as 'bacupari'. It is a tree of medium size that blooms from August to September and presents a yellow fruit with a white and edible mucilaginous pulp. In folk medicine, the leaves of *G. brasiliensis* are used to treat tumors, inflammation of the urinary tract, arthritis and to relieve pain.[3]

Guttiferone-A (**1**) is one of the most abundant natural polyisoprenylated benzophenone isolated from *G. brasiliensis*, and many pharmacological activities have been reported for this metabolite, including anti-HIV,[4] cytotoxic,[5] trypanocidal, antiplasmodial, antioxidant,[6] inhibitory activity of cysteine and serine proteases,[7] and antimicrobial.[8,9] In a continuing search for new antimicrobial drug candidates, we elected compound **1**, that had already showed antimicrobial activities,[8,9] as a model for molecular modifications aiming the preparation of more potent derivatives and the establishment of a structure–activity relationship and evaluate the contributions of functional group modifications in the lipophilicity of the target derivatives. Hydroxyl groups at C-13 and C-14 positions were focused for chemical modifications based on their higher reactivity and on previous data[10] that indicates the importance of the chelatogenic system on ring B for biological activity. Thus, in this report we present the semisynthetic preparation of a series of six new derivatives of natural guttiferone-A (**1**), isolated from the seeds of *G. brasiliensis*, and the results of their antimicrobial evaluation.

## 2. Results and discussion

### 2.1. Semisynthesis of guttiferone-A derivatives

The synthesis of guttiferone-A (**1**) derivatives LFQM-79 (**2**), LFQM-80 (**3**), LFQM-81 (**4**), LFQM-82 (**5**), LFQM-113 (**6**) and LFQM-114 (**7**) are shown in Figure 1. The isolation of starting material was optimized from the ethyl acetate extract of seeds of *G. brasiliensis*

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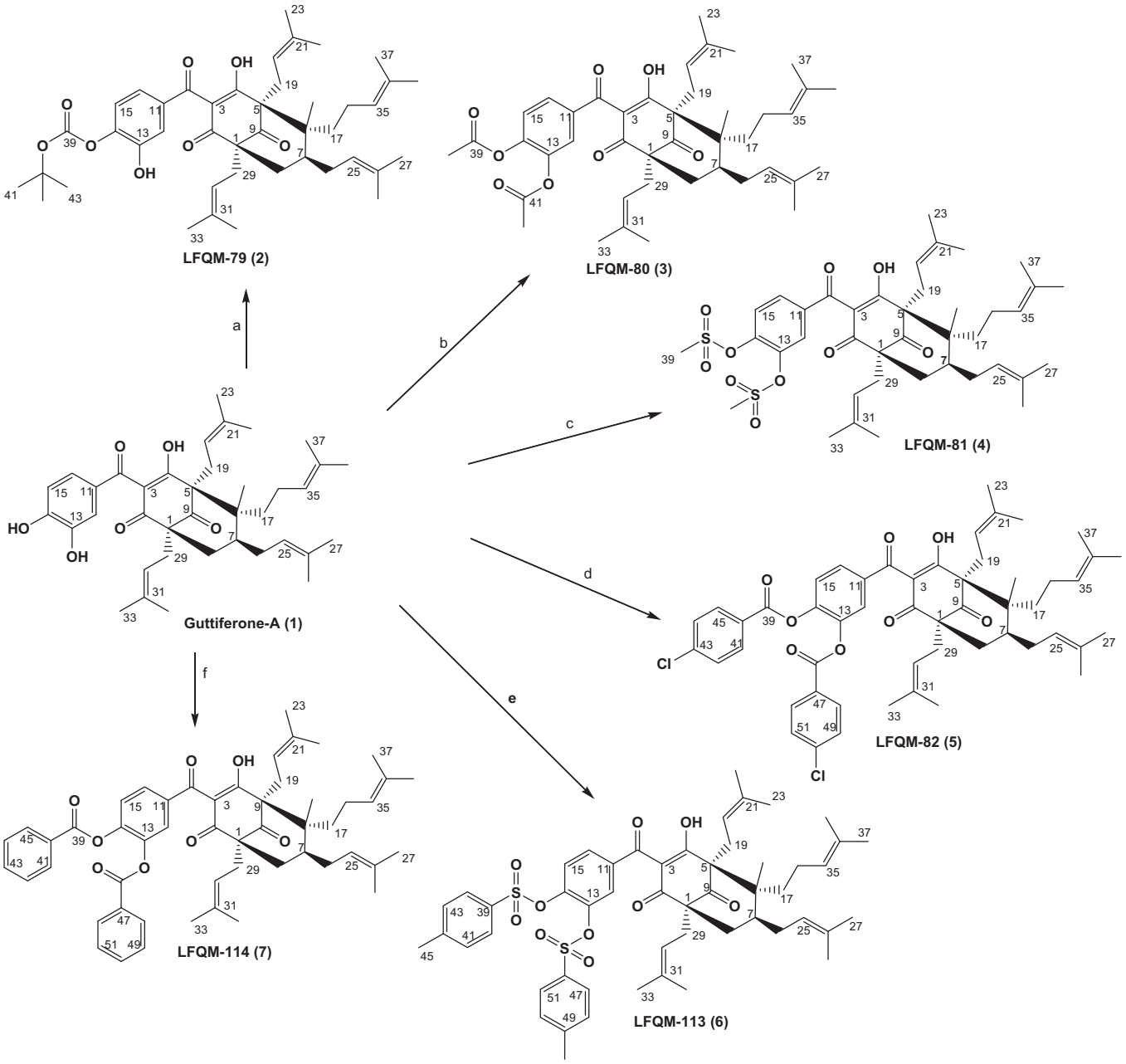


**Figure 1.** Synthesis of guttiferone-A derivatives (**2**–**7**). Reagents and conditions: (a) $Boc_2O$/DMAP in $CH_2Cl_2$; (b) $Ac_2O$/DMAP in $CH_2Cl_2$ (c) MsCl/$Et_3N$ in $CH_2Cl_2$ (d) Chlorobenzoylchoride/$K_2CO_3$ in acetone; (e) TsCl/$Et_3N$ in $CH_2Cl_2$; (f) benzoylchoride/$K_2CO_3$ in acetone.

affording compound **1** in yield from the seeds (8% yield form ethyl acetate extract). Complete $^1H$ and $^{13}C$ NMR data are displayed on Tables 5 and 6.

In order to synthesize compound LFQM-79 (**2**), guttiferone-A (**1**) was reacted with di-*tert*-butoxycarbonyl anhydride and 4-DMAP in $CH_2Cl_2$. The molecular weight of compound **2** was determined by HRESI-MS to be 725.4033 $[M+Na]^+$, consistent with a molecular formula of $C_{43}H_{58}O_8Na$.

Analysis by $^1H$ and $^{13}C$ NMR, revealed a singlet for 9H at $\delta$ 1.11, related to three equivalent methyl groups, and the presence of three carbon signals at $\delta$ 140.76, 83.18 and 26.58, attributed to the carbonyl, quaternary carbon and methyl group, respectively, confirmed the desired conversion of only one hydroxyl group into the correspondent di-*tert*-butoxycarbonyl ester.

Furthermore, the IR spectrum exhibited absorption bands at 3446 $cm^{-1}$, indicating that there was no substitution of all hydroxyl and strong absorption bands at 1766 and 1257 $cm^{-1}$, consistent, to the presence of an ester group bands. The UV spectrum displayed absorptions with $\lambda_{máx}$ ($\log\varepsilon$) at 275 nm (3.45), 250 nm (3.46) for the pure compound. Addition of $AlCl_3$ produced absorptions at

**Table 1**
Calculated lipophilicity of compounds **1**–**7** expressed as *c*Log*P* (oct/wat)

| Compound | *c*Log*P* (oct/wat) |
|---|---|
| Guttiferone-A (**1**) | 9.24 |
| LFQM-79 (**2**) | 10.82 |
| LFQM-80 (**3**) | 9.06 |
| LFQM-81 (**4**) | 5.20 |
| LFQM-82 (**5**) | 11.11 |
| LFQM-113 (**6**) | 8.75 |
| LFQM-114 (**7**) | 11.85 |

326 nm (3.35), 251 nm (3.41) and 223 nm (3.55). Addition of HCl produced absorptions at 328 nm (3.37), 252 (3.42) and 223 nm (3.59). These shift changes observed in the UV spectra produced by adding $AlCl_3$ and HCl indicated the presence of chelatogenic hydroxyl group, confirming that the reaction does not occurred on the hydroxyl group at C-4 position. According to the UV data, jointly with NMR and MS analysis, compound LFQM-79 (**2**) was deduced as 14-*O*-*tert*-butoxycarbonyl-guttiferone-A.

The reaction of **1** with acetic anhydride and triethylamine in $CH_2Cl_2$ at room temperature gave compound LFQM-80 (**3**). The molecular weight of compound **3** was determined by HRESI-MS to be 709.3709 $[M+Na]^+$, consistent with a molecular formula of $C_{42}H_{54}O_8Na$. As observed for all semisynthetic derivatives, most signals in $^1H$ and $^{13}C$ NMR spectra of 3 were similar to compound **1**, except for an extra signal at $\delta$ 2.22 (6H, s) in the $^1H$ NMR spectrum related to the two methyl groups and the signals for two methylic carbons at $\delta$ 19.55 and 19.72, and two carbonyl carbons at $\delta$ 166.45 and 166.75, observed in the $^{13}C$ NMR spectrum, indicated that two hydroxyl groups were converted to the correspondent acetate ester groups. In the IR spectrum of compound **3**, the absence of a hydroxyl absorption band at 3267 $cm^{-1}$ and the appearance of an absorption band at 1778 $cm^{-1}$, a typical stretch of C=O ester, suggested completion of the substitution reaction. The UV spectrum displayed absorptions with $\lambda_{máx}$ ($\log\varepsilon$) at 293 nm (3.84), 248 nm (3.97) and 202 nm (4.23) for the pure compound. The addition of $AlCl_3$ produced a shift at 324 nm (3.86), 252 nm (3.97) and 222 nm (4.00). The addition of HCl does not restore the spectrum, showing absorptions at 327 nm (3.88), 252 nm (3.97) and 223 nm (4.03). On the basis of these spectroscopic data, compound LFQM-80 was elucidated as 13,14-di-acetyl-guttiferone-A (**3**).

Reaction of compound **1** with methanesulfonyl chloride and triethylamine in $CH_2Cl_2$ resulted in compound LFQM-81 (**4**). The molecular weight of compound **4** was determined by HRESI-MS to be 781.3057 $[M+Na]^+$, consistent with a molecular formula of $C_{40}H_{54}O_{10}S_2Na$. The $^1H$ NMR signals at $\delta$ 3.18 and 3.21 were assigned as H-39 and H-40, respectively, and the chemical shifts of C-39 and C-40 at $\delta$ 32.63, observed in the $^{13}C$ NMR spectrum, supported the desired mesylation on positions 13 and 14 of guttiferone-A (**1**). Analysis of IR spectrum of derivative **4** showed two characteristic bands at 1373 and 1371 $cm^{-1}$, attributed to asymmetric deformation of the $S(=O)_2$ group, one band at 1180 $cm^{-1}$, related to angular symmetric deformation of $S(=O)_2$ and two bands at 1103.28 and 738.74 $cm^{-1}$ attributed to the axial strain of the group SOC. The absence of the band related to phenolic hydroxyl groups at 3485 $cm^{-1}$ suggested the replacement of two hydroxyl groups of the substrate **1**. On the basis of these spectroscopic data, compound LFQM-81 was identified as 13,14-di-methanesulfonyl-guttiferone-A (**4**).

Compound LFQM-82 (**5**) was prepared by reaction of compound **1** with chlorobenzoyl chloride and $K_2CO_3$ in acetone. The molecular weight of compound **5** was determined by HRESI-MS to be 901.3214 $[M+Na]^+$, consistent with a molecular formula of $C_{52}H_{56}Cl_2O_8Na$. The chlorobenzoylation of guttiferone-A was supported by the presence of additional signals of aromatic hydrogen at $\delta$ 7.96 (H-41, H-45, H-48 and H-52) and $\delta$ 7.38 (H-42, H-44, H-49 and H-51), related to the chlorobenzoyl subunit. The presence of chlorobenzoyl group was also confirmed in the $^{13}C$ NMR spectrum, by the signals at $\delta$ 128.47 attributed to C-42, C-44, C-49 and C-51; $\delta$ 129.87 attributed to C-41, C-45, C-48 and C-52, $\delta$ 163.91 (C-39), $\delta$ 133.77 (C-43), $\delta$ 124.10 (C-47), $\delta$ 163.47 (C-46), $\delta$ 124.48 (C-40), and $\delta$ 133.47 (C-50). Based on the absence of bands for axial deformation of OH group in the IR spectrum, it was presumed that all phenolic hydroxyl groups were substituted. Characteristic bands were observed at 1749 $cm^{-1}$ and 1253 $cm^{-1}$, corresponding to axial deformations of C=O and C–O–C=O of the ester group.

The UV spectrum displayed absorptions with $\lambda_{máx}$ ($\log\varepsilon$) at 244 nm (4.21) for the pure compound. Addition of $AlCl_3$ led to absorptions at 468 nm (2.27) and 244 nm (4.18). Addition of HCl produced absorptions at 468 nm (2.04) and 227 nm (4.12). Again, these shift changes in the UV absorption produced by adding $AlCl_3$ and HCl revealed that the chelatogenic hydroxyl group at C-4 position was preserved. According to these spectroscopic data, compound LFQM-82 was confirmed as 13,14-di-chlorobenzoyl-guttiferone-A (**5**).

Compound LFQM-113 (**6**) was obtained by reaction of compound **1** with *p*-toluenesulfonyl chloride and triethylamine in $CH_2Cl_2$. The molecular weight of compound **6** was determined by HRESI-MS to be 933.3645 $[M+Na]^+$, consistent with a molecular formula of $C_{52}H_{62}O_{10}S_2Na$. The formation of tosyl ester was evidenced in the IR spectrum by the absorption bands at 1749 $cm^{-1}$ and 1246 $cm^{-1}$ characteristic of axial deformations of C=O of ester and C–O–C=O, respectively. The formation of the tosyl-guttiferone derivative was also confirmed by $^1H$ and $^{13}C$ NMR. In the lower field region of $^{13}C$ NMR spectrum, it was observed characteristic signals for tosyl subunit at $\delta$ 130.42 (C-39), 128.57 (C-40), 129.84 (C-41), 144.05 (C-42), 129.84 (C-43), 128.57 (C-44), 127.87 (C-46), 128.57 (C-47), 129.84 (C-48), 145.77 (C-49), 129.84 (C-50), 128.57 (C-51) and the methyl carbon attached to aromatic ring 21.78 (C-45), 21.78 (C-52). The presence of aromatic hydrogen signals at $\delta$ 7.65 (H-40, 44, 47, 51) and 7.29 (H-41, 43, 48, 50), along with hydrogen signal of the methyl group bound to the benzene ring at 2.45 (H-45 and 52). The UV spectrum displayed absorptions with $\lambda_{máx}$ ($\log\varepsilon$) at 484 nm (2.10) and 227 nm (4.35), for the pure compound. Absorptions at 468 nm (2.13), 312 nm (3.55) and 222 nm (4.36) were produced when $AlCl_3$ was added. The system could not be regenerated with the addition of HCl, producing absorptions at 468 nm (1.89) and 313 nm (3.50) that was indicative of the presence of chelatogenic hydroxyl group on ring B. On the basis of these spectroscopic data, compound LFQM-113 was elucidated as 13,14-di-toluenesulphonyl-guttiferone-A (**6**).

The reaction of compound **1** with benzoyl chloride and $K_2CO_3$ in acetone gave compound LFQM-114 (**7**). The molecular weight of compound **6** was determined by HRESI-MS to 833.4012 $[M+Na]^+$, consistent with a molecular formula of $C_{52}H_{58}O_8Na$. The characteristic monosubstituted aromatic ring signals observed in the $^1H$ NMR spectra at $\delta$ 7.35, 7.45 and 8.02 ppm, and the carbon signals in $^{13}C$ NMR spectra at $\delta$ 130.20, 128.49, 133.78 showed indicated that the two phenolic hydroxyl groups of the substrate were converted to the di-benzoyl ester derivative. Furthermore, this conversion was also supported by the carbonyl peak at 1719 $cm^{-1}$ observed in the IR spectrum.

Shifts in the UV spectrum produced by adding $AlCl_3$ and HCl indicated the presence of a chelatogenic hydroxyl group. The UV spectrum displayed absorptions with $\lambda_{máx}$ ($\log\varepsilon$) at 339 nm (1.88), 233 nm (3.76) and 206 nm (3.56), for the pure compound. Adding $AlCl_3$ produced absorptions at 333 nm (2.52), 232 nm (3.84) and 207 nm (3.64). Addition of HCl produced absorptions at 335 nm (2.44), 231 nm (3.87) and 206 nm (3.73) indicating that the reaction did not occur at the hydroxyl group attached to C-4. On the basis of these spectroscopic data compound LFQM-114 was identified as 13,14-di-benzoyl-guttiferone-A (**7**).

## 2.2. Evaluation of lipophilicity

The lipophilicity of compounds **1**–**7** were estimated by theoretical calculation of partition coefficient octanol/water (*c*Log*P* oct/wat). The values are shown in Table 1, and clearly indicate that substituted sulfonyl groups, independent of their volume and size, contributed to reduce *c*Log*P* (oct/wat).

### 2.3. Antimicrobial assay

Guttiferone-A (**1**) and its derivatives **2**–**7** were evaluated for their antimicrobial activities. The results of antimicrobial assays against a panel of selected Gram-positive, Gram-negative bacteria and fungi are reported in Table 2 and 3, along with those of reference drugs chloramphenicol and amphotericin B. DMSO used as vehicle did not show any effect on bacteria and fungi.

The semisynthetic derivatives **2**–**7** exhibited a significant action against Gram-positive *S. aureus* and *B. cereus*. For *S. aureus*, compounds guttiferone-A (**1**), LFQM-79 (**2**), LFQM-80 (**3**), LFQM-81 (**4**), and LFQM-113 (**6**) were the most active, with prominent results for compounds **1**, **3**, 4 and **6** exhibiting a potency of 1.45-, 3.31-, 3.66- and 4.39-fold, respectively, more active than the standard drug chloramphenicol. Furthermore, compounds **3**, 4 and **6** were about 2.5 times more active than the natural prototype 1, showing that the modifications carried out on these derivatives were very important for activity modulation. Against *S. epidermidis,* only compound **6** showed activity 3.5-fold greater than that of chloramphenicol. For *B. cereus,* only compound **7** was not active, and all other derivatives were more active than chloramphenicol, with compounds **3** and **4** showing to be 9.13 and 1.25-fold more active, respectively, than the natural prototype 1.

For Gram-negative microorganisms, all compounds tested showed a lower activity profile, with the majority of them exhibiting only bacteriostatic properties. Compounds **2** and **7** were active against *P. aeruginosa*, while compounds **1**, 2 and **5** showed better fungistatic results against *S. typhimurium*, in comparison to chloramphenicol used as standard drug. On the other hand, compounds **2**, **4**, 5 and **7** were the most active against *E. coli*, showing to be bacteriostatic agents, while only compounds **1** and **5** were active against *P. mirabilis*, in spite of derivative **5** have showed lower activity than chloramphenicol.

Differences in the composition of the cell wall of Gram-positive and Gram-negative bacteria and a possible relationship with the lipophilic characteristics of the target molecules could be helpful to explain the difference of these antibacterial compounds. Gram-positive bacteria possess only one discrete membrane composition: acidic polysaccharides (teichoic acids) and negatively charged phospholipids. In contrast, Gram-negative bacteria present two membranes with different chemical constituents: the outer membrane containing lipopolysaccharides and the inner membrane containing negatively charged phospholipids.[11]

Considering the results obtained for guttiferone-A (**1**) and its derivatives **2**–**7**, it is possible to note that compounds **3**, 4 and **6** were the most active against Gram-positive bacteria, and that

**Table 2**
Antibacterial activity of guttiferone-A (**1**) and its derivatives **2**–**7**

| MO | μmol/mL | GUT-A (**1**) | LFQM-79 (**2**) | LFQM-80 (**3**) | LFQM-81 (**4**) | LFQM-82 (**5**) | LFQM-113 (**6**) | LFQM-114 (**7**) | CHL |
|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | $CIM_{50}$ | 8.293* | 18.000* | 0.056* | 0.051* | 0.177* | 0.043* | 1.541* | 6.034* |
| | $CIM_{100}$ | 8.293** | 142.300** | 3.639** | 3.293** | — | 2.743** | — | 12.060** |
| *S. epidermides* | $CIM_{50}$ | — | — | — | — | — | 2.743* | — | 193.420* |
| | $CIM_{100}$ | — | — | — | — | — | 109.7* | — | 386.840** |
| *B. cereus* | $CIM_{50}$ | 0.064* | 1.800* | — | 1.647* | 0.3545* | 0.171* | 123.300* | 4.820* |
| | $CIM_{100}$ | 2.073** | 28.400** | 0.227** | 1.647** | 45.459 ** | 21.940 ** | — | 77.360** |
| *P. aeruginosas* | $CIM_{50}$ | — | 58.200* | — | — | — | — | 109.700* | 48.277* |
| | $CIM_{100}$ | — | — | — | — | — | — | — | 386.841 |
| *S. typhimurium* | $CIM_{50}$ | 0.065* | 0.057* | — | — | 0.044* | — | 49.320* | 15.071* |
| | $CIM_{100}$ | — | — | — | — | — | 109.700** | — | 6.034** |
| *E. coli* | $CIM_{50}$ | — | 0.555* | 1.818* | 0.205* | 0.044* | — | 0.048* | 1.507* |
| | $CIM_{100}$ | — | — | — | — | — | — | — | 12.060 |
| *P. mirabis* | $CIM_{50}$ | 165.870* | — | — | — | 113.649* | | — | 24.130* |
| | $CIM_{100}$ | — | — | — | — | — | — | — | 48.270* |

—, Showed no significant activity.
CHL, Chloramphenicol.
* Statistically significant difference between $CIM_{50}$ results. $p < 0.05$.
** Statistically significant difference between $CIM_{100}$ results. $p < 0.05$.

**Table 3**
Antifungal activity of guttiferone-A (**1**) and its semisynthetic derivatives **2**–**7**

| MO | μmol/mL | GUT-A (**1**) | LFQM-79 (**2**) | LFQM-80 (**3**) | LFQM-81 (**4**) | LFQM-82 (**5**) | LFQM-113 (**6**) | LFQM-114 (**7**) | FLC | AMB |
|---|---|---|---|---|---|---|---|---|---|---|
| *C. albicans* | $CIM_{50}$ | 66.350* | — | — | — | — | — | — | 6.530* | |
| | $CIM_{100}$ | — | — | — | — | — | — | — | — | 0.540 |
| *C. krusei* | $CIM_{50}$ | — | 56.900* | 3.639 * | 52.700* | — | — | — | 208.960* | — |
| | $CIM_{100}$ | — | 142.300** | — | — | — | — | — | — | 1.080** |
| *C. parapsilosis* | $CIM_{50}$ | 33.175* | 142.300* | — | — | — | — | — | 52.240* | — |
| | $CIM_{100}$ | — | — | — | — | — | — | — | — | 0.540 |
| *C. grabrata* | $CIM_{50}$ | 8.293* | 56.900* | 7.279* | 131.700* | — | — | — | 208.960* | — |
| | $CIM_{100}$ | — | — | — | — | — | — | — | — | 0.540 |
| *C. tropicalis* | $CIM_{50}$ | 33.175* | — | — | — | — | — | — | 3.260* | — |
| | $CIM_{100}$ | — | — | — | — | — | — | — | | 1.080 |
| *C. neoformans* | $CIM_{50}$ | 8.293* | 0.555* | 0.056* | 52.700* | 45.459* | 43.899* | 49.320* | 6.530* | — |
| | $CIM_{100}$ | 16.587 ** | 1.818** | 3.639** | — | — | — | — | | 1.080** |
| *T. mentagrophytes* | $CIM_{50}$ | | 0.055* | | 3.293* | — | — | — | 52.240* | — |
| | $CIM_{100}$ | 33.175** | 1.818** | 3.639** | — | — | — | — | | 0.540** |
| *M. gypseum* | $CIM_{50}$ | 165.870* | 1.818* | — | — | — | — | — | 52.240* | — |
| | $CIM_{100}$ | — | 28.400** | 7.279** | — | — | — | — | | 1.080** |

—, Showed no significant activity.
FLC, fluconazole.
AMB, amphotericin B.
* Statistically significant difference between $CIM_{50}$ results. $p < 0.05$.
** Statistically significant difference between $CIM_{100}$ results. $p < 0.05$.

**Table 4**
Cytotoxic concentration ($CC_{50}$) of compounds **1–7**

| Compound | $CC_{50}$ (μmol/L) |
|---|---|
| GUTTIFERONE-A (**1**) | 116.00 |
| LFQM-79 (**2**) | >142.25 |
| LFQM-80 (**3**) | 113.55 |
| LFQM-81 (**4**) | >131.87 |
| LFQM-82 (**5**) | >113.85 |
| LFQM-113 (**6**) | >113.33 |
| LFQM-114 (**7**) | >123.39 |

these molecules exhibit the lowest *c*Log*P* (oct/wat) value, with lipophilicity of 9.06, 5.20 and 8.75, respectively. As for Gram-negative compounds LFQM-79, 82 and 114 that were active against some Gram-positive bacteria showed higher bactericidal activity than the other compounds. Despite of the reduced number of analogs, a comparative analysis of the theoretical lipophilicity of antibacterial derivatives was indicative that variation in lipophilicity could be an important requisite for modulation of the activity and, as reported in the literature,[12] compounds with higher hydrophilicity are more active against Gram-positive, while a higher lipophilicity favors the activity against Gram-negative. However, the correlation between lipophilicity and antimicrobial activity should be confirmed experimentally and does not exclude the importance of other structural factors that may be responsible for the activity of this series of compounds.

Among all compounds tested against yeast, only the natural prototype **1** showed significant fungistatic activity in comparison to Fluconazole and Amphotericin B used as standard drugs. All other derivatives were not active or showed poor activity, revealing that the phenolic hydroxyl group is a pharmacophoric subunit. For *C. neoformans* and dermatophytes *T. mentagrophytes* and *M. gypseum* the prominent derivatives were compounds **2** and **3** that showed better antifungal activity than the natural benzophenone **1**, showing that the insertion of carbonyl functional groups, like an ester or carbonate, could be auxophoric for the activity. There was not evidenced any correlation between *c*Log*P* (oct/wat) and antifungal activity among all tested derivatives.

### 2.4. Cell viability assay

Cell viability was assessed by MTT reduction assay that is a rapid and objective assay used to evaluate cellular cytotoxicity, based on a colorimetric reaction. The values of the cytotoxic concentration ($CC_{50}$) are showed in Table 4 and revealed that all derivatives exhibited higher values of minimum inhibitor concentration (MIC) required for inhibiting the growth of bacteria and fungi tested, demonstrating that compound **1** and the derivatives **2–7** are selective and less toxic compounds.

## 3. Experimental section

### 3.1. General

The 1D NMR spectra were performed on a Varian MR-400 spectrometer, operating at 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR, using $CDCl_3$ as solvent. Chemical shifts are reported in parts per million (ppm) with reference to tetramethylsilane (TMS). The coupling constants are reported in hertz (Hz) and signal multiplicities are reported as singlet (s), doublet (d), doublet of doublet (dd),

**Table 5**
$^1$H NMR data of guttiferone-A (**1**) and compounds **2–7** (400 MHz, $\delta$ ppm, $CDCl_3$)

| Position | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | GUT-A (**1**) | LFQM-79 (**2**) | LFQM-80 (**3**) | LFQM-81 (**4**) | LFQM-82 (**5**) | LFQM-113 (**6**) | LFQM-114 (**7**) |
| 7 | 2.51, m, 1H | 2.49, m, 1H | 2.45, m, 1H | 2.20, m, 1H | 2.45, m, 1H | 2.55, m, 1H | 2.45, m, 1H |
| 8 | 2.16, m, 2H | 2.15, m, 2H | 2.12, m, 2H | 2.16, m, 2H | 2.17, m, 2H | 2.19, m, 2H | 2.15, m, 2H |
| 12 | 7.05, d, 1H, *J* = 8.5 | 7.44, d, 1H, *J* = 8.2 | 7.39, d, 1H, *J* = 8.1 | 7.84, d, 1H, *J* = 8.3 | 7.57, d, 1H, *J* = 8.4 | 7.50, d, 1H, *J* = 8.6 | 7.88, d, 1H, *J* = 7.3 |
| 15 | 6.60, d, 1H, *J* = 2.1 | 7.36, d, 1H *J* = 1.9 | 7.13, d, 1H, *J* = 1.9 | 7.44, d, 1H, *J* = 2.0 | 7.51, d, 1H, *J* = 3.7 | 7.26, d, 1H, *J* = 6.0 | 7.59, d, 1H, *J* = 2.0 |
| 16 | 6.99, dd, 1H, *J* = 8.5, 2.1 | 7.41, dd, 1H, *J* = 8.2, 1.9 | 7.33, dd, 1H, *J* = 8.1, 1.9 | 7.46, dd, 1H, *J* = 8.3, 2.0 | 7.95, dd, 1H, *J* = 8.4, 3.7 | 7.18, dd, 1H, *J* = 8.6, 6.0 | 7.64, dd, 1H, *J* = 7.3, 2.0 |
| 17 | 1.96, m, 2H | 1.90, m, 2H | 1.96, m, 2H | 1.78, m, 2H | 1.85, m, 2H | 1.96, m, 2H | 2.03, m, 2H |
| 18 | 1.20, s, 3H | 1.24, s, 3H | 1.07, s, 3H | 1.01, s, 3H | 1.26, s, 3H | 1.30, s, 3H | 1.23, s, 3H |
| 19 | 2.61, m, 2H | 2.57, m, 2H | 2.52, m, 2H | 2.46, m, 2H | 2.61, m, 2H | 2.59, m, 2H | 2.56, m, 2H |
| 20 | 5.24, m, 1H | 5.20, m, 1H | 5.24, m, 1H | 5.11, m, 1H | 5.16, m, 1H | 5.20, m, 1H | 5.16, m, 1H |
| 22 | 1.70, s, 3H | 1.68, s, 3H | 1.59, s, 3H | 1.63, s, 3H | 1.67, s, 3H | 1.67, s, 3H | 1.72, s, 3H |
| 23 | 1.48, s, 3H | 1.46, s, 3H | 1.24, s, 3H | 1.45, s, 3H | 1.57, s, 3H | 1.48, s, 3H | 1.46, s, 3H |
| 24 | 2.74, m, 2H | 2.64, m, 2H | 2.59, m, 2H | 2.55, m, 2H | 2.67, m, 2H | 2.74, m, 2H | 2.59, m, 2H |
| 25 | 5.04, m, 1H | 5.14, m, 1H | 4.97, m, 1H | 4.94, m, 1H | 5.04, m, 1H | 5.04, m, 1H | 5.03, m, 1H |
| 27 | 1.57, s, 3H | 1.60, s, 3H | 1.48, s, 3H | 1.60, s, 3H | 1.64, s, 3H | 1.61, s, 3H | 1.67, s, 3H |
| 28 | 1.79, s, 3H | 1.68, s, 3H | 1.67, s, 3H | 1.65, s, 3H | 1.68, s, 3H | 1.69, s, 3H | 1.74, s, 3H |
| 29 | 2.61, m, 2H | 1.74, m, 2H | 2.48, m, 2H | 2.26, m, 2H | 2.54, m, 2H | 2.59, m, 2H | 2.52, m, 2H |
| 30 | 5.15, m,1H | 5.02, m, 1H | 5.08, m, 1H | 4.96, m, 1H | 5.03, m, 1H | 4.88, m, 1H | 4.99, m, 1H |
| 32 | 1.53, s, 3H | 1.52, s, 3H | 1.34, s, 3H | 1.48, s, 3H | 1.58, s, 3H | 1.56, s, 3H | 1.58, s, 3H |
| 33 | 1.55, s, 3H | 1.56, s, 3H | 1.41, s, 3H | 1.57, s, 3H | 1.60, s, 3H | 1.58, s, 3H | 1.60, s, 3H |
| 34 | 2.03, m, 2H | 2.06, m, 2H | 2.03, m, 2H | 2.04, m, 2H | 1.99, m, 2H | 2.07, m, 2H | 2.07, m, 2H |
| 35 | 4.92, m, 1H | 4.85, m, 1H | 4.79, m, 1H | 4.92, m, 1H | 4.88, m, 1H | 4.77, m, 1H | 4.87, m, 1H |
| 37 | 1.68, s, 3H | 1.65, s, 3H | 1.50, s, 3H | 1.60, s, 3H | 1.64, s, 3H | 1.64, s, 3H | 1.67, s, 3H |
| 38 | 1.79, s, 3H | 1.74, s, 3H | 1.67, s, 3H | 1.65, s, 3H | 1.70, s, 3H | 1.69, s, 3H | 1.74, s, 3H |
| 39 | – | – | – | 3.18, s, 6H | – | – | – |
| 40 | – | – | 2.22, s, 6H | 3.21, s, 6H | – | 7.65, d, 4H, *J* = 8.1 | – |
| 41 | – | 1.11, s, 9H | – | – | 7.99, d, 4H, *J* = 8.5 | 7.29, d, 4H, *J* = 7.0 | 8.02, d, 4H, *J* = 7.9 |
| 42 | – | 1.11, s, 9H | 2.22, s, 6H | – | 7.39, d, 4H, *J* = 9.3 | – | 7.35, t, 4H, *J* = 7.8 |
| 43 | – | 1.11, s, 9H | – | –– | – | 7.29, d, 4H, *J* = 7.0 | 7.52, t, 2H, *J* = 7.9 |
| 44 | – | – | – | – | 7.39, d, 4H, *J* = 9.3 | 7.65, d, 4H, *J* = 8.1 | 7.35, t, 4H, *J* = 7.8 |
| 45 | – | – | – | – | 7.99, d, 4H, *J* = 8.5 | 2.45, s, 6H | 8.02, d, 4H, *J* = 7.9 |
| 47 | – | – | – | – | – | 7.65, d, 4H, *J* = 8.1 | – |
| 48 | – | – | – | – | 7.99, d, 4H, *J* = 8.5 | 7.29, d, 4H, *J* = 7.0 | 8.02, d, 4H, *J* = 7.9 |
| 49 | – | – | – | – | 7.39, d, 4H, *J* = 9.3 | – | 7.35, t, 4H, *J* = 7.8 |
| 50 | – | – | – | – | – | 7.29, d, 4H, *J* = 7.0 | 7.52, t, 2H, *J* = 7.9 |
| 51 | – | – | – | – | 7.39, d, 4H, *J* = 9.3 | 7.65, d, 4H *J* = 8.1 | 7.35, t, 4H, *J* = 7.8 |

**Table 6**
$^{13}C$ NMR data of guttiferone-A (**1**) and its derivatives **2–7** (100 MHz, $\delta$ ppm, $CDCl_3$)

| Position | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | GUTTIFERONE-A (**1**) | LFQM-79 (**2**) | LFQM-80 (**3**) | LFQM-81 (**4**) | LFQM-82 (**5**) | LFQM-113 (**6**) | LFQM-114 (**7**) |
| 1 | 61.87 | 62.07 | 62.06 | 55.53 | 60.36 | 63.29 | 55.37 |
| 2 | 196.23 | 193.46 | 193.40 | 191.93 | 195.45 | 194.16 | 195.71 |
| 3 | 114.93 | 115.93 | 114.91 | 114.57 | 115.91 | 114.82 | 115.19 |
| 4 | 193.83 | 192.63 | 192.70 | 190.14 | 190.27 | 193.58 | 190.56 |
| 5 | 68.58 | 68.58 | 68.60 | 70.66 | 69.60 | 69.64 | 70.71 |
| 6 | 50.44 | 50.64 | 50.63 | 51.01 | 51.71 | 51.67 | 51.01 |
| 7 | 39.02 | 38.97 | 38.93 | 40.06 | 39.96 | 39.54 | 39.68 |
| 8 | 37.40 | 37.60 | 37.49 | 38.96 | 38.52 | 38.56 | 37.71 |
| 9 | 206.77 | 206.78 | 205.70 | 205.66 | 207.74 | 207.37 | 206.78 |
| 10 | 197.74 | 196.81 | 196.91 | 196.18 | 198.03 | 197.75 | 197.64 |
| 11 | 132.00 | 131.93 | 133.62 | 132.65 | 131.94 | 132.03 | 132.00 |
| 12 | 115.50 | 114.92 | 115.82 | 115.24 | 116.85 | 115.99 | 116.83 |
| 13 | 142.78 | 144.98 | 140.49 | 141.09 | 141.89 | 140.81 | 142.56 |
| 14 | 148.66 | 149.37 | 144.61 | 144.80 | 145.04 | 144.05 | 145.50 |
| 15 | 113.68 | 113.76 | 113.93 | 113.04 | 113.69 | 113.34 | 113.70 |
| 16 | 126.98 | 126.75 | 127.00 | 129.0 | 122.29 | 124.94 | 125.31 |
| 17 | 34.68 | 34.82 | 34.82 | 35.13 | 35.55 | 35.57 | 35.20 |
| 18 | 17.13 | 17.13 | 17.13 | 18.03 | 18.05 | 18.09 | 17.75 |
| 19 | 21.72 | 21.72 | 21.72 | 21.51 | 21.00 | 22.77 | 22.82 |
| 20 | 117.87 | 119.05 | 117.90 | 118.40 | 118.91 | 118.83 | 119.30 |
| 21 | 134.24 | 133.99 | 134.00 | 136.17 | 135.03 | 136.60 | 135.56 |
| 22 | 24.73 | 24.71 | 24.98 | 26.88 | 25.70 | 25.82 | 24.83 |
| 23 | 17.82 | 17.80 | 17.62 | 18.18 | 18.61 | 18.63 | 18.18 |
| 24 | 30.34 | 30.18 | 29.98 | 30.33 | 29.66 | 30.59 | 31.01 |
| 25 | 123.01 | 123.11 | 123.42 | 124.04 | 120.15 | 123.58 | 123.83 |
| 26 | 133.99 | 133.82 | 133.93 | 135.85 | 132.92 | 133.01 | 132.09 |
| 27 | 24.83 | 24.81 | 25.02 | 26.88 | 25.80 | 26.02 | 24.95 |
| 28 | 16.83 | 16.85 | 16.84 | 17.54 | 17.85 | 17.89 | 17.03 |
| 29 | 27.56 | 28.68 | 27.57 | 29.59 | 28.59 | 28.82 | 29.73 |
| 30 | 118.90 | 117.93 | 119.31 | 118.87 | 118.47 | 119.94 | 119.32 |
| 31 | 134.11 | 133.89 | 133.99 | 137.71 | 134.77 | 135.00 | 135.10 |
| 32 | 24.97 | 24.99 | 25.05 | 26.24 | 25.95 | 26.12 | 24.98 |
| 33 | 18.45 | 18.55 | 18.54 | 18.34 | 19.54 | 19.62 | 18.38 |
| 34 | 24.41 | 24.61 | 24.65 | 25.64 | 25.45 | 25.59 | 24.66 |
| 35 | 122.66 | 122.73 | 122.73 | 123.46 | 199.66 | 122.99 | 123.29 |
| 36 | 133.88 | 133.77 | 133.84 | 135.64 | 132.42 | 134.87 | 131.85 |
| 37 | 24.66 | 24.65 | 24.80 | 25.82 | 25.63 | 25.68 | 24.73 |
| 38 | 16.64 | 16.56 | 16.50 | 17.99 | 17.55 | 17.64 | 16.49 |
| 39 | – | 140.76 | 166.45 | 32.63 | 163.91 | 130.42 | 167.11 |
| 40 | – | 83.18 | 19.55 | 32.63 | 124.48 | 128.57 | 127.96 |
| 41 | – | 26.58 | 166.75 | – | 129.87 | 129.84 | 130.20 |
| 42 | – | 26.58 | 19.72 | – | 128.47 | 144.05 | 128.49 |
| 43 | – | 26.58 | – | – | 133.77 | 129.84 | 133.78 |
| 44 | – | – | – | – | 128.47 | 128.57 | 128.49 |
| 45 | – | – | – | – | 129.87 | 21.78 | 130.20 |
| 46 | – | – | – | – | 163.47 | 127.87 | 161.34 |
| 47 | – | – | – | – | 124.10 | 128.57 | 127.96 |
| 48 | – | – | – | – | 129.87 | 129.84 | 130.20 |
| 49 | – | – | – | – | 128.47 | 145.77 | 128.49 |
| 50 | – | – | – | – | 133.47 | 129.84 | 134.22 |
| 51 | – | – | – | – | 128.47 | 128.57 | 128.49 |
| 52 | – | – | – | – | 129.87 | 21.78 | 130.20 |

mulitplet (m). Absorption spectra in the infrared region (IR) were obtained with a Prestige-21 spectrometer using KBr pellets. Separations by column chromatography were carried out with Silica Gel 60 (60–200 mesh, Merck) and TLC experiments were carried out on Silica gel 60 F254, supported in aluminum plates (0.2 mm, Merck). Absorption spectra in the ultraviolet region were collected with a Shimadzu-2550 dual beam UV–visible spectrophotometer, as described by Mabry et al.[13] with modifications. The phenolic constituents were dissolved in ethanol (0.1%) and analyzed by scanning over the range $\lambda$ = 500–200 nm, followed by the addition of $AlCl_3$ and HCl. All chemicals and solvents were used without prior treatment, except for anhydrous reactions when solvents were dried according to the literature.

### 3.2. General

The fruits of *G. brasiliensis* were collected from species cultivated at University of Viçosa, Minas Gerais, Brazil, where its voucher specimen is deposited under the number VIC2604.

### 3.3. Extraction and purification of guttiferone-A (1)

The seeds of *G. brasiliensis* were dried in air, powdered and extracted by percolation with ethyl acetate at room temperature for 24 h. The process was repeated until all the material has been extracted and the solvent was removed under reduced pressure to furnish a crude ethyl acetate extract (SEAE). SEAE was then

submitted to column chromatography on silica gel, eluted with crescent polarity mixtures of hexanes/ethyl acetate to furnish pure guttiferone-A as a yellow solid. Its structure was confirmed by several spectroscopic techniques (IR, UV, MS and NMR) in comparison to literature data.[14]

### 3.4. Semisynthesis of guttiferone-A derivatives

#### 3.4.1. Compound LFQM-79 (2)

0.17 mmol (100 mg) of guttiferone-A (**1**) was dissolved in 5 mL of $CH_2Cl_2$. Then 0.34 mmol (0.05 mL) of $Et_3N$, 0.34 mmol (0.08 mL) of $(Boc)_2O$ and a catalytic amount of 4-DMAP were added. The mixture was stirred for a 12 h period at room temperature, until TLC analysis indicated total conversion. Next, 20 mL of $H_2O$ was added and the reaction mixture was extracted with $CH_2Cl_2$ ($3 \times 10$ mL) and dried over $MgSO_4$. The combined organic layers were evaporated and the reaction product was purified by column chromatography on silica gel using hexanes (9): ethyl acetate (1) as eluent. Compound **2** was obtained in 87% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 3446, 2981, 2931 (C–H), 1766, 1647, 1579, 1257. HRMS Calcd for $C_{43}H_{58}O_8$ $[M+Na]^+$, 725.4023, Found 725.4033. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

#### 3.4.2. Compound LFQM-80 (3)

To a solution of 0.17 mmol (100 mg) guttiferone-A (**1**) in $CH_2Cl_2$ (5 mL), were added 1 mL (10.5 mmol) of acetic anhydride and catalytic amount of 4-DMAP. The system was kept under stirring at room temperature for 4 h, monitored by TLC. After reaction was complete, water were added (20 mL) to the mixture, the aqueous phase was extracted with ethyl acetate ($3 \times 20$ mL) and dried with sodium sulfate. The combined organic layers were concentrated and the crude product was purified by column chromatography on silica gel using hexanes (9): ethyl acetate (2) as eluent. Pure compound **3** was obtained in 81% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 2970, 2922, 1778, 1666, 1608, 1201. HRMS Calcd for $C_{42}H_{54}O_8$ $[M+Na]^+$, 709.3710, Found 709.3709. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

#### 3.4.3. Compound LFQM-81 (4)

0.17 mmol (100 mg) of guttiferone-A (**1**) was dissolved in dried $CH_2Cl_2$ (5 mL) under $N_2$ atmosphere. After cooling to 5 °C, 0.34 mmol (0.05 mL) of $Et_3N$ and 0.34 mmol (0.03) of MsCl were added. The reaction was carried out with stirring, at room temperature for a 8 h period. After reaction was complete (TLC), water were added (20 mL), the aqueous phase was extracted with ethyl acetate ($3 \times 20$ mL), dried with sodium sulfate and concentrated under reduced pressure, affording the mesylate **4** in 55% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 2968, 2929, 1732, 1662, 1575, 1180, 1103. HRMS Calcd for $C_{40}H_{54}O_{10}S_2$ $[M+Na]^+$, 781.3050, Found 781.3057. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

#### 3.4.4. Compound LFQM-82 (5)

Guttiferone-A (**1**) (0.17 mmol) was dissolved in 5 mL acetone. Then, 0.34 mmol (47 mg) of $K_2CO_3$ and 0.34 mmol (0.04 mL) of 4-ClBzCl were added in sequence. The reaction mixture was stirred at room temperature for a period of 4 h. After total conversion of the starting material (TLC), the reaction material was neutralized with aqueous saturated $NaHCO_3$ and extracted with ethyl acetate ($3 \times 10$ mL). The combined organic extracts were dried over $MgSO_4$, evaporated and purified by preparative TLC using hexanes (9): EtOAc (1) as eluent, furnishing compound **5** as a pale yellow oil in 60% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 2976, 2926, 1770, 1668, 1652, 1252. HRMS Calcd for $C_{52}H_{56}Cl_2O_8$ $[M+Na]^+$, 901.3244, Found 901.3214. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

#### 3.4.5. Compound LFQM-113 (6)

0.17 mmol (100 mg) of guttiferone-A (**1**) was dissolved in 5 mL of dried $CH_2Cl_2$ (5 mL) under $N_2$ atmosphere. After cooling to 5 °C, 0.51 mmol (0.07 mL) of $Et_3N$ and 0.51 mmol (65 mg) of TsCl were added. The reaction was carried out with stirring at room temperature for 8 h, monitored by TLC. When reaction was complete, it was neutralized with aqueous saturated $NaHCO_3$ and extracted with ethyl acetate ($3 \times 10$ mL). The combined organic extracts were dried over $MgSO_4$, evaporated and purified by preparative TLC using hexanes (7): EtOAc (3) as eluent, furnishing compound **6** as a pale yellow oil in 65% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 2960, 1668, 1478, 1433, 1260, 1184, 1118, 1021, 799, 749, 716, 540. HRMS Calcd for $C_{52}H_{62}O_{10}S_2$ $[M+Na]^+$, 933.3676, Found 933.3645. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

#### 3.4.6. Compound LFQM-114 (7)

0.17 mmol (100 mg) of guttiferone-A (**1**) was dissolved in 5 mL of dried $CH_2Cl_2$. Then, 0.34 mmol (47.00 mg) of $K_2CO_3$ and 0.34 mmol (0.04 mL) of BzCl were added in sequence. The reaction mixture was stirred at room temperature for a 12 h period. After total conversion of the starting material observed by TLC, two portions of 30 mL of $CH_2Cl_2$ were added and the resultant solution was washed with aqueous saturated $NaHCO_3$. The organic extract was dried over $MgSO_4$, evaporated and purified by preparative TLC using hexanes (9.5): EtOAc (0.5) as eluent, affording compound **7** in 51% yield. IR spectrum $\nu_{max}$ $CH_2Cl_2$ $cm^{-1}$: 2964, 2916, 1749, 1600, 1681, 1246. HRMS Calcd for $C_{52}H_{58}O_8$ $[M+Na]^+$, 833.4023, Found 833.4012. $^1H$ and $^{13}C$ NMR data are presented in Tables 5 and 6.

### 3.5. The evaluation of lipophilicity by *c*Log*P* (oct/water)

Lipophilicity values were estimated through determination theoretical of *c*Log*P* (oct/wat) by using the QikProp program.[15] The QikProp program calculates the *c*Log*P* (oct/wat) values from regression equations using experimental data and molecule physical descriptors (hydrogen bond counts, atom types and charges, rotor counts, etc.) through Monte Carlo statistical mechanics simulations.[16] Calculated lipophilicity expressed by *c*Log*P* (oct/wat) of compounds **1–7** are showed on Table 1.

### 3.6. Antimicrobial activity evaluation

Guttiferone-A (**1**) and its derivatives **2–7** were evaluated for their antimicrobial activities against the fungi through a standard RPMI medium with L-glutamine and morpholinepropanesulfonic acid buffer broth microdilution method proposed by document M27A3 (CLSI, 2008)[17] and supplemented with 2% dextrose to yeasts and document M38A2 (CLSI, 2003)[18] to filamentous fungi and a standard Mueller Hinton broth microdilution method for bacteria proposed by document M7A6 (CLSI, 2003).[19] The stock solutions of compounds **1–7** were prepared in DMSO 1% at final concentration and tested at concentrations (μg/mL) 100; 62.5; 31.2; 15.6; 7.8; 3.9; 1.95; 0.48; 0.24; 0.06. The standard drug chloramphenicol was applied as a control of antibacterial action at concentrations (μg/mL) 8; 4; 2; 1; 0.5; 0.25; 0.12; 0.06; 0.03; 0.015; Amphotericin B as a control of fungicidal action at concentrations (μg/mL) 8; 4; 2; 1; 0.5; 0.25; 0.12; 0.06; 0.03; 0.015; and Fluconazole as a control of fungistatic action at concentration (μg/mL) 64; 32; 16; 8; 4; 2; 1; 0.5; 0.25; 0.125; 0.0625; 0.03125. The microplates were incubated at 35 °C for 24 h for bacterial, 37 °C for 24 h for yeast and 30 °C for 48 h for filamentous fungi. Results were visualized and analyzed by spectrophotometry. The inhibitory concentration of microbial growth was determined at 50% ($IC_{50}$) and 100% ($IC_{100}$) in μmol/mL and compared for each compound and microorganism. The tests were all done in duplicates.

### 3.7. Cell viability assay

Cell viability was determined using a modified MTT assay with peripheral human blood mononuclear cells (PBMC) obtained from healthy volunteers by Ficoll-Hypaque density gradient centrifugation. The cell suspension of normal PBMC at a concentration of $2.4 \times 10^6$ cells/mL was distributed in a 96-well plate, 90 μL in each well with 10 μL of test compounds at different concentrations, incubated at 37 °C in an incubator at 5% $CO_2$. In this assay, concentrations used were 100, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95, 0.48, 0.24, 0.06 μg/mL of the compounds tested and the plate was incubated for 48 h. After the incubation period, the morphology of cells of control and test wells were observed microscopically. After, it was added 10 μL of the dye MTT (5 mg/mL) and the cells were incubated again for an additional 4 h period. Then, the medium was carefully removed and added to 100 μL of DMSO for solubilization of formazan crystals. The plates were shaken for 5 min and absorbance for each sample was measured in a spectrophotometric microplate reader at 560 nm. The absorbance obtained from control cells, untreated, was taken as 100% cellular viability.[20] Data were analyzed using linear regression to obtain values for $CC_{50}$ (cytotoxic concentration for 50% of cells).

### 3.8. Statistical analysis

Statistical Analyses were performed using the software Prism 5.0 (GraphPad, 2007). Statistical analysis for antibacterial and antifungal activities was performed by one way ANOVA and Kruskal–Wallis. The results were shown in the number of times of increase or decrease of activity analyzed and statistical significance expressed by $p$ with a confidence interval of 95%.

## 4. Conclusions

The natural guttiferone-A (**1**) and all semisynthetic derivatives **2–7** exhibited a remarkable inhibition of a broad spectrum of Gram-positive and Gram-negative bacteria, in spite of a moderate antifungal activity of compounds **1–3**. The most highlighted results seems to be for compounds **4**–**6** that were most active against the pathogenic Gram-positive *S. aureus* and *B. cereus*, with antimicrobial effects higher than chloramphenicol, used in clinics. In a comparative analysis of all results, it was evidenced that variation in lipophilicity of the target molecules was an important requisite for modulation of antimicrobial activity. Thus, these new series of semisynthetic derivatives could be explored as new drug prototype candidates to antimicrobial agents with easy access from an abundant natural product, with low toxicity and good selectivity. Further studies should be conducted to better understand the mechanism of action of these novel molecules and their effectiveness in in vivo models.

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