Inhibition of Enterococcus faecalis biofilm formation by highly active lactones and lactams analogues of rubrolides

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MAY 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2014.05.035 · Source: PubMed

6 AUTHORS, INCLUDING:

Ulisses Alves Pereira
Universidade Federal de Viçosa (UFV)
15 PUBLICATIONS **71** CITATIONS
SEE PROFILE

Luiz Claudio De Almeida Barbosa
Federal University of Minas Gerais
355 PUBLICATIONS **2,127** CITATIONS
SEE PROFILE

Andréa Pimenta
Université de Cergy-Pontoise
25 PUBLICATIONS **226** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 82 (2014) 127–138

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Inhibition of *Enterococcus faecalis* biofilm formation by highly active lactones and lactams analogues of rubrolides

Ulisses A. Pereira [a], Luiz C.A. Barbosa [a,b,*], Célia R.A. Maltha [a], Antônio J. Demuner [a], Mohammed A. Masood [c], Andréa L. Pimenta [d,e]

[a] Department of Chemistry, Federal University of Viçosa, Av. PH Rolfs, Viçosa, MG 36570-000, Brazil
[b] Department of Chemistry, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos 6627, Campus Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil
[c] Gene Solutions, LLC, One Boars Head Pointe, Suite 131, Charlottesville, VA 22903, USA
[d] Department of Odontology, Federal University of Santa Catarina, Florianópolis, SC 88040-900, Brazil
[e] Department of Biologie, University of Cergy Pontoise, 2 Av. Adolphe Chauvin, 95302 Cergy Pontoise, France

ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form
6 May 2014
Accepted 11 May 2014
Available online 16 May 2014

Keywords:
γ-Alkylidene-γ-lactones
γ-Hydroxy-γ-lactams
γ-Alkylidene-γ-lactams
Quorum sensing antagonist
Rubrolides
Anti-biofilm activity

ABSTRACT

Seven β-aryl substituted γ-alkylidene-γ-lactones analogues of rubrolides were synthesized from mucobromic acid and converted through a lactamization with isobutylamine into their corresponding γ-hydroxy-γ-lactams (76–85%). These lactams were converted into (*Z*)- and (*E*)-γ-alkylidene-γ-lactams (23–45%). All compounds were fully characterized by IR, NMR (^{1}H and ^{13}C), COSY and HETCOR bidimensional experiments, and NOE difference spectroscopy experiments when necessary. Evaluation of these three different classes of compounds against *Enterococcus faecalis* biofilm formation showed that all classes are active and the highest biofilm inhibition activity was caused by lactam **13f** (IC_{50} = 0.76 μg/mL). Moreover, in almost all cases at least one of the lactams is more active than its correspondent γ-alkylidene-γ-lactone. The use of rubrolides as a lead structure has proven successful for the identification of new compounds displaying novel antibacterial activities, namely biofilm inhibition, which have the potential for the development of antimicrobial drugs targeted to inhibition of the initial stages of bacterial infections, rather than bacterial viability. Such drugs are less prompt to induce bacterial resistance, being therefore a more cost-effective investment for pharmaceutical research.

1. Introduction

Resistance to antibiotics has become a major clinical and public health problem affecting the lives of a large percentage of the world population. Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded by the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria has also contributed to the problem. We currently face multi-resistant infectious disease organisms that are difficult and, sometimes, impossible to treat successfully. Misuse of antibiotics has almost certainly abrogated the situation [1–3].

Biofilms are highly organized surface-associated communities of bacteria encased within an extracellular matrix [4]. Some reports on bacterial resistance have demonstrated that, when organized in biofilms, bacteria are able to survive to antibiotics treatments at concentrations up to a thousand times higher than those used to kill their planktonic counterparts [4,5]. It has been estimated that 80% of the world's microbial biomass exists in the biofilm state and that 65–80% of the microbial infections occurring in the human body are biofilm-mediated [3]. Moreover, bacterial biofilm impacts countless environments, from water pipes to indwelling devices installed within or attached in hospitalized patients. Therefore, identifying substances that are able to inhibit bacterial adherence and biofilm formation constitutes a promising approach for the development of a new generation of antimicrobial drugs targeted to bacterial virulence rather than bacterial viability, which would impose a low selection pressure on the bacterial populations, thus avoiding development of resistance [6].

From a drug discovery perspective, little research has been done in this arena for the past 25 years and there is a now resurgence in

* Corresponding author. Department of Chemistry, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos 6627, Campus Pampulha, CEP 31270-901, Belo Horizonte, MG, Brazil.
E-mail addresses: lcab@ufmg.br, lcab@ufv.br (L.C.A. Barbosa).

http://dx.doi.org/10.1016/j.ejmech.2014.05.035
0223-5234/

research activities directed to finding novel mechanisms that would circumvent bacterial resistance. Therefore, due to rapid increase of bacterial resistance to currently available antimicrobial drugs, the discovery of new molecules active against bacterial pathogens is an important area of research in academy and in the pharmaceutical industry [7]. In this context and in line with our continuous effort in the search of bioactive molecules [8–11], we embarked on a program to prepare and explore the potential bioactivity of new natural product analogues having the rubrolides (**1**) (Fig. 1) as a model compound [12–18]. This work was motivated by recent reports on the biofilm inhibitor properties of some natural halogenated butenolides (**2**) (Fig. 1), isolated from the marine red algae *Delisea pulchra* [19]. These compounds have been shown to prevent biofilm formation in *Escherichia coli* and *Bacillus subtilis* [20,21]. A similar activity was reported for the synthetic furanone **3** which is able to inhibit *E. coli* biofilm formation by 80% at 141 μM [22]. Examination of structures depicted in Fig. 1, indicates that natural and synthetic compounds **2** and **3** shares some structural features with rubrolides and they all correspond to γ-alkylidene-γ-lactones (**4**). We then hypothesized that the rubrolide analogues could also have some inhibitory effect on bacterial biofilm formation. In addition, we wanted to gain understanding of the biological mechanism by which these bacterial biofilms were prevented from forming.

Further insight into this work proposal came from the knowledge that natural products bearing a γ-hydroxy-γ-lactam (**5**) and γ-alkylidene-γ-lactam units (**6**) (Fig. 1), mainly produced by fungi [23], also display a large array of biological activities such as antifungal activity [24], cytotoxicity [25], angiogenesis inhibition properties [26], etc.

Considering that lactones could be easily transformed into the corresponding lactams [27], we envisaged that the rubrolide analogues could be converted into the corresponding γ-hydroxy-γ-lactams and γ-alkylidene-γ-lactams. To the best of our knowledge, there is no report on the bacterial biofilm inhibition caused by these classes of compounds.

In light of the needs for new substances able to inhibit the bacterial biofilm formation, we report here the results of our investigation in this area, demonstrating for the first time that rubrolide analogues and their corresponding lactams are amongst the most actives micromolecules in inhibiting biofilm formation by *Enterococcus faecalis*, an important bacteria that resides in the human gastrointestinal tract. This Gram-positive bacteria, frequently isolated from patients with endocarditis, bacteremia, urinary-tract infections, and septicemia in general, is a multi-antibiotic resistant pathogen [28]. Although there is no recent compilation data, it is known that several types of hospitalized patients suffer a great negative impact due to bacterial resistance to antibiotics and as a consequence new therapeutic agents are constantly required. We believe that the results reported here can open a new perspective for the development of a new quorum sensing antagonists that could help in the control of this pathogen.

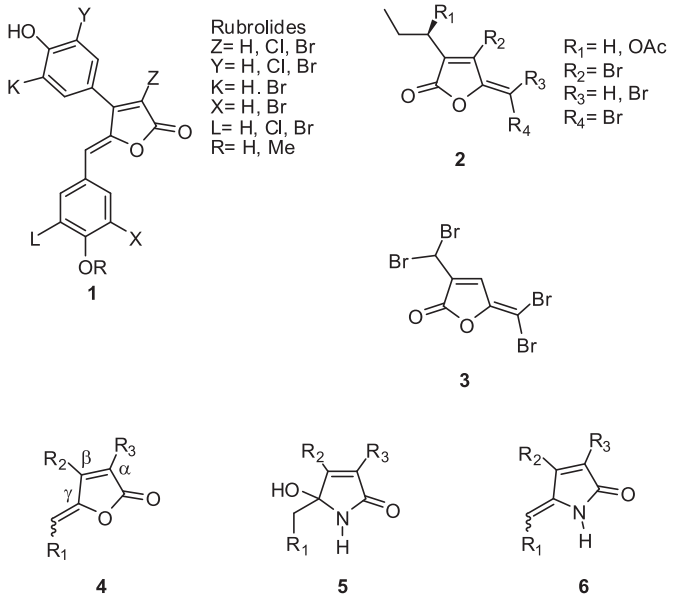


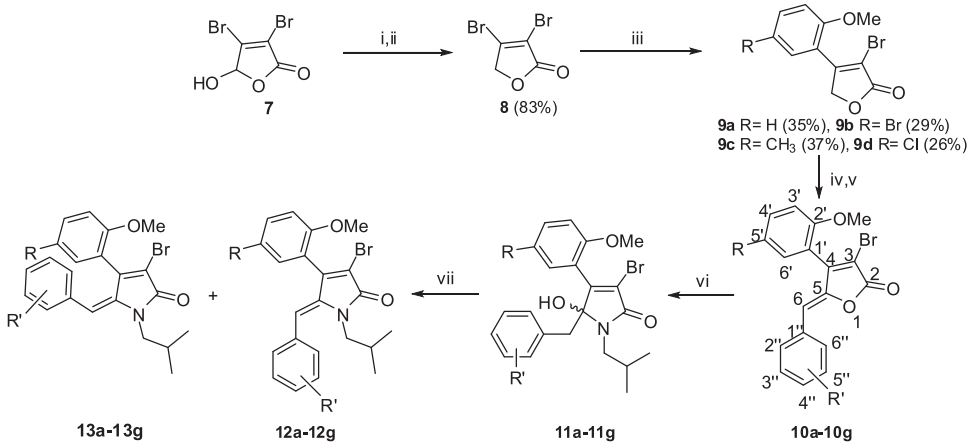
Fig. 1. General structure for naturally occurring bioactive rubrolides (**1**), furanonas displaying anti-biofilm activity (**2** and **3**), γ-alkylidene-γ-lactones (**4**), γ-hydroxy-γ-lactams (**5**) and γ-alkylidene-γ-lactams (**6**).

2. Results and discussion

2.1. Synthesis

The rubrolide analogues (**10a**–**g**) were prepared from commercially available mucobromic acid (**7**) as shown in Scheme 1. In this procedure compound **7** was converted into 3,4-dibromofuranone **8** in 83% [10]. A subsequent regioselective Suzuki–Miyaura cross-coupling between **8** and arylboronic acids in the presence of Ag_2O, $AsPh_3$ and catalytic amount of $PdCl_2(MeCN)_2$ [16] resulted in the formation of 4-aryl-3-bromofuran-2(5*H*)-ones (**9a**–**9d**) in low yields (26–37%). The reactions with boronic acids with Br or Cl at the 5′ position resulted in the required product in slightly lower yield than the corresponding analogues without electron withdrawing groups. Usually electron deficient arylboronic acids tend to be difficult coupling partners, as they are less nucleophilic and, hence transmetalate more slowly than electron-neutral and -rich analogues [29]. In addition, the low conversions of these Suzuki cross-couplings reactions can be explained in part by the formation of the biphenyls 2,2′-dimethoxybiphenyl (**9a**′), 5,5′-dibromo-2,2′-dimethoxybiphenyl (**9b**′), 5,5′-dimethyl-2,2′-dimethoxybiphenyl (**9c**′), and 5,5′-dichloro-2,2′-dimethoxybiphenyl (**9d**′) produced in 14–23% as a result of the homocoupling of the boronic acids. Structural characterization of biphenyls compounds **9a**′–**9d**′ has already been described [30–32]. Although the required products were obtained in low yields (26–37%), no effort to optimize the reaction conditions was made since at this stage we focused our attention in obtaining the final products for biological evaluation, as no data on bacterial biofilm inhibition for this class of compound has been reported yet.

On the next step 4-aryl-3-bromofuran-2(5*H*)-ones (**9a**–**9d**) were treated with aromatic aldehyde, *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf), diisopropylethylamine (DIPEA) followed by treatment of the silyl ether generated *in situ* with DBU. Initially some experiments were carried out in order to get the best reaction conditions and the β-aryl substituted γ-alkylidene-γ-lactones (**10a**–**10g**) analogues to rubrolides were prepared stereoselectively in yields ranging from 44% to 68%. All compounds were fully characterized by detailed IR, NMR, and MS analyses. The configuration of the C5–C6 double bound was clarified by NOE difference spectroscopy experiments. Irradiation of hydrogen H-6 resulted in an enhancement of H-6′ aromatic hydrogen signal. In addition enhancement of H-2″ and H-6″ aromatic hydrogen signals were also observed. These results prove the *Z* geometry for exocyclic double bound for compounds **10a**–**10g**. The stereoselectivity bias of these reactions can, in part, be related to the steric hindrance of the aromatic group on the β position. While this is a possible explanation other factors can also be involved since preferential *Z* geometry products have also been reported for similar structures without β substituent [33].



Reagents and conditions: (i) 1,5 equiv. $NaBH_4$, MeOH, 0 °C; (ii) H_2SO_4 conc., MeOH, 0 °C; (iii) boronic acid, $AsPh_3$, $PdCl_2(MeCN)_2$, Ag_2O, THF, 65 °C, 24h; (iv) aldehyde, TBDMSOTf, DIPEA, DCM; (v) DBU, reflux; (vi) isobutylamine, DCM, 0 °C, 3h; (vii) *p*-TsOH,$CHCl_3$, reflux, 2h.

R	R'	Compound (%)	Compound (%)	Compound (%)	Compound (%)
H	*p*-CF_3	**10a** (63)	**11a** (85)	**12a** (36)	**13a** (39)
Br	*m*-Cl	**10b** (44)	**11b** (78)	**12b** (28)	**13b** (31)
Br	*p*-Br	**10c** (45)	**11c** (85)	**12c** (23)	**13c** (33)
CH_3	*m*-Cl	**10d** (63)	**11d** (84)	**12d** (35)	**13d** (45)
CH_3	*o*-Cl	**10e** (68)	**11e** (84)	**12e** (29)	**13e** (39)
Cl	*o*-Br	**10f** (58)	**11f** (76)	**12f** (29)	**13f** (43)
Cl	*m*-OCH_3	**10g** (65)	**11g** (76)	**12g** (41)	**13g** (35)

Scheme 1. Preparation of γ-alkylidene-γ-lactones (**10a**–**10g**) and their corresponding γ-hydroxy-γ-lactams (**11a**–**11g**) and γ-alkylidene-γ-lactams (**12a**–**12g** and **13a**–**13g**).

A subsequent treatment of compounds **10a**–**10g** with an excess of isobutylamine resulted in the formation of the corresponding γ-hydroxy-γ-lactams (**11a**–**11g**) in good yields (76–85%) [27]. The hydroxylactams were then dehydrated with PTSA under reflux affording a mixture of *Z*- and *E*-γ-alkylidene-γ-lactams (**12a**–**12g** and **13a**–**13g**) that were isolated from all reaction after purification using column chromatography, a separation that failed for similar lactams as reported by Gupton and co-workers [34].

Detailed spectroscopic analyses (IR, ^{1}H NMR, ^{13}C NMR, and MS) analyses confirmed the structures of all compounds and stereochemistry characterization of the exocyclic double bond was achieved by NOE experiments. For example irradiation at H-6 signal resulted in NOE enhancements at H-7/H-8/H-9/H-10 absorptions, consistent with *E* configuration in the case of compounds **13a**–**13g**, once H-6 is in close proximity to isobutyl group. As shown on Scheme 1, the *E*-compounds were obtained in better yields (31–45%) than the *Z*-form (23–41%). At this point we have no final explanation for such results, but it seems to be related to a balanced steric interaction between the arylidene moiety and the *N*-isobutyl and aryl group at C-4.

Even though some reactions were low yielding, at this stage we managed to prepare a series of 28 compounds closely related to natural rubrolides, ready for the first time evaluation of their effects on biofilm formation by *E. faecalis*, an important microorganism responsible for endodontic infections [35].

2.2. Effects on E. faecalis planktonic growth

In order to select the maximum concentration for the biofilm inhibition assays all compound series (**10**–**13**) had their effect on the planktonic growth of *E. faecalis* evaluated. This is important in order to establish if eventual effects on biofilm formation were specifically due to an inhibition of bacterial adherence or could be associated to an indirect effect of the compounds on bacterial viability. For this, bacterial growth was quantified by absorbance at 630 nm readings using a microtiter plate reader device in the presence of each compound at concentrations of 87.5, 43.8, 21.9, 10.9, 5.5, 2.73, 1.37, 0.68, 0.34 and 0.17 μg/mL. The results obtained (Table S1) showed that γ-hydroxy-γ-lactam **11a** (at 87.5 μg/mL) inhibited the planktonic growth of *E. faecalis* above 20%, while all other compounds did not show any significant inhibitory effect on *E. faecalis* growth to a maximum concentration of 87.5 μg/mL. Consequently the biofilm inhibition assays were carried out with all compounds, except for **11a**, in a maximum concentration of 87.5 μg/mL.

2.3. Inhibition of E. faecalis biofilm formation

The antagonist effect of compounds **10a**–**10g**, **11a**–**11g**, **12a**–**12g** and **13a**–**13g** on biofilm formation of *E. faecalis* was tested using crystal violet staining assay and the results are shown in Table 1. For the lactone series, **10d** was the most active compound (IC_{50} = 1.51 μg/mL), followed by **10b** (IC_{50} = 6.91 μg/mL) and **10c** (IC_{50} = 18.7 μg/mL). A comparison between the effect of lactones **10a**–**g** and their corresponding nitrogen-analogues γ-lactams, **11a**, **11b**, **11d**, and **11g** did not show good inhibition of biofilm formation of *E. faecalis* (IC_{50} > 87.5 μg/mL). Compound **11c** caused some effect (IC_{50} > 33 μg/mL), while **11e** and **11f** were highly active (IC_{50} = 1.07 and 1.29 μg/mL, respectively). Among these two series of lactones (**10a**–**g**, **11a**–**g**) the structural modifications were restricted to the benzylidene ring and due to the limited number of analogues no meaningful insight on the structure–activity relationship can be obtained.

Table 1
Antagonistic effect of compounds **10a**–**10g**, **11a**–**11g**, **12a**–**12g** and **13a**–**13g** against *E. faecalis* biofilm formation.

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
10a	[a]	**11a**	[a]	**12a**	12.01 ± 4.62	**13a**	2.96 ± 0.67
10b	6.91 ± 1.73	**11b**	>87.5	**12b**	3.31 ± 1.34	**13b**	3.46 ± 0.24
10c	18.7 ± 5.1	**11c**	33.41 ± 16.96	**12c**	6.62 ± 0.35	**13c**	3.35 ± 0.43
10d	1.51 ± 0.11	**11d**	>87.5	**12d**	62.64 ± 9.68	**13d**	59.34 ± 11.13
10e	[a]	**11e**	1.07 ± 0.06	**12e**	1.09 ± 0.30	**13e**	1.04 ± 0.23
10f	>87.5	**11f**	1.29 ± 0.19	**12f**	1.48 ± 0.10	**13f**	0.76 ± 0.19
10g	53.11 ± 16.72	**11g**	>87.5	**12g**	1.46 ± 0.33	**13g**	3.26 ± 1.52

IC_{50}: concentration of compound needed to inhibit biofilm formation by 50%.
[a] The IC_{50} was not possible to be calculated due to irregular response of inhibition of *E. faecalis* biofilm in function of concentration of the solution of the compound tested.

Considering the lactams (series **12** and **13**) obtained by dehydration of the series **11**, it can be observed that 10 out of 14 compounds were highly active ($IC_{50} < 3.5$ μg/mL). For comparison, only 3 out of 14 compounds from the previously discussed series **10a**–**g** and **11a**–**g** had $IC_{50} < 3.5$ μg/mL.

A comparison between the bioactivity of isomers (*Z*)- and (*E*)-γ-alkylidene-γ-lactams indicates that both series of compounds have, in general, the same effect on *E. faecalis* biofilm formation.

Considering all lactams, those bearing chlorine or bromine at the *ortho* position on the benzylidene ring were the most active.

As the physicochemical properties of the molecules are an important aspect to be considered during a drug discovery process, we have calculated some properties for all compounds using MedChem Designer software (Table 2) [36]. The compounds were designed to determine if any structure–activity relationship could be found at this stage. Considering all 28 compounds, the "rule of five" was violated twice by 10 of them (**10c**, **11b**, **11c**, **11f**, **12b**, **12c**, **12f**, **13b**, **13c** and **13f**). Among such compounds are the most active ones as **11f**, **12f**, **13f**. These data suggest that the compounds might have poor oral bioavailability [37], and further work has to be done in order to better evaluate such hypothesis, especially considering that compounds like **10a** that was inactive did not violate any parameter of the Lipinsky's rule of five. The low polar surface area of all the compounds synthesized and tested was below 75 and there was only 1 hydrogen bond donor group or less and these features have been shown to give drugs the ability to penetrate the blood brain barrier potentially leading to harmful side effects [38].

To the best of our knowledge, none of these synthetic compounds have been tested previously as antagonists of quorum sensing regulated biofilm formation in *E. faecalis*. In the case of the Gram-positive bacteria *E. faecalis*, the quorum sensing autoinducers are constituted by cyclic peptides. One of the autoinducers named gelatinase biosynthesis-activating pheromone (GBAP) is constituted by 11 aminoacids residues forming a macrolactone ring involving the α-carboxyl group at the C-terminal methionine residue and the hydroxyl group of a serine at the third position on the other side of the peptide chain. The lactone unit was shown to be essential for the activity [39]. Based on the structure of GBAP, Nakayama, et al. (2013) [40] have used a unique drug design approach to produce a series of polypeptides capable of inhibiting the GBAP signals. The complex peptide named ZBzl-YAA5911 showed the strongest quorum sensing of *E. faecalis* antagonist activity ($IC_{50} = 262$ nM). Although a direct quantitative comparison of the levels of activities of small molecules can be misleading due to a lack of standardization between the assays [41], the most active lactam **13f** ($IC_{50} = 760$ nM) was only three time less active then the

Table 2
Predicted physicochemical properties of compounds **10**–**13**.

Compound	S + logP	S + logD	MlogP	MWt	HBDH	M-NO	T-PSA	Rule of 5
10a	5.705	5.705	3.883	425.209	0	3	35.530	0
10b	6.253	6.253	4.151	470.557	0	3	35.530	1
10c	6.353	6.353	4.259	515.013	0	3	35.530	2
10d	5.884	5.884	3.774	405.683	0	3	35.530	0
10e	5.852	5.852	3.774	405.683	0	3	35.530	0
10f	6.133	6.133	4.151	470.557	0	3	35.530	1
10g	5.715	5.715	3.234	421.682	0	4	44.760	0
11a	5.184	5.184	4.395	498.348	1	4	49.770	1
11b	5.307	5.307	4.663	543.695	1	4	49.770	2
11c	5.439	5.439	4.764	588.151	1	4	49.770	2
11d	4.964	4.964	4.293	478.821	1	4	49.770	1
11e	4.909	4.909	4.293	478.821	1	4	49.770	1
11f	5.223	5.223	4.663	543.695	1	4	49.770	2
11g	4.824	4.824	3.774	494.821	1	5	59.000	0
12a	6.281	6.281	4.721	480.332	0	3	29.540	1
12b	6.657	6.657	4.989	525.680	0	3	29.540	2
12c	6.773	6.773	5.090	570.136	0	3	29.540	2
12d	6.321	6.321	4.619	460.806	0	3	29.540	1
12e	6.277	6.277	4.619	460.806	0	3	29.540	1
12f	6.555	6.555	4.989	525.680	0	3	29.540	2
12g	6.153	6.153	4.079	476.806	0	4	38.770	0
13a	6.281	6.281	4.721	480.332	0	3	29.540	1
13b	6.657	6.657	4.989	525.680	0	3	29.540	2
13c	6.773	6.773	5.090	570.136	0	3	29.540	2
13d	6.321	6.321	4.619	460.806	0	3	29.540	1
13e	6.277	6.277	4.806	460.806	0	3	29.540	1
13f	6.555	6.555	4.989	525.680	0	3	29.540	2
13g	6.153	6.153	4.079	476.806	0	4	38.770	0

complex peptide ZBzl-YAA5911. Considering the simplicity of the molecule compared with polypeptides, and the possibility of easily increasing the molecular diversity in these rubrolide derived lactams, we believe that such compounds can be used as a new scaffold for the discovery of even more active antagonist to GBAP.

Among the quorum sensing antagonists reported, several brominated furanones share some structural features with the rubrolide derivatives reported here [22]. The authors suggested that a conjugated exocyclic vinylbromide on the furanone ring of several analogues of **3** is the most important structural feature for the biofilm inhibition formation by *E. coli*. In this work the most active compounds reported here, **11e** and **11f**, lack such functionality. We should also note that none of the active lactones or lactams has a bromine atom attached to the exocyclic C=C double bond. Han et al. (2008) [22] suggested that one bromine atom directly attached to the furanone ring seems not to be a requirement for the activity. Although all active compounds tested by us have one bromine atom on the furan ring, we cannot draw any conclusion about the influence of such group on the activity since no derivative deprived of this group was assayed.

3. Conclusion

In the present study we synthesized γ-hydroxy-γ-lactams and (*Z*)- and (*E*)-γ-alkylidene-γ-lactams from the correspondent rubrolides analogues. The potential of these compounds as inhibitors of bacterial biofilm formation was tested against *E. faecalis*, and results showed that these classes of compounds are very active, with twelve of them exhibiting IC_{50} less than 3.5 μg/mL.

Moreover, by comparing the activity of these classes of compounds against *E. faecalis* biofilm formation, we showed that the majority of the γ-alkylidene-γ-lactams were more active than the precursor lactones. Although the data obtained did not allow us to draw a clear structure–activity relationship, it has successfully allowed us to show that the nature of the substituents on both aromatic rings has a dramatic effect on the bioactivity, such effects being different for each class of compound tested. Considering that many of the compounds prepared are amongst the most active biofilm inhibitors described in the literature, further studies toward the identification of the structural elements important for the toxicity of these substances against *E. faecalis*, may result in the development of new anti-infective agents to attenuate virulence expression in this opportunistic important pathogen.

4. Material and methods

4.1. Synthesis

Reagents and solvents were purified, when necessary, according to procedures described by Perrin and Armarego [42]. The 3,4-dibromofuran-2(5*H*)-ona **8** was obtained by sodium borohydride reduction of mucobromic acid (**7**) commercially available (Aldrich – Milwaukee, USA) according to a procedure described in the literature [43]. It is important to mention that compound **7** can alternatively be prepared from furfural as previously described [44]. All reactions were carried out under a protective atmosphere of dry nitrogen. The ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 instrument (300 MHz and 75 MHz, respectively), using deuterated chloroform as a solvent and tetramethylsilane (TMS) as internal standard ($\delta = 0$). Infrared spectra were recorded on a Varian 660-IR, equipped with GladiATR scanning from 4000 to 500 cm^{-1}. Mass spectra were recorded on a Shimadzu GCMS-QP5050A instrument under electron impact (70 eV) conditions. Melting points are uncorrected and were obtained from MQAPF-301 melting point apparatus (Microquimica, Brazil). High resolution mass spectra were recorded on a Bruker MicroTof (resolution = 10,000 FWHM) under electrospray ionization (ESI) and are given to four decimal places. Analytical thin layer chromatography analysis was conducted on aluminum packed precoated silica gel plates. Column chromatography was performed over silica gel (60–230 mesh).

4.2. Synthesis

4.2.1. General procedure for the synthesis of **9a–9d**

*4.2.1.1. 3-Bromo-4-(2-methoxyphenyl)furan-2(5H)-one (**9a**).* To a two neck round-bottomed flask (100 mL) were added 3,4-dibromofuran-2(5*H*)-ona **8** (1.00 g; 4.13 mmol), 2-methoxyphenylboronic acid (0.69 g; 4.54 mmol), bis(acetonitrile) dichloropalladium(II) ($PdCl_2(CH_3CN)_2$) (0.054 g; 0.21 mmol), triphenylarsine (0.254 g; 0.83 mmol) and silver oxide (2.871 g; 12.39 mmol). Under nitrogen was added anhydrous THF (18 mL). This system was maintained under magnetic stirring at 65 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and filtered through a Celite pad. The filtrate was concentrated in a rotary evaporator to afford the resulting material of the reaction, which was purified by column chromatography on silica gel employing elutions with mixtures of dichloromethane–hexane 3:1, 2:1, 1: 1, and 1:2 v/v. This procedure afforded compound **9a** as a white solid in 35% yield (0.436 g; 1.62 mmol), m.p. 169.2–171.5 °C. $R_f = 0.28$ (hexane:dichloromethane, 1:1.5, v/v). IR (ATR) ν_{max} 3072, 2958, 1769, 1614, 1598, 1486, 1264, 1248, 1178, 1012, 978, 744, 721 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, $-OCH_3$), 5.26 (s, 2H, H-5), 6.99 (dd, 1H, $J = 8.4$, 0.9 Hz, H-3′), 7.09 (ddd, 1H, $J = 7.5$, 7.5, 0.9 Hz, H-5′), 7.48 (ddd, 1H, $J = 8.4$, 7.5, 1.5 Hz, H-4′), 7.80 (dd, 1H, $J = 7.5$, 1.5 Hz, H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8 ($-OCH_3$), 73.8 (C-5), 108.2 (C-3), 111.6 (C-3′), 118.9 (C-1′), 121.1 (C-5′), 130.3 (C-6′), 132.8 (C-4′), 157.4 (C-2′)*, 157.7 (C-4)*, 170,0 (C-2). *These assignments could be reversed; MS, *m/z* (%): 270 ($[M+2]^{+\bullet}$, 35); 268 ($[M^{\bullet}]^+$, $C_{11}H_9BrO_3$, 36), 241 (32), 239 (33), 189 (100), 161 (19), 145 (27), 133 (38), 132 (18), 131 (52), 118 (17), 115 (24), 105 (87), 103 (21), 102 (39), 89 (39), 88 (28), 71 (49), 76 (19), 75 (28), 74 (25), 63 (37), 62 (30), 51 (39), 50 (28), 39 (43), 38 (18). HRMS (ESI TOF-MS) calcd. for $[C_{11}H_9BrNaO_3]^+$: 290.9627, found 290.9628.

*4.2.1.2. 3-Bromo-4-(5-bromo-2-methoxyphenyl)furan-2(5H)-one (**9b**).* Compound **9b** was synthesized using a method similar to that of **9a** and was isolated as a white solid in 29% yield; purified by column chromatography, eluent hexane/dichloromethane (1:1.5 v/v), m.p. 169.2–171.5 °C. $R_f = 0.28$ (hexane:dichloromethane, 1:1.5, v/v). IR (ATR) ν_{max} 2997, 2964, 1769, 1618, 1591, 1482, 1470, 1401, 1266, 1245, 1181, 1139, 1062, 1012, 983, 880, 805, 749, 621 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 3H, $-OCH_3$), 5.21 (s, 2H, H-5), 6.88 (d, 1H, $J = 8.8$ Hz, H-3′), 7.56 (dd, 1H, $J = 8.8$, 2.4 Hz, H-4′), 7.88 (d, 1H, $J = 2.4$ Hz, H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.1 ($-OCH_3$), 73.5 (C-5), 109.5 (C-3), 113.3 (C-5′), 113.4 (C-3′), 120.7 (C-1′), 132.6 (C-6′), 135.2 (C-4′), 156.0 (C-2′)*, 156.5 (C-4)*, 169.5 (C-2). *These assignments could be reversed; MS, *m/z* (%): 350 ($[M+4]^{+\bullet}$, 3), 348 ($[M+2]^{+\bullet}$, 9), 346 ($[M]^{+\bullet}$, $C_{11}H_8Br_2O_3$, 2), 269 (21), 267 (19), 132 (100), 131 (32), 102 (27), 101 (15), 99 (11), 89 (14), 88 (14), 87 (33), 77 (11), 75 (26), 74 (20), 63 (24), 62 (14), 51 (33), 50 (34), 44 (22), 38 (14). HRMS (ESI TOF-MS) calcd. for $[C_{11}H_8Br_2NaO_3]^+$: 368.8732, found 368.8731.

*4.2.1.3. 3-Bromo-4-(5-methyl-2-methoxyphenyl)furan-2(5H)-one (**9c**).* Compound **9c** was synthesized using a method similar to that of **9a** and was isolated as a white solid in 37% yield; purified by column chromatography, eluent hexane/dichloromethane (1:1.5 v/v); m.p. 97.9–99.5 °C; $R_f = 0.20$ (hexane:dichlorometane, 1:1 v/v);

IR (ATR) ν_{max} 3026, 2989, 2955, 2910, 2854, 1749, 1610, 1575, 1494, 1444, 1346, 1260, 1190, 989, 822, 752, 726, 660, 560 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, 5′-CH_3), 3.83 (s, 3H, 2′-OCH_3), 5.24 (s, 2H, H-5), 6.90 (d, 1H, J = 8.4 Hz, H-3′), 7.26 (dd, 1H, J = 8.4, 2.0 Hz, H-4′), 7.60 (d, 1H, J = 2.0 Hz, H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (5′-CH_3), 55.8 (2′-OCH_3), 73.8 (C-5), 107.9 (C-3), 111.6 (C-3′), 118.5 (C-1′), 130.4 (C-5′), 130.5 (C-6′), 133.3 (C-4′), 155.4 (C-2′)*, 157.8 (C-4)*, 170.1 (C-2). *These assignments could be reversed; MS, *m/z* (%) 284 ($[M+2]^{+\bullet}$, 46), 282 ($[M]^{+\bullet}$, $C_{12}H_{11}BrO_3$, 45), 255 (27), 253 (29), 238 (17), 203 (87), 175 (25), 174 (15), 159 (42), 147 (84), 146 (23), 145 (60), 132 (26), 131 (33), 131 (33), 129 (30), 119 (90), 117 (28), 116 (42), 115 (100), 103 (33), 102 (57), 91 (77), 89 (32), 87 (16), 78 (22), 76 (29), 75 (34), 74 (31), 63 (56), 62 (24), 52 (32), 51 (91), 50 (48); HRMS (ESI TOF-MS) calcd. for $[C_{12}H_{12}BrO_3]^+$: 282.9964, found 282.9882.

*4.2.1.4. 3-Bromo-4-(5-chloro-2-methoxyphenyl)furan-2(5H)-one (**9d**).* Compound **9d** was synthesized using a method similar to that of **9a** and was isolated as white solid in 26% yield; purified by column chromatography, eluent hexane/dichloromethane (1:1 v/v). m.p. 159.4–160.6 °C. R_f = 0.23 (hexane:dichloromethane, 1:1, v/v). IR (ATR) ν_{max} 2997, 2961, 2855, 1769, 1615, 1596, 1485, 1471, 1441, 1407, 1266, 1245, 1182, 1012, 982, 880, 808, 749, 679, 640 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, $-OCH_3$), 5.23 (s, 2H, H-5), 6.95 (d, 1H, J = 9.0 Hz, H-3′), 7.43 (dd, 1H, J = 9.0, 2.7 Hz, H-4′), 7.77 (d, 1H, J = 2.7 Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.2 ($-OCH_3$), 73.5 (C-5), 109.4 (C-3), 113.0 (C-3′), 120.2 (C-1′), 126.2 (C-5′), 129.8 (C-6′), 132.3 (C-4′), 156.0 (C-2′)*, 156.1 (C-4)*, 169.6 (C-2). *These assignments could be reversed; MS, *m/z* (%): 306 ($[M+4]^{+\bullet}$, 21), 304 ($[M+2]^{+\bullet}$, 85), 302 ($[M]^{+\bullet}$, $C_{11}H_8BrClO_3$, 64), 275 (71), 273 (54), 260 (30), 225 (35), 223 (100), 179 (42), 167 (57), 165 (62), 139 (48), 136 (35), 132 (49), 101 (54), 87 (46), 75 (53). HRMS (ESI TOF-MS) calcd. for $[C_{11}H_9BrClO_3]^+$: 302.9418, found 302.9367.

*4.2.2. General procedure for the synthesis of **10a–10g***

*4.2.2.1. (Z)-3-bromo-4-(2-methoxyphenyl)-5-(4-trifluoromethylbenzylidene)furan-2(5H)-one (**10a**).* To a two-neck round-bottom flask under nitrogen atmosphere were added 3-bromo-4-(2-methoxyphenyl)furan-2(5*H*)-one **9a** (900 mg, 3.34 mmol), dichloromethane (20 mL), TBDMSOTf (1.688 mL, 7.35 mmol), DIPEA (1.745 mL, 10.02 mmol), and *p*-trifluoromethylbenzaldehyde (0.651 mL, 3.74 mmol). The resulting mixture was stirred at room temperature over 1 h. After DBU (1.099 mL, 7.35 mmol) was added, the reaction mixture was refluxed for an additional 3 h before the addition of dichloromethane (70 mL). The resulting organic layer was washed with aqueous HCl 3 mol L^{-1} solution (2 × 40 mL) and brine (3 × 40 mL). After separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting material was purified by column chromatography on silica gel eluted with hexane/dichloromethane (3:2 v/v) to afford compound **10a** in 63% yield (896 mg, 2.11 mmol). m.p. 147.6–149.3 °C. IR (ATR) ν_{max} 3006, 1779, 1608, 1492, 1322, 1249, 1164, 1066, 1020, 975, 872, 840, 597 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 3H, $-OCH_3$), 5.94 (s, 1H, H-6), 7.09 (dd, 1H, J = 8.4, 0.9 Hz, H-3′), 7.12 (ddd, 1H, J = 7.5, 7.5, 0.9 Hz, H-5′), 7.27 (dd, 1H, J = 7.5, 1.8 Hz, H-6′), 7.54 (ddd, 1H, J = 8.4, 7.5, 1.8 Hz, H-4′), 7.61 (d, 2H, J = 8.4 Hz, H-3″ and H-5″), 7.85 (d, 2H, J = 8.4 Hz, H-2″ and H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.9 ($-OCH_3$); 111.6 (C-6), 111.9 (C-3′), 112.2 (C-3), 117.7 (C-1′), 121.0 (C-5′), 124.1 (q, J = 270.6 Hz, $-CF_3$), 125.9 (q, J = 3.8 Hz, C-3″ and C-5″), 130.5 (C-6′), 130.5 (q, J = 32.2 Hz, C-4″), 130.9 (C-2″ and C-6″), 132.4 (C-4′), 136.3 (C-1″), 149.3 (C-5), 152.5 (C-4)*, 156.8 (C-2′)*, 165 (C-2). *These assignments could be reversed. MS, *m/z* (%) 426 ($[M+2]^{+\bullet}$, 68), 424 ($[M]^{+\bullet}$, $C_{19}H_{12}BrF_3O_3$, 66), 345 (41), 317 (33), 167 (22), 163 (37), 159 (31), 158 (43), 135 (18), 131 (81), 119 (54), 115 (45), 103 (30), 102 (25), 101 (17), 91 (27), 89 (53), 77 (100), 63 (24), 62 (24), 39 (35). HRMS (ESI TOF-MS) calcd. for $[C_{19}H_{12}BrF_3NaO_3]^+$: 446.9814, found 446.9816.

*4.2.2.2. (Z)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(3-chlorobenzylidene)furan-2(5H)-one (**10b**).* Compound **10b** was synthesized using a method similar to that of **10a** and was isolated as pale green solid in 44% yield; purified by column chromatography, eluent hexane/dichloromethane (3:2 v/v). m.p. 149.5–150.6 °C. R_f = 0.31 (hexane:dichloromethane, 3:2, v/v). IR (ATR) ν_{max} 3057, 2963, 2937, 1764, 1600, 1488, 1459, 1260, 1226, 1119, 1019, 984, 960, 883, 780, 679 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H, $-OCH_3$), 5.84 (s, 1H, H-6), 6.95 (d, 1H, J = 9.0 Hz, H-3′), 7.30–7.32 (m, 2H, H-4″ and H-5″), 7.37 (d, 1H, J = 2.4 Hz, H-6′), 7.63 (dd, 1H, J = 9.0, 2.4 Hz, H-4′); 7.66–7.67 (m, 1H, H-6″), 7.71–7.72 (m, 1H, H-2″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.2 ($-OCH_3$), 112.2 (C-6), 112.3 (C-3), 113.0 (C-5′), 113.6 (C-3′), 119.6 (C-1′), 129.0 (C-6″), 129.7 (C-5″)*, 130.3 (C-4″)*, 130.6 (C-2″), 132.8 (C-6′), 134.4 (C-1″)**, 134.9 (C-4′), 135.0 (C-3″)**, 148.2 (C-5), 151.0 (C-4), 156.0 (C-2′), 164.8 (C-2). *, **These assignments could be reversed; MS, *m/z* (%) 474 ($[M+6]^{+\bullet}$, 1), 472 ($[M+4]^{+\bullet}$, 7), 470 ($[M+2]^{+\bullet}$, 11), 468 ($[M]^{+\bullet}$, $C_{18}H_{11}Br_2ClO_3$, 4), 310 (16), 211 (11), 209 (14), 176 (12), 152 (18), 130 (25), 124 (21), 118 (28), 102 (61), 89 (100), 88 (26), 87 (47), 75 (21), 63 (35), 39 (28), 36 (15). HRMS (ESI TOF-MS) calcd. for $[C_{18}H_{12}Br_2ClO_3]^+$: 468.8836, found 468.8886.

*4.2.2.3. (Z)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(4-bromobenzylidene)furan-2(5H)-one (**10c**).* Compound **10c** was synthesized using a method similar to that of **10a** and was isolated as white solid in 45% yield; purified by column chromatography, eluent hexane/dichloromethane (3:2 v/v). m.p. 157.5–158.6 °C. R_f = 0.32 (hexane:dichloromethane, 3:2, v/v). IR (ATR) ν_{max} 3022, 2941, 2843, 1773, 1644, 1575, 1481, 1403, 1266, 1250, 1178, 1121, 1008, 978, 874, 836, 800, 693, 750, 622 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 3H, $-OCH_3$), 5.85 (s, 1H, H-6), 6.96 (d, 1H, J = 9.0 Hz, H-3′), 7.37 (d, 1H, J = 2.4 Hz, H-6′), 7.50 (d, 2H, J = 8.4 Hz, H-3″ and H-5″), 7.62 (d, 2H, J = 8.4 Hz, H-2″ and H-6″), 7.63 (dd, 1H, J = 9.0, 2.4 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.2 ($-OCH_3$), 111.9 (C-3), 112.6 (C-6), 112.9 (C-5′), 113.6 (C-3′), 119.7 (C-1′)*, 124.2 (C-4″)*, 131.7 (C-1″), 132.3 (C-2″, C-3″, C-5″ and C-6″), 132.8 (C-6′), 134.9 (C-4′), 147.9 (C-5), 150.9 (C-4), 156.0 (C-2′), 164.9 (C-2). *These assignments could be reversed; MS, *m/z* (%) 518 ($M+6]^{+\bullet}$, 4), 516 ($[M+4]^{+\bullet}$, 11), 514 ($[M+2]^{+\bullet}$, 12), 512 ($[M]^{+\bullet}$, $C_{18}H_{11}Br_3O_3$, 4), 197 (11), 196 (15), 168 (12), 130 (13), 118 (13), 102 (30), 89 (100), 87 (26), 63 (32), 39 (22). HRMS (ESI TOF-MS) calcd. for $[C_{18}H_{12}Br_3O_3^+$: 512.8331, found 512.8310.

*4.2.2.4. (Z)-3-bromo-5-(3-chlorobenzylidene)-4-(5-methyl-2-methoxyphenyl)furan-2(5H)-one (**10d**).* Compound **10d** was synthesized using a method similar to that of **10a** and was isolated as pale yellow solid in 63% yield; purified by column chromatography, eluent hexane/dichloromethane (3:2 v/v). m.p. 138.2–139.6 °C. R_f = 0.25 (hexane:dichloromethane, 3:2, v/v). IR (ATR) ν_{max} 3008, 2929, 1769, 1644, 1497, 1457, 1248, 1230, 986, 928, 882, 810, 780, 697, 679 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.37 (s, 3H, Ar–CH_3), 3.81 (s, 3H, $-OCH_3$), 5.87 (s, 1H, H-6), 6.95 (d, 1H, J = 8.4 Hz, H-3′); 7.04 (d, 1H, J = 1.8 Hz, H-6′), 7.28–7.31 (m, 2H, H-4″ and H-5″), 7.30–7.33 (m, 1H, H-4′), 7.63–7.67 (m, 1H, H-6″), 7.71 (m, 1H, H-2″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (Ar–CH_3), 55.9 ($-OCH_3$), 111.5 (C-3), 111.8 (C-3′), 112.1 (C-6), 117.4 (C-1′), 128.9 (C-6″), 129.4 (C-4″)*, 130.2 (C-5″)*, 130.3 (C-5′), 130.5 (C-2″), 130.7 (C-6′), 132.7 (C-4′), 134.7 (C-1″), 135.0 (C-3″), 148.7 (C-5), 152.7 (C-4), 154.6 (C-2′), 165.3 (C-2). *These assignments could be reversed; MS, *m/z* (%) 408 ($[M+4]^{+\bullet}$, 20), 406 ($[M+2]^{+\bullet}$, 76), 404 ($[M]^{+\bullet}$, $C_{19}H_{14}BrClO_3$, 58), 325 (26), 297 (29), 279 (17), 219 (21), 177 (67), 165 (17), 149 (42), 145

(76), 133 (64), 129 (29), 116 (35), 115 (75), 105 (31), 102 (86), 111 (49), 91 (48), 89 (100), 63 (43), 51 (23). HRMS (ESI TOF-MS) calcd. for $[C_{19}H_{15}BrClO_3]^+$: 404.9888, found 404.9779.

*4.2.2.5. (Z)-3-bromo-5-(2-chlorobenzylidene)-4-(5-methyl-2-methoxyphenyl)furan-2(5H)-one (**10e**).* Compound **10e** was synthesized using a method similar to that of **10a** and was isolated as pale yellow solid in 68% yield; purified by column chromatography, eluent hexane/dichloromethane (3:2 v/v). m.p. 121.3–122.6 °C. R_f = 0.27 (hexane:dichloromethane, 3:2, v/v). IR (ATR) ν_{max} 3063, 2998, 2964, 2832, 1766, 1613, 1584, 1499, 1436, 1250, 1109, 996, 979, 814, 737 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.37 (s, 3H, Ar–C$\underline{H}_3$), 3.83 (s, 3H, –OC$\underline{H}_3$), 6.49 (s, 1H, H-6), 6.97 (d, 1H, J = 8.4 Hz, H-3′), 7.11 (d, 1H, J = 2.4 Hz, H-6′), 7.25 (ddd, 1H, J = 7.8, 7.5, 1.8 Hz, H-5″), 7.32 (ddd, 1H, J = 7.8, 7.5, 1.8 Hz, H-4″), 7.32 (dd, 1H, J = 8.4, 2.4 Hz, H-4′), 7.37 (dd, 1H, J = 7.8, 1.8 Hz, H-3″), 8.24 (dd, 1H, J = 7.8, 1.8 Hz, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (Ar–$\underline{C}H_3$), 55.9 (–O$\underline{C}H_3$), 109.2 (C-6), 111.3 (C-3), 111.8 (C-3′), 117.4 (C-1′), 127.5 (C-4″), 129.9 (C-3″), 130.3 (C-5′), 130.4 (C-5″), 130.9 (C-1″), 131.0 (C-6′), 132.1 (C-6″), 132.8 (C-4′), 134.9 (C-2″), 148.7 (C-5), 153.0 (C-4), 154.7 (C-2′), 165.5 (C-2). *These assignments could be reversed; MS, *m/z* (%) 408 ($[M+4]^{+\bullet}$, 26), 406 ($[M+2]^{+\bullet}$, 100), 404 ($[M]^{+\bullet}$, $C_{19}H_{14}BrClO_3$, 77), 325 (31), 297 (30), 279 (15), 253 (20), 251 (19), 189 (17), 177 (48), 173 (16), 149 (35), 145 (61), 133 (39), 129 (23), 116 (26), 115 (54), 102 (65), 95 (40), 89 (73), 63 (27). HRMS (ESI TOF-MS) calcd. for $[C_{19}H_{15}BrClO_3]^+$: 404.9888, found 404.9878.

*4.2.2.6. (Z)-3-bromo-5-(2-bromobenzylidene)-4-(5-chloro-2-methoxyphenyl)furan-2(5H)-one (**10f**).* Compound **10f** was synthesized using a method similar to that of **10a** and was isolated as pale yellow solid in 58% yield, purified by column chromatography, eluent hexane/dichloromethane (3:2 v/v). m.p. 154.3–155.7 °C. R_f = 0.25 (hexane:dichloromethane, 3:2, v/v). IR (ATR) ν_{max} 3097, 3061, 3010, 2968, 2845, 1768, 1645, 1487, 1462, 1249, 1184, 1025, 981, 825, 751, 671 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, –OC$\underline{H}_3$), 6.44 (s, 1H, H-6), 7.02 (d, 1H, J = 9.0 Hz, H-3′), 7.18 (ddd, 1H, J = 7.8, 7.8, 1.8 Hz, H-4″), 7.32 (dd, 1H, J = 2.7 Hz, H-6′), 7.37 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-5″), 7.49 (dd, 1H, J = 9.0, 2.7 Hz, H-4′), 7.58 (dd, 1H, J = 7.8, 1.2 Hz, H-3″), 8.20 (dd, 1H, J = 7.8, 1.8 Hz, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.2 (–O$\underline{C}H_3$), 112.0 (C-3), 112.1 (C-6), 113.1 (C-3′), 119.1 (C-1′), 125.8 (C-2″), 126.0 (C-5′), 128.1 (C-5″), 130.3 (C-6′), 130.8 (C-4″), 132.0 (C-4′), 132.3 (C-6″), 132.3 (C-1″), 133.3 (C-3″), 148.1 (C-5), 151.3 (C-4), 155.4 (C-2′), 165.1 (C-2); MS, *m/z* (%) 474 ($[M+6]^{+\bullet}$, 8), 472 ($[M+4]^{+\bullet}$, 38), 470 ($[M+2]^{+\bullet}$, 48), 468 ($[M]^{+\bullet}$, $C_{18}H_{11}Br_2ClO_3$, 23), 391 (20), 273 (19), 198 (16), 197 (18), 196 (21), 176 (17), 169 (17), 165 (27), 102 (32), 101(29), 94 (18), 89 (100), 88 (24), 87 (25), 63 (28). HRMS (ESI TOF-MS) calcd. for $[C_{18}H_{12}Br_2ClO_3]^+$: 468.8836, found 468.8822.

*4.2.2.7. (Z)-3-bromo-4-(5-chloro-2-methoxyphenyl)-5-(3-methoxybenzylidene)furan-2(5H)-one (**10g**).* Compound **10g** was synthesized using a method similar to that of **10a** and was isolated as pale green solid in 65% yield, purified by column chromatography, eluent hexane/dichloromethane (1:2 v/v). m.p. 137.8–139.4 °C. R_f = 0.44 (hexane:dichloromethane, 1:2, v/v). IR (ATR) ν_{max} 3049, 3009, 2831, 1767, 1756, 1640, 1598, 1487, 1459, 1250, 1161, 1123, 989, 869, 822, 789, 686, 641 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 6H, 2 × –OC$\underline{H}_3$), 5.90 (s, 1H, H-6), 6.89–6.93 (m, 1H, H-4″), 7.01 (d, 1H, J = 9.0 Hz, H-3′), 7.25 (d, 1H, J = 2.4 Hz, H-6′), 7.26–7.35 (m, 3H, H-2″, H-5″ and H-6″), 7.48 (dd, J = 9.0, 2.7 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (–O$\underline{C}H_3$), 56.6 (–O$\underline{C}H_3$), 111.6 (C-3), 113.2 (C-3′), 113.9 (C-6), 115.6 (C-2″), 116.1 (C-4″), 119.4 (C-1′), 123.8 (C-6″), 125.9 (C-5′), 130.0 (C-5″), 130.1 (C-6′), 131.8 (C-4′), 133.9 (C-1″), 147.6 (C-5)*, 151.1 (C-4), 155.5 (C-2′), 160.0 (C-3″), 165.1 (C-2); MS, *m/z* (%) 424 ($[M+4]^{+\bullet}$, 26), 422 ($[M+2]^{+\bullet}$, 100), 420 ($[M]^{+\bullet}$, $C_{19}H_{14}BrClO_4$, 75), 341 (24), 278 (38), 250 (14), 225 (22), 223 (23), 189 (16), 165 (22), 161 (29), 149 (24), 148 (67), 121 (26), 102 (26), 101 (24), 91 (58), 89 (25), 77 (34), 51 (41). HRMS (ESI TOF-MS) calcd. for $[C_{19}H_{14}BrClNaO_4]^+$: 442.9656, found 446.9661.

*4.2.3. General procedure for the synthesis of **11a–11g***

*4.2.3.1. 3-Bromo-5-(4-trifluoromethylbenzyl)-5-hydroxy-1-isobutyl-4-(2-methoxyphenyl)pyrrol-2(5H)-one (**11a**).* To a two-neck round-bottom flask were added (*Z*)-3-bromo-4-(2-methoxyphenyl)-5-(4-trifluoromethylbenzylidene)furan-2(5*H*)-one (**10a**) (650 mg, 1.53 mmol) dissolved in dichloromethane (8 mL) and the solution was cooled to 0 °C. Then the isobutylamine (560 mg, 7.65 mmol) dissolved in dichloromethane was added in a dropwise fashion and the resulting mixture was stirred at 0 °C for 3 h before the addition of dichloromethane (70 mL). After this time, the mixture was quenched with HCl aqueous solution (2 mol L^{-1}, 2 × 55 mL), and washed with saturated $NaHCO_3$ solution (2 × 55 mL) and brine (2 × 55 mL). After separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting material was purified by column chromatography on silica gel eluted with hexane/dichloromethane (1:7 v/v). The procedure described afforded compound **11a** in 85% yield (646 mg, 1.30 mmol). m.p. 160.4–161.2 °C. IR (ATR) ν_{max} 3450–3180, 2963, 2873, 1695, 1682, 1492, 1418, 1324, 1250, 1158, 1115, 1067, 1019, 927, 862, 753, 642, 618, 500 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, 3H, J = 6.6 Hz, H-9), 0.98 (d, 3H, J = 6.6 Hz, H-10), 2.25–2.39 (m, 1H, H-8), 3.12 (d, 1H, J = 14.4 Hz, H-6a), 3.13 (dd, 1H, J = 13.8, 7.8 Hz, H-7a), 3.20 (d, 1H, J = 14.4 Hz, H-6b), 3.53 (dd, 1H, J = 13.8, 8.1 Hz, H-7b), 3.76 (s, 3H, –OC$\underline{H}_3$), 5.13 (s, 1H, –OH), 6.83 (dd, 1H, J = 8.4, 0.9 Hz, H-3′), 6.98 (ddd, 1H, J = 7.5, 7.5, 0.9 Hz, H-5′), 7.05 (dd, 1H, J = 7.5, 1.8 Hz, H-6′), 7.07 (d, 2H, J = 8.1 Hz, H-2″ and H-6″), 7.34 (d, 2H, J = 8.1 Hz, H-3″ and H-5″), 7.36–7.31 (m, 1H, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (C-9), 20.9 (C-10), 27.9 (C-8), 42.9 (C-6), 48.3 (C-7), 56.2 (–O$\underline{C}H_3$), 93.3 (C-5), 111.7 (C-3′), 120.7 (C-3)**, 121.7 (C-5′), 122.1 (C-1′)**, 124.3 (q, J = 270.5 Hz, –$\underline{C}F_3$), 124.9 (q, J = 3.7 Hz, C-3″ and C-5″), 129.2 (q, J = 32.3 Hz, C4″), 130.4 (C-2″ and C-6″), 131.4 (C-6′), 131.5 (C-4′), 138.5 (C-1″), 153.3 (C-2′)*, 155.8 (C-4)*, 164 (C-2). *, **These assignments could be reversed; MS, *m/z* (%) 481 (7), 479 (6), 356 (9), 341 (17), 340 (100), 339 (19), 338 (99), 284 (25), 282 (25), 241 (11), 239 (11), 159 (43), 132 (13), 131 (26), 115 (14), 109 (12), 89 (11), 77 (19), 57 (23), 55 (12), 43 (21), 41 (61), 39 (18), 30 (17). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{23}BrF_3NNaO_3]^+$: 520.0706, found 520.0705.

*4.2.3.2. 3-Bromo-4-(5-bromo-2-methoxyphenyl)-5-(3-chlorobenzyl)-5-hydroxy-1-isobutylpyrrol-2(5H)-one (**11b**).* Compound **11b** was synthesized using a method similar to that of **11a** and was isolated as white solid in 78% yield, purified by column chromatography, eluent hexane/dichloromethane (1:9 v/v). m.p. 171.4–173.0 °C. R_f = 0.17 (hexane:dichloromethane, 1:9, v/v). IR (ATR) ν_{max} 3385–3205, 3007, 2960, 2928, 2868, 2843, 1693, 1680, 1485, 1415, 1386, 1245, 1183, 1083, 1025, 780, 701, 682, 650, 621 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.95 (d, 3H, J = 6.9 Hz, H-9), 0.97 (d, 3H, J = 6.9 Hz, H-10), 2.22–2.36 (m, 1H, H-8), 2.97 (d, 1H, J = 14.4 Hz, H-6a), 3.15 (dd, 1H, J = 14.1, 7.5 Hz, H-7a), 3.16 (d, 1H, J = 14.4 Hz, H-6b), 3.52 (dd, 1H, J = 14.1, 7.8 Hz, H-7b), 3.77 (s, 3H, –OC$\underline{H}_3$), 6.73 (d, 1H, J = 9.0 Hz, H-3′), 6.86 (ddd, 1H, J = 7.8, 1.6, 1.6 Hz, H-6″), 6.96–6.98 (m, 1H, H-2″), 7.05 (dd, 1H, J = 7.8, 7.8 Hz, H-5″), 7.09 (ddd, 1H, J = 7.8, 1.6, 1.6 Hz, H-4″), 7.18 (d, 1H, J = 2.4 Hz, H-6′), 7.40 (dd, 1H, J = 9.0, 2.4 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8 (C-9), 20.9 (C-10), 28.0 (C-8), 42.7 (C-6), 48.2 (C-7), 56.4 (–O$\underline{C}H_3$), 93.4 (C-5), 113.1 (C-3′), 113.6 (C-5′), 122.6 (C-3 and C-1′), 127.4 (C-4″), 128.1 (C-6″), 129.3 (C-5″), 130.4 (C-2″), 133.4 (C-6′), 133.9 (C-4′ and C-3″), 136.1 (C-1″), 151.6 (C-2′), 155.0 (C-4), 164.5 (C-2); MS, *m/z* (%) 420 (48), 418 (100), 416 (50), 362 (25), 360 (13), 283 (15), 281

(15), 202 (13), 127 (15), 125 (41), 89 (14), 57 (55), 56 (17), 55 (18). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{23}Br_2ClNO_3]^+$: 541.9728, found 541.9572.

*4.2.3.3. 3-Bromo-4-(5-bromo-2-methoxyphenyl)-5-(4-bromobenzyl)-5-hydroxy-1-isobutylpyrrol-2(5H)-one (**11c**).* Compound **11c** was synthesized using a method similar to that of **11a** and was isolated as white solid in 85% yield, purified by column chromatography, eluent hexane/dichloromethane (1:7 v/v). m.p. 172.5–173.5 °C. R_f = 0.22 (hexane:dichloromethane, 1:7, v/v). IR (ATR) ν_{max} 3399–3154, 2957, 2869, 2844, 1676, 1645, 1486, 1385, 1247, 1050, 1026, 809, 653, 620, 506 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, 3H, J = 6.6 Hz, H-9), 0.98 (d, 3H, J = 6.6 Hz, H-10), 2.23–2.37 (m, 1H, H-8), 3.02 (d, 1H, J = 14.7 Hz, H-6a), 3.11 (d, 1H, J = 14.7 Hz, H-6b), 3.13 (dd, 1H, J = 14.1, 7.2 Hz, H-7a), 3.53 (dd, 1H, J = 14.1, 8.1 Hz, H-7b), 3.73 (s, 3H, −OCH$_3$), 6.74 (d, 1H, J = 8.7 Hz, H-3′), 6.83 (d, 2H, J = 8.4 Hz, H-2″ and H-6″), 7.10 (d, 1H, J = 2.7 Hz, H-6′), 7.25 (d, 2H, J = 8.4 Hz, H-3″ and H-5″), 7.45 (dd, 1H, J = 8.7, 2.7 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (C-9), 21.0 (C-10), 28.0 (C-8), 42.3 (C-6), 48.3 (C-7), 56.5 (−OCH$_3$), 93.5 (C-5), 113.2 (C-3′), 113.6 (C-5′), 121.4 (C-4″), 122.6 (C-1′ and C-3), 131.2 (C-3″ and C-5″), 131.6 (C-2″ and C-6″), 133.0 (C-1″), 133.6 (C-6′), 133.7 (C-4′), 151.7 (C-2′), 155.0 (C-4), 164.4 (C-2); MS, *m/z* (%) 420 (47), 418 (100), 416 (50), 364 (10), 362 (20), 360 (10), 283 (13), 281 (13), 171 (12), 169 (16), 90 (15), 89 (12), 57 (26). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{22}Br_3NNaO_3]^+$: 607.9042, found 607.9052.

*4.2.3.4. 3-Bromo-5-(3-chlorobenzyl)-5-hydroxy-1-isobutyl-4-(5-methyl-2-methoxyphenyl)pyrrol-2(5H)-one (**11d**).* Compound **11d** was synthesized using a method similar to that of **11a** and was isolated as white solid in 84% yield, purified by column chromatography, eluent hexane/dichloromethane (1:8 v/v). m.p. 140.0–140.5 °C. R_f = 0.30 (hexane:dichloromethane, 1:8, v/v). IR (ATR) ν_{max} 3376–3226, 2957, 2928, 2869, 1691, 1678, 1638, 1500, 1434, 1414, 1248, 1064, 1017, 882, 801, 785, 686, 656 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.96 (d, 3H, J = 6.9 Hz, H-10), 0.99 (d, 3H, J = 6.9 Hz, H-9), 2.30 (s, 3H, Ar−CH$_3$), 2.26–2.38 (m, 1H, H-8), 3.05 (s, 2H, H-6), 3.14 (dd, 1H, J = 13.8, 7.2 Hz, H-7a), 3.55 (dd, 1H, J = 13.8, 8.1 Hz, H-7b), 3.78 (s, 3H, −OCH$_3$), 5.37 (s, 1H, −OH), 6.75–6.77 (m, 1H, H-2″), 6.78 (d, 1H, J = 9.0 Hz, H-3′), 6.85 (ddd, 1H, J = 8.1, 1.5, 1.5 Hz, H-6″), 6.91 (d, 1H, J = 2.1 Hz, H-6′), 7.05 (dd, 1H, J = 8.1, 8.1 Hz, H-5″), 7.10 (ddd, 1H, J = 8.1, 1.5, 1.5 Hz, H-4″), 7.14 (dd, J = 9.0, 2.1 Hz, H-4′) ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (C-8), 20.7 (C-9), 20.9 (C-10), 28.0 (Ar−CH$_3$), 42.9 (C-6), 48.3 (C-7), 56.5 (−OCH$_3$), 93.4 (C-5), 111.7 (C-3′), 120.4 (C-3)**, 121.7 (C-1′)**, 127.3 (C-4″), 127.8 (C-6″), 129.3 (C-5″), 130.8 (C-6′), 131.3 (C-5′), 131.9 (C-4′), 132.0 (C-2″), 133.7 (C-3″), 136.3 (C-1″), 153.4 (C-2′)*, 153.7 (C-4)*, 164.6 (C-2). *, **These assignments could be reversed. MS, *m/z* (%) 354 (99), 352 (100), 298 (18), 296 (19), 146 (11), 145 (17), 125 (21), 115 (13), 57 (27). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{26}BrClNO_3]^+$: 478.0779, found 478.0798.

*4.2.3.5. 3-Bromo-5-(2-chlorobenzyl)-5-hydroxy-1-isobutyl-4-(5-methyl-2-methoxyphenyl)pyrrol-2(5H)-one (**11e**).* Compound **11e** was synthesized using a method similar to that of **11a** and was isolated as white solid in 84% yield, purified by column chromatography, eluent hexane/dichloromethane (1:8 v/v). m.p. 128.3–129.8 °C. R_f = 0.28 (hexane:dichloromethane, 1:8, v/v). IR (ATR) ν_{max} 3401–3174, 2959, 2928, 2668, 1689, 1675, 1411, 1385, 1244, 1056, 806, 730, 686, 656, 620 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (d, 3H, J = 6.9 Hz, H-9), 0.95 (d, 3H, J = 6.9 Hz, H-10), 2.21 (s, 3H, Ar−CH$_3$), 2.25–2.35 (m, 1H, H-8), 3.05 (dd, 1H, J = 14.1, 7.2 Hz, H-7a), 3.26 (d, 1H, J = 15.0 Hz, H-6a), 3.35 (d, 1H, J = 15.0 Hz, H-6b), 3.50 (dd, 1H, J = 14.1, 7.8 Hz, H-7b), 3.77 (s, 3H, −OCH$_3$), 5.22 (s, 1H, −OH), 6.71–6.72 (m, 1H, H-6′), 6.73 (d, 1H, J = 8.7 Hz, H-3′), 7.01–7.04 (m, 2H, H-4″ and H-5″), 7.08 (dd, 1H, J = 8.7, 2.1 Hz, H-4′), 7.17 (dd, 1H, J = 6.6, 2.7 Hz, H-3″), 7.25 (dd, 1H, J = 6.9, 2.7 Hz, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.5 (Ar−CH$_3$), 20.7 (C-9), 20.8 (C-10), 27.8 (C-8), 38.7 (C-6), 48.2 (C-7), 56.5 (−OCH$_3$), 93.4 (C-5), 111.5 (C-3′), 120.3 (C-3)**, 121.7 (C-1′)**, 126.6 (C-5″), 128.3 (C-4″), 129.3 (C-3″), 130.4 (C-6″), 131.5 (C-6′), 131.5 (C-4′), 131.2 (C-5′), 132.8 (C-2″), 135.2 (C-1″), 153.6 (C-2′)*, 154.1 (C-4)*, 164.7 (C-2). *, **These assignments could be reversed; MS, *m/z* (%) 354 (98), 352 (100), 298 (19), 296 (19), 145 (17), 127 (11), 125 (27), 115 (12), 57 (19). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{26}BrClNO_3]^+$: 478.0779, found 478.0804.

*4.2.3.6. 3-Bromo-5-(2-bromobenzyl)-4-(5-chloro-2-methoxyphenyl)-5-hydroxy-1-isobutylpyrrol-2(5H)-one (**11f**).* Compound **11f** was synthesized using a method similar to that of **11a** and was isolated as white solid in 76% yield, purified by column chromatography, eluent hexane/dichloromethane (1:9 v/v). m.p. 159.3–160.5 °C. R_f = 0.31 (hexane:dichloromethane, 1:9, v/v). IR (ATR) ν_{max} 3221–3397, 3068, 2936, 2864, 2842, 1688, 1673, 1486, 1411, 1390, 1239, 1137, 1046, 890, 806, 751 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (d, 3H, J = 6.6 Hz, H-9), 0.95 (d, 3H, J = 6.6 Hz, H-10), 2.19–2.36 (m, 1H, H-8), 3.07 (dd, 1H, J = 13.8, 7.8 Hz, H-7a), 3.30 (d, 1H, J = 15 Hz, H-6a), 3.41 (d, 1H, J = 15 Hz, H-6b), 3.50 (dd, 1H, J = 13.8, 7.8 Hz, H-7b), 3.76 (s, 3H, −OCH$_3$), 4.86 (s, 1H, −OH), 6.75 (d, 1H, J = 9.0 Hz, H-3′), 6.96 (ddd, 1H, J = 7.8, 7.8, 1.8 Hz, H-4″), 6.99 (d, 1H, J = 2.4 Hz, H-6′), 7.06 (ddd, 1H, J = 7.8, 7.8, 1.5 Hz, H-5″), 7.23 (dd, 1H, J = 9.0, 2.4 Hz, H-4′), 7.25 (dd, 1H, J = 7.8, 1.8 Hz, H-6″), 7.41 (dd, 1H, J = 7.8, 1.5 Hz, H-3″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (C-9), 20.9 (C-10), 27.8 (C-8), 41.5 (C-6), 48.2 (C-7), 56.6 (−OCH$_3$), 93.6 (C-5), 112.6 (C-3′), 122.0 (C-3)*, 122.6 (C-1′)*, 126.4 (C-2″): 126.5 (C-5′), 127.3 (C-5″), 128.8 (C-4″), 130.3 (C-6″), 130.6 (C-6′), 130.7 (C-4′), 132.8 (C-3″), 134.1 (C-1″), 152.2 (C-2′), 154.4 (C-4), 164.6 (C-2). *These assignments could be reversed; MS, *m/z* (%) 529 ($[M+6]^{+\bullet} - H_2O$, 3), 527 ($[M+4]^{+\bullet} - H_2O$, 16), 525 ($[M+2]^{+\bullet} - H_2O$, 24), 523 ($[M]^{+\bullet} - H_2O$, 10), 376 (17), 375 (16), 374 (100), 372 (73), 317 (22), 316 (16), 171 (10), 169 (14), 165 (12), 57 (18), 41 (41). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{23}Br_2ClNO_3]^+$: 541.9728, found 541.9700.

*4.2.3.7. 3-Bromo-4-(5-chloro-2-methoxyphenyl)-5-hydroxy-1-isobutyl-5-(3-methoxybenzyl)pyrrol-2(5H)-one (**11g**).* Compound **11g** was synthesized using a method similar to that of **11a** and was isolated as white solid in 76% yield, purified by column chromatography, eluent dichloromethane. m.p. 152.0–153.3 °C. R_f = 0.20 (dichloromethane). IR (ATR) ν_{max} 3187–3416, 3069, 3003, 2960, 2931, 2868, 2837, 1685, 1671, 1592, 1490, 1434, 1412, 1253, 1104, 701, 655, 640 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.97 (d, 3H, J = 6.6 Hz, H-9), 0.98 (d, 3H, J = 6.6 Hz, H-10), 2.25–2.39 (m, 1H, H-8), 3.07 (d, 1H, J = 14.7 Hz, H-6a), 3.14 (d, 1H, J = 14.7 Hz, H-6b), 3.14 (dd, 1H, J = 13.8, 7.2 Hz, H-7a), 3.56 (dd, 1H, J = 13.8, 8.1 Hz, H-7b), 3.73 (s, 3H, −OCH$_3$), 3.80 (s, 3H, −OCH$_3$), 4.63 (s, 1H, −OH), 6.50 (s, 1H, H-2″), 6.56 (d, 1H, J = 7.8 Hz, H-4″), 6.71 (m, 1H, H-6″), 6.84 (d, 1H, J = 8.7 Hz, H-3′), 6.94 (d, 1H, J = 2.4 Hz, H-6′), 7.09 (dd, 1H, J = 7.8, 7.8 Hz, H-5″), 7.30 (dd, 1H, J = 8.7, 2.4 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7 (C-9), 20.9 (C-10), 27.9 (C-8), 42.5 (C-6), 48.4 (C-7), 55.4 (−OCH$_3$), 56.7 (−OCH$_3$), 93.8 (C-5), 112.9 (C-3′), 113.3 (C-6″), 115.1 (C-2″), 122.2 (C-4″), 122.3 (C-3)*, 122.7 (C-1′)*, 126.5 (C-5′), 129.3 (C-5″), 130.8 (C-4′), 131.0 (C-6′), 135.5 (C-1″), 151.9 (C-2′), 154.6 (C-3″), 159.5 (C-4), 164.3 (C-2). *These assignments could be reversed; MS, *m/z* (%) 479 ($[M+4]^{+\bullet} - H_2O$, 10), 477 ($[M+2]^{+\bullet} - H_2O$, 37), 475 ($[M]^{+\bullet} - H_2O$, 29), 396 (15), 375 (17), 374 (100), 373 (11), 372 (76), 340 (29), 318 (25), 316 (18), 122 (25), 121 (25), 91 (15), 57 (26), 41 (44). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{26}BrClNO_4]^+$: 494.0728, found 494.0837.

4.2.4. General procedure for the synthesis of **12a–12g** and **13a–13g**

4.2.4.1. Synthesis of (Z)-3-bromo-5-(4-trifluoromethylbenzylidene)-1-isobutyl-4-(2-methoxyphenyl)pyrrol-2(5H)-one (**12a**) and (E)-3-bromo-5-(4-trifluoromethylbenzylidene)-1-isobutyl-4-(2-methoxyphenyl)pyrrol-2(5H)-one (**13a**).

To a round-bottom flask under nitrogen atmosphere were added 3-bromo-5-(4-trifluoromethylbenzyl)-5-hydroxy-1-isobutyl-4-(2-methoxyphenyl)pyrrol-2(5*H*)-one **11a** (250 mg, 0.502 mmol) dissolved in dry $CHCl_3$ (20 mL) and *p*-toluenesulfonic acid (14 mg, 0.075 mmol). After reflux for 2 h, the reaction was diluted with additional chloroform (20 mL) and quenched with saturated aqueous $NaHCO_3$ solution (3 × 20 mL) and washed with brine (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting material was purified by column chromatography on silica gel eluted with hexane/dichloromethane (1:2.5 v/v). The procedure described afforded compound **12a** in 36% yield (87 mg, 0.18 mmol) and **13a** in 39% yield (95 mg, 0.20 mmol).

Data for **12a**: pale yellow solid. m.p. 100.3–101.3 °C. R_f = 0.38 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 3050, 2958, 2921, 2850, 1698, 1615, 1491, 1455, 1407, 1322, 1254, 1161, 1120, 1065, 908, 843, 751, 655 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.53 (d, 6H, J = 6.9 Hz, H-9 and H-10), 1.33–1.45 (m, 1H, H-8), 3.42 (dd, 1H, J = 14.1, 7.5 Hz, H-7a), 3.47 (dd, 1H, J = 14.1, 7.5 Hz, H-7b), 3.84 (s, 3H, $-OC\underline{H}_3$), 6.07 (s, 1H, H-6), 7.04 (dd, 1H, J = 8.4, 0.9 Hz, H-3′), 7.07 (ddd, 1H, J = 7.5, 7.5, 0.9 Hz, H-5′), 7.25 (dd, 1H, J = 7.5, 1.8 Hz, H-6′), 7.37 (d, 2H, J = 8.1 Hz, H-2″ and H-6″), 7.47 (ddd, 1H, J = 8.4, 7.5, 1.8 Hz, H-4′), 7.61 (d, 2H, J = 8.1 Hz, H-3″ and H-5″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.5 (C-9), 19.6 (C-10), 27.9 (C-8), 49.6 (C-7), 55.9 ($-O\underline{C}H_3$), 111.8 (C-3′), 113.0 (C-6), 116.6 (C-3), 119.7 (C-1′), 120.7 (C-5′), 124.1 (q, J = 270 Hz, $-\underline{C}F_3$), 125.2 (q, J = 3.7 Hz, C-3″ and C-5″), 129.9 (C-2″ and C-6″), 130.1 (q, J = 32.5 Hz, C-4″), 131.2 (C-6′), 131.3 (C-4′), 138.8 (C-1″), 140.1 (C-5), 146.9 (C-4), 157.2 (C-2′), 167.1 (C-2); MS, *m/z* (%) 481 ($[M+2]^{+\bullet}$, 74), 479 ($[M]^{+\bullet}$, $C_{23}H_{21}BrF_3NO_2$, 74), 401 (14), 400 (51), 358 (15), 357 (57), 356 (100), 354 (24), 345 (17), 344 (80), 342 (15), 322 (23), 320 (23), 314 (19), 313 (24), 286 (14), 272 (15), 241 (15), 207 (42), 189 (16), 159 (51), 131 (28), 115 (31), 77 (38), 57 (20): 55 (21), 41 (97), 39 (40). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{22}BrF_3NO_2]^+$: 480.0781, found 480.0834.

Data for **13a**: amorphous green solid. R_f = 0.27 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 2960, 2929, 2873, 1704, 1614, 1490, 1463, 1322, 1163, 1122, 1089, 1066, 1018, 753 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.02 (d, 3H, J = 6.9 Hz, H-9); 1.03 (d, 3H, J = 6.9 Hz, H-10), 2.10–2.23 (m, 1H, H-8), 3.50 (s, 3H, $-OC\underline{H}_3$), 3.63 (dd, 1H, J = 14.4, 7.5 Hz, H-7a), 3.69 (dd, 1H, J = 14.4, 7.5 Hz, H-7b), 6.41 (dd, 1H, J = 8.4, 0.9 Hz, H-3′), 6.51 (s, 1H, H-6), 6.74 (ddd, 1H, J = 7.5, 7.5, 0.9 Hz, H-5′), 6.93–6.96 (m, 3H, H-2″, H-6′ and H-6″), 7.07–7.13 (m, 3H, H-3″, H-4′ and H-5″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.2 (C-9 and C-10), 28.2 (C-8), 47.6 (C-7), 54.9 ($-O\underline{C}H_3$), 110.1 (C-3′), 113.1 (C-6), 120.1 (C-5′), 120.6 (C-3)*, 121.3 (C-1′)*, 123.5 (q, J = 3.7 Hz, C-3″ and C-5″), 123.9 (q, J = 270.6 Hz, $-\underline{C}F_3$), 129.0 (q, J = 32.3 Hz, C-4″), 129.4 (C-2″ and C-6″), 130.4 (C-6′), 130.5 (C-4′), 137.3 (C-1″), 139.5 (C-5), 142.3 (C-4), 155.8 (C-2′), 164.5 (C-2). *These assignments could be reversed; MS, *m/z* (%) 481 ($[M+2]^{+\bullet}$, 43), 479 ($[M]^{+\bullet}$, $C_{23}H_{21}BrF_3NO_2$, 44), 400 (23), 357 (26), 356 (50), 344 (38), 159 (28), 77 (22), 57 (31), 55 (29), 41 (33), 41 (100). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{22}BrF_3NO_2]^+$: 480.0781, found 480.0833.

4.2.4.2. (Z)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(3-chlorobenzylidene)-1-isobutylpyrrol-2(5H)-one (**12b**) and (E)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(3-chlorobenzylidene)-1-isobutylpyrrol-2(5H)-one (**13b**).

Compounds **12b** and **13b** were synthesized using a method similar to that of **12a** and **13a** were isolated in 28% and 31% yield, respectively.

Data for **12b**: pale yellow solid, purified by column chromatography, eluent hexane/dichloromethane (1:3 v/v). m.p. 120.2–122.0 °C. R_f = 0.41 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 3060, 2957, 2927, 2870, 1702, 1632, 1484, 1250, 1080, 1049, 1024, 914, 810, 736, 688, 621 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.54 (d, 3H, J = 6.9 Hz, H-9), 0.56 (d, 3H, J = 6.9 Hz, H-10), 1.36–1.49 (m, 1H, H-8), 3.47 (d, 2H, J = 7.0 Hz, H-7), 3.82 (s, 3H, $-OC\underline{H}_3$), 5.99 (s, 1H, H-6), 6.90 (d, 1H, J = 9.0 Hz, H-3′), 7.13–7.16 (m, 1H, H-6″), 7.26–7.27 (m, 1H, H-2″), 7.29–7.31 (m, 2H, H-4″ and H-5″), 7.35 (d, 1H, J = 2.7 Hz, H-6′), 7.55 (dd, 1H, J = 9.0, 2.7 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.6 (C-9), 19.7 (C-10), 28.0 (C-8), 49.6 (C-7), 56.2 ($-O\underline{C}H_3$), 112.7 (C-5′), 113.4 (C-6), 113.5 (C-3′), 117.0 (C-3), 121.8 (C-1′), 127.7 (C-6″), 128.3 (C-2″)*, 129.5 (C-4″)*, 129.6 (C-5″)*, 133.5 (C-6′), 134.0 (C-4′), 134.3 (C-3″), 136.5 (C-1″), 139.4 (C-5), 145.3 (C-4), 156.4 (C-2′), 166.8 (C-2); MS, *m/z* (%) 529 ($[M+6]^{+\bullet}$, 6), 527 ($[M+4]^{+\bullet}$, 32), 525 ($[M+2]^{+\bullet}$, 42), 523 ($[M]^{+\bullet}$, $C_{22}H_{20}Br_2ClNO_2$, 19), 446 (29), 444 (16), 403 (14), 402 (27), 401 (13), 400 (30), 390 (31), 388 (29), 125 (20), 114 (20), 102 (33), 101 (29), 100 (15), 94 (17), 57 (33). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_2ClNO_2]^+$: 523.9622, found 523.9589.

Data for **13b**: amorphous green solid, purified by column chromatography, eluent hexane/dichloromethane (1:3 v/v). R_f = 0.31 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 2958, 2929, 2871, 1702, 1484, 1462, 1370, 1254, 1136, 1074, 1048, 1025, 915, 809, 740, 688, 621 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.01 (d, 3H, J = 6.6 Hz, H-9), 1.02 (d, 3H, J = 6.6 Hz, H-10), 2.07–2.21 (m, 1H, H-8), 3.59 (dd, 1H, J = 14.4, 7.2 Hz, H-7a), 3.64 (s, 3H, $-OC\underline{H}_3$), 3.67 (dd, 1H, J = 14.4, 8.1 Hz, H-7b), 6.48 (s, 1H, H-6), 6.49 (d, 1H, J = 8.7 Hz, H-3′), 6.81–6.84 (m, 2H, H-2″ and H-6″), 6.95–6.98 (m, 3H, H-6′, H-4″ and H-5″), 7.20 (dd, 1H, J = 8.7, 2.4 Hz, H-4′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4 (C-9), 20.5 (C-10), 28.4 (C-8), 47.9 (C-7), 55.6 ($-O\underline{C}H_3$), 112.2 (C-3′), 112.4 (C-5′), 113.6 (C-6), 121.6 (C-3), 123.4 (C-1′), 127.5 (C-6″)**, 127.6 (C-4″)*, 128.6 (C-5″)*, 129.6 (C-2″)**, 132.6 (C-6′)*, 133.1 (C-4′), 133.4 (C-3″), 135.5 (C-1″), 139.3 (C-5), 141.0 (C-4), 155.4 (C-2′), 164.4 (C-2). *, **These assignments could be reversed; MS, *m/z* (%) 529 ($[M+6]^{+\bullet}$, 6), 527 ($[M+4]^{+\bullet}$, 29), 525 ($[M+2]^{+\bullet}$, 40), 523 ($[M]^{+\bullet}$, $C_{22}H_{20}Br_2ClNO_2$, 17), 446 (27), 444 (13), 434 (15), 403 (14), 402 (27), 401 (12), 400 (28), 390 (29), 388 (26), 202 (13), 189 (15), 187 (14), 125 (18), 115 (14), 114 (18), 102 (34), 101 (26), 100 (15), 57 (36), 41 (100). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_2ClNO_2]^+$: 523.9622, found 523.9589.

4.2.4.3. (Z)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(4-bromobenzylidene)-1-isobutylpyrrol-2(5H)-one (**12c**) and (E)-3-bromo-4-(5-bromo-2-methoxyphenyl)-5-(4-bromobenzylidene)-1-isobutylpyrrol-2(5H)-one (**13c**).

Compounds **12c** and **13c** were synthesized using a method similar to that of **12a** and **13a** were isolated in 23% and 33% yield, respectively.

Data for **12c**: pale yellow solid, purified by column chromatography, eluent hexane/dichloromethane (1:3 v/v). m.p. 191.6–192.4 °C. R_f = 0.37 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 3059, 2958, 2932, 2871, 1701, 1483, 1386, 1278, 1250, 1070, 1010, 913, 808, 736, 664, 620 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.54 (d, 3H, J = 6.6 Hz, H-9), 0.55 (d, 3H, J = 6.6 Hz, H-10), 1.36–1.50 (m, 1H, H-8), 3.47 (d, 2H, J = 7.5 Hz, H-7), 3.81 (s, 3H, $-OC\underline{H}_3$), 5.96 (s, 1H, H-6), 6.91 (d, 1H, J = 8.7 Hz, H-3′), 7.13 (d, 2H, J = 8.7 Hz, H-2″ and H-6″), 7.33 (d, 1H, J = 2.4 Hz, H-6′), 7.50 (d, 2H, J = 8.7 Hz, H-3″ and H-5″), 7.54 (dd, 1H, J = 8.7, 2.4 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.6 (C-9), 19.7 (C-10), 27.9 (C-8), 49.7 (C-7), 56.2 ($-O\underline{C}H_3$), 112.7 (C-5′), 113.5 (C-3′), 113.8 (C-6), 116.9 (C-3), 121.9 (C-1′), 122.4 (C-4″), 131.2 (C-2″ and C-6″), 131.5 (C-3″ and C-5″), 133.5 (C-6′), 133.6 (C-1″), 133.9 (C-4′), 139.1 (C-5), 145.3 (C-4), 156.4 (C-2′), 166.9 (C-2); MS, *m/z* (%) 573 ($[M+6]^{+\bullet}$, 10), 571 ($[M+4]^{+\bullet}$, 29), 569 ($[M+2]^{+\bullet}$, 28),

567 ([M]$^{+\bullet}$, $C_{22}H_{20}Br_3NO_2$, 9), 448 (19), 447 (19), 446 (32), 444 (15), 436 (18), 434 (38), 432 (22), 355 (25), 353 (31), 352 (27), 350 (23), 189 (17), 187 (15), 102 (37), 101 (26), 94 (21), 57 (32). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_3NO_2]^+$: 567.9117, found 567.9079.

Data for **13c**: amorphous green solid, purified by column chromatography, eluent hexane/dichloromethane (1:3 v/v). R_f = 0.26 (hexane:dichloromethane, 1:3, v/v). IR (ATR) ν_{max} 2958, 2928, 2871, 1700, 1484, 1461, 1389, 1262, 1070, 1010, 912, 848, 801, 743, 663 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (d, 3H, J = 6.6 Hz, H-9), 1.01 (d, 3H, J = 6.6 Hz, H-10), 2.07–2.21 (m, 1H, H-8), 3.56 (s, 3H, –OC$\underline{H}_3$), 3.60 (dd, 1H, J = 14.1, 7.8 Hz, H-7a), 3.67 (dd, 1H, J = 14.1, 7.8 Hz, H-7b), 6.42 (d, 1H, J = 9.0 Hz, H-3′), 6.47 (s, 1H, H-6), 6.73 (d, 2H, J = 8.1 Hz, H-2″ and H-6″), 6.98 (d, 1H, J = 2.7 Hz, H-6′), 7.05 (d, 2H, J = 8.1 Hz, H-3″ and H-5″), 7.26 (dd, 1H, J = 9.0, 2.7 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4 (C-9), 20.5 (C-10), 28.4 (C-8), 47.9 (C-7), 55.7 (–O$\underline{C}H_3$), 112.3 (C-3′), 112.4 (C-5′), 114.1 (C-6), 121.3 (C-3), 121.9 (C-1′), 123.5 (C-4″), 130.4 (C-3″ and C-5″), 130.8 (C-2″ and C-6″), 132.6 (C-1″), 132.8 (C-4′), 133.2 (C-6′), 138.8 (C-5), 140.8 (C-4), 155.4 (C-2′), 164.4 (C-2); MS, *m/z* (%) 573 ([M+6]$^{+\bullet}$, 9), 571 ([M+4]$^{+\bullet}$, 28), 569 ([M+2]$^{+\bullet}$, 28), 567 ([M]$^{+\bullet}$, $C_{22}H_{20}Br_3NO_2$, 9), 447 (20), 446 (32), 444 (15), 436 (17), 434 (37), 432 (21), 400 (15), 355 (24), 353 (29), 352 (27), 350 (22), 202 (15), 189 (16), 115 (18), 114 (19), 102 (36), 101 (25), 100 (15), 89 (20), 57 (35), 41 (100). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_3NO_2]^+$: 567.9117, found 567.9085.

*4.2.4.4. (Z)-3-bromo-5-(3-chlorobenzylidene)-1-isobutyl-4-(5-methyl-2-methoxyphenyl)pyrrol-2(5H)-one (**12d**) and (E)-3-bromo-5-(3-chlorobenzylidene)-1-isobutyl-4-(5-methyl-2-methoxyphenyl) pyrrol-2(5H)-one (**13d**).* Compounds **12d** and **13d** were synthesized using a method similar to that of **12a** and **13a** were isolated in 35% and 45% yield, respectively.

Data for **12d**: yellow solid, purified by column chromatography, eluent hexane/dichloromethane (1:4 v/v). m.p. 150.7–151.7 °C. R_f = 0.35 (hexane:dichloromethane, 1:4, v/v). IR (ATR) ν_{max} 3056, 2957, 2926, 2870, 2836, 1701, 1632, 1498, 1462, 1249, 1084, 1050, 1029, 809, 736, 689 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.56 (d, 6H, J = 6.9 Hz, H-9 and H-10), 1.37–1.50 (m, 1H, H-8), 2.35 (s, 3H, Ar–C$\underline{H}_3$), 3.45 (dd, 1H, J = 14.1, 7.5 Hz, H-7a), 3.50 (dd, 1H, J = 14.1, 7.5 Hz, H-7b), 3.80 (s, 3H, –OC$\underline{H}_3$), 6.02 (s, 1H, H-6), 6.92 (d, 1H, J = 8.4 Hz, H-3′), 7.01 (d, 1H, J = 1.8 Hz, H-6′), 7.12–7.16 (m, 1H, H-6″), 7.22–7.30 (m, 4H, H-4′, H-2″, H-4″ and H-5″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.6 (C-9), 19.7 (C-10), 20.7 (Ar–$\underline{C}H_3$), 28.0 (C-8), 49.6 (C-7), 56.1 (–O$\underline{C}H_3$), 111.8 (C-3′), 113.3 (C-6), 116.2 (C-3), 119.6 (C-1′), 127.7 (C-6″), 128.1 (C-4″)*, 129.5 (C-2″)*, 129.5 (C-4′)*, 130.0 (C-5′), 131.5 (C-6′), 131.7 (C-5″)*, 134.3 (C-3″), 136.8 (C-1″), 139.7 (C-5), 147.1 (C-4), 155.1 (C-2′), 167.2 (C-2). *These assignments could be reversed; MS, *m/z* (%) 463 ([M+4]$^{+\bullet}$, 27), 461 ([M+2]$^{+\bullet}$, 100), 459 ([M]$^{+\bullet}$, $C_{23}H_{23}BrClNO_2$, 74), 382 (39), 381 (27), 380 (72), 337 (39), 336 (71), 334 (29), 326 (28), 324 (87), 293 (21), 286 (31), 173 (34), 145 (25), 116 (20), 115 (42), 102 (24), 101 (24), 57 (31). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_2]^+$: 460.0673, found 460.0740.

Data for **13d**: amorphous green solid, purified by column chromatography, eluent hexane/dichloromethane (1:4 v/v). R_f = 0.26 (hexane:dichloromethane, 1:4, v/v). IR (ATR) ν_{max} 2958, 2926, 2872, 1706, 1631, 1500, 1464, 1251, 1087, 1051, 1030, 810, 690 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (d, 3H, J = 6.6 Hz, H-9), 1.02 (d, 3H, J = 6.6 Hz, H-10), 2.11 (s, 3H, Ar–C$\underline{H}_3$), 2.14 (m, 1H, H-8), 3.59 (s, 3H, –OC$\underline{H}_3$), 3.59 (dd, 1H, J = 14.1, 7.2 Hz, H-7a), 3.67 (dd, 1H, J = 14.1, 7.8 Hz, H-7b), 6.45 (s, 1H, H-6), 6.47 (d, 1H, J = 8.4 Hz, H-3′), 6.69–6.94 (m, 6H, H-4′, H-6′, H-2″, H-4″, H-5″ and H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4 (Ar–$\underline{C}H_3$), 20.5 (C-9 and C-10), 28.5 (C-8), 47.8 (C-7), 55.4 (–O$\underline{C}H_3$), 110.4 (C-3′), 113.6 (C-6), 120.6 (C-3), 121.0 (C-1′), 127.2 (C-6′)*, 127.4 (C-6″)*, 128.3 (C-4″)*, 129.6 (C-5′), 129.7 (C-2″)*, 130.6 (C-4′)*, 131.0 (C-5″)*, 133.1 (C-3″), 135.7 (C-1″), 139.5 (C-5), 142.7 (C-4), 154.1 (C-2′), 164.7 (C-2). *These assignments could be reversed; MS, *m/z* (%) 463 ([M+4]$^{+\bullet}$, 27), 461 ([M+2]$^{+\bullet}$, 100), 459 ([M]$^{+\bullet}$, $C_{23}H_{23}BrClNO_2$, 74), 430 (18), 383 (18), 382 (39), 381 (27), 380 (72), 370 (22), 368 (23), 338 (25), 337 (39), 336 (71), 334 (29), 326 (28), 324 (87), 286 (31), 202 (20), 173 (34), 145 (25), 115 (42), 57 (31), 41 (99). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_2]^+$: 460.0673, found 460.0745.

*4.2.4.5. (Z)-3-bromo-5-(2-chlorobenzylidene)-1-isobutyl-4-(5-methyl-2-methoxyphenyl)pyrrol-2(5H)-one (**12e**) and (E)-3-bromo-5-(2-chlorobenzylidene)-1-isobutyl-4-(5-methyl-2-methoxyphenyl) pyrrol-2(5H)-one (**13e**).* Compounds **12e** and **13e** were synthesized using a method similar to that of **12a** and **13a** were isolated in 29% and 39% yield, respectively.

Data for **12e**: amorphous yellow solid, purified by column chromatography, eluent hexane/dichloromethane (1:3.5 v/v). R_f = 0.34 (hexane:dichloromethane, 1:3.5, v/v). IR (ATR) ν_{max} 3056, 2957, 2927, 2870, 1702, 1498, 1249, 1087, 1048, 1030, 938, 902, 809, 753, 735 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.52 (d, 3H, J = 6.9 Hz, H-9), 0.54 (d, 3H, J = 6.9 Hz, H-10), 1.37–1.51 (m, 1H, H-8), 2.34 (s, 3H, Ar–C$\underline{H}_3$), 3.36 (dd, 1H, J = 14.1, 7.5 Hz, H-7a), 3.43 (dd, 1H, J = 14.1, 7.5 Hz, H-7b), 6.04 (s, 1H, H-6), 6.91 (d, 1H, J = 8.4 Hz, H-3′), 7.08 (d, 1H, J = 2.4 Hz, H-6′), 7.22 (dd, J = 8.4, 2.4 Hz, H-4′), 7.26–7.28 (m, 3H, H-4″, H-5″ and H-6″), 7.39 (m, 1H, H-3″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7 (C-9), 19.8 (C-10), 20.7 (Ar–$\underline{C}H_3$), 27.9 (C-8), 49.4 (C-7), 56.0 (–O$\underline{C}H_3$), 111.7 (C-3′), 112.2 (C-6), 116.0 (C-3), 119.5 (C-1′), 126.3 (C-6″)*, 129.5 (C-3″)*, 129.6 (C-5″)*, 130.0 (C-1″), 131.7 (C-6′)*, 131.8 (C-4′)*, 131.8 (C-4″)*, 133.7 (C-5′), 134.2 (C-2″), 139.5 (C-5), 146.9 (C-4), 155.2 (C-2′), 167.1 (C-2). *These assignments could be reversed; MS, *m/z* (%) 463 ([M+4]$^{+\bullet}$, 26), 461 ([M+2]$^{+\bullet}$, 100), 459 ([M]$^{+\bullet}$, $C_{23}H_{23}BrClNO_2$, 83), 383 (31), 382 (63), 381 (45), 380 (93), 370 (61), 368 (58), 337 (37), 336 (56), 324 (34), 289 (44), 287 (16), 286 (53), 173 (27), 116 (16), 115 (37), 102 (21), 101 (19), 57 (31). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_2]^+$: 460.0673, found 460.0604.

Data for **13e**: green solid, purified by column chromatography, eluent hexane/dichloromethane (1:3.5 v/v). m.p. 123.0–124.2 °C. R_f = 0.26 (hexane:dichloromethane, 1:3.5, v/v). IR (ATR) ν_{max} 3054, 2959, 2927, 2871, 2835, 1702, 1499, 1402, 1264, 1246, 1090, 1048, 1013, 806, 747, 737, 689 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (d, 6H, J = 6.6 Hz, H-9 and H-10), 2.11 (s, 3H, Ar–C$\underline{H}_3$), 2.14–2.27 (m, 1H, H-8), 3.56 (s, 3H, –OC$\underline{H}_3$), 3.63 (dd, 1H, J = 14.4, 7.5 Hz, H-7a), 3.69 (dd, 1H, J = 14.1, 7.5 Hz, H-7b), 6.35 (d, 1H, J = 8.4 Hz, H-3′), 6.48 (s, 1H, H-6), 6.52 (ddd, 1H, J = 7.8, 7.8, 1.2 Hz, H-5″), 6.66 (dd, 1H, J = 7.8, 1.5 Hz, H-6″), 6.74 (d, 1H, J = 2.1 Hz, H-6′), 6.84 (dd, 1H, J = 8.4, 2.1 Hz, H-4′), 6.89 (ddd, 1H, J = 7.8, 7.8, 1.5 Hz, H-4″), 7.19 (dd, 1H, J = 7.8, 1.2 Hz, H-3″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.3 (Ar–$\underline{C}H_3$), 20.5 (C-9 and C-10), 28.5 (C-8), 47.8 (C-7), 55.3 (–O$\underline{C}H_3$), 110.1 (C-3′), 112.8 (C-6), 120.4 (C-3), 121.0 (C-1′), 125.2 (C-5″), 128.1 (C-3″), 128.8 (C-4″), 129.3 (C-1″), 130.8 (C-4′), 130.9 (C-6′), 131.9 (C-6″), 132.7 (C-5′), 133.8 (C-2″), 139.2 (C-5), 142.7 (C-4), 154.2 (C-2′), 164.7 (C-2); MS, *m/z* (%) 463 ([M+4]$^{+\bullet}$, 16), 461 ([M+2]$^{+\bullet}$, 72), 459 ([M]$^{+\bullet}$, $C_{23}H_{23}BrClNO_2$, 56), 383 (27), 382 (52), 381 (40), 380 (71), 370 (50), 368 (48), 338 (18), 336 (43), 324 (29), 287 (15), 286 (51), 202 (30), 115 (53), 102 (29), 101 (29), 57 (36), 41 (100). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_2]^+$: 460.0673, found 460.0629.

*4.2.4.6. (Z)-3-bromo-5-(2-bromobenzylidene)-4-(5-chloro-2-methoxyphenyl)-1-isobutylpyrrol-2(5H)-one (**12f**) and (E)-3-bromo-5-(2-bromobenzylidene)-4-(5-chloro-2-methoxyphenyl)-1-isobutylpyrrol-2(5H)-one (**13f**).* Compounds **12f** and **13f** were synthesized using a method similar to that of **12a** and **13a** were isolated in 29% and 43% yield, respectively.

Data for **12f**: pale yellow solid, purified by column chromatography, eluent hexane/dichloromethane (1:2 v/v). m.p. 162.7–

164.2 °C. R_f = 0.22 (hexane:dichloromethane, 1:2, v/v). IR (ATR) ν_{max} 3058, 2959, 2871, 1706, 1643, 1486, 1463, 1281, 1251, 1026, 919, 811, 755, 670 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.52 (d, 3H, J = 6.9 Hz, H-9), 0.54 (d, 3H, J = 6.9 Hz, H-10), 1.36–1.50 (m, 1H, H-8), 3.35 (dd, 1H, J = 14.1, 7.5 Hz, H-7a), 3.42 (dd, 1H, J = 14.1, 7.5 Hz, H-7b), 3.83 (s, 3H, $-OCH_3$), 5.98 (s, 1H, H-6), 6.96 (d, 1H, J = 8.7 Hz, H-3′), 7.20 (ddd, 1H, J = 8.1, 6.9, 2.4 Hz, H-5″), 7.29 (d, 1H, J = 2.7 Hz, H-6′), 7.27–7.36 (m, 2H, H-3″ and H-4″), 7.41 (dd, 1H, J = 8.7, 2.7 Hz, H-4′), 7.59 (dd, 1H, J = 8.1, 1.2 Hz, H-6″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.7 (C-9), 19.9 (C-10), 28.0 (C-8), 49.5 (C-7), 56.3 ($-OCH_3$), 113.0 (C-3′), 114.4 (C-6), 116.8 (C-3), 121.2 (C-1′), 124.5 (C-2″), 125.6 (C-5′), 127.0 (C-4″)*, 129.9 (C-5″), 131.0 (C-4′), 131.0 (C-6′)*, 131.7 (C-3″)*, 132.7 (C-6″), 135.3 (C-1″), 138.8 (C-5), 145.2 (C-4), 155.9 (C-2′), 166.7 (C-2). *These assignments could be reversed; MS, *m/z* (%) 529 ($[M+6]^{+\bullet}$, 9), 527 ($[M+4]^{+\bullet}$, 53), 525 ($[M+2]^{+\bullet}$, 70), 523 ($[M]^{+\bullet}$, $C_{22}H_{20}Br_2ClNO_2$, 31), 446 (27), 404 (23), 403 (36), 402 (59), 401 (30), 400 (42), 390 (54), 388 (42), 309 (42), 306 (47), 189 (15), 102 (21), 101 (19), 57 (24), 55 (23). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_2ClNO_2]^+$: 523.9622, found 523.9599.

Data for **13f**: green solid, purified by column chromatography, eluent hexane/dichloromethane (1:2 v/v). m.p. 140.7–142.4 °C. R_f = 0.16 (hexane:dichloromethane, 1:2, v/v). IR (ATR) ν_{max} 3061, 2959, 2929, 2872, 1699, 1628, 1485, 1462, 1399, 1261, 1245, 1088, 1024, 912, 742, 710, 668 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.03 (d, 6H, J = 6.6 Hz, H-9 and H-10), 2.22 (m, 1H, H-8), 3.58 (s, 3H, $-OCH_3$), 3.67 (d, 2H, J = 7.5 Hz, H-7), 6.36 (d, 1H, J = 8.7 Hz, H-3′), 6.46 (s, 1H, H-6), 6.63–6.73 (m, 2H, H-5″ and H-6″), 6.84–6.89 (m, 1H, H-4″), 6.98 (d, 1H, J = 2.7 Hz, H-6′), 7.02 (dd, 1H, J = 8.7, 2.7 Hz, H-4′), 7.42 (dd, 1H, J = 7.5, 1.2 Hz, H-3″). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6 (C-9 and C-10), 28.5 (C-8), 47.9 (C-7), 55.8 ($-OCH_3$), 111.4 (C-3′), 115.1 (C-6), 121.2 (C-3), 122.9 (C-1′), 124.4 (C-2″), 125.1 (C-5′), 125.9 (C-6″), 129.3 (C-4″), 130.0 (C-4′), 130.2 (C-6′), 131.6 (C-3″), 131.9 (C-5″), 134.2 (C-1″), 138.7 (C-5), 141.2 (C-4), 154.8 (C-2′), 164.4 (C-2); MS, *m/z* (%) 529 ($[M+6]^{+\bullet}$, 15), 527 ($[M+4]^{+\bullet}$, 70), 525 ($[M+2]^{+\bullet}$, 99), 523 ($[M]^{+\bullet}$, $C_{22}H_{20}Br_2ClNO_2$, 43), 446 (36), 404 (28), 403 (52), 402 (76), 400 (54), 392 (19), 391 (16), 390 (71), 389 (16), 388 (57), 356 (25), 354 (20), 309 (58), 306 (58), 102 (23), 101 (26), 57 (25), 41 (100). HRMS (ESI TOF-MS) calcd. for $[C_{22}H_{21}Br_2ClNO_2]^+$: 523.9622, found 523.9576.

*4.2.4.7. (Z)-3-bromo-4-(5-chloro-2-methoxyphenyl)-1-isobutyl-5-(3-methoxybenzylidene)pyrrol-2(5H)-one (**12g**) and (E)-3-bromo-4-(5-chloro-2-methoxyphenyl)-1-isobutyl-5-(3-methoxybenzylidene) pyrrol-2(5H)-one (**13g**).* Compounds **12g** and **13g** were synthesized using a method similar to that of **12a** and **13a** were isolated in 41% and 35% yield, respectively.

Data for **12g**: amorphous green solid, purified by column chromatography, eluent hexane/dichloromethane (1:6 v/v). R_f = 0.22 (hexane:dichloromethane, 1:6, v/v). IR (ATR) ν_{max} 3066, 3002, 2957, 2871, 2838, 1702, 1595, 1485, 1459, 1272, 1250, 1158, 1046, 920, 812, 700, 643 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.53 (d, 3H, J = 6.6 Hz, H-9), 0.55 (d, 3H, J = 6.6 Hz, H-10), 1.41–1.55 (m, 1H, H-8), 3.52 (d, 2H, J = 7.5 Hz, H-7), 3.81 (s, 3H, $-OCH_3$), 3.82 (s, 3H, $-OCH_3$), 6.01 (s, 1H, H-6), 6.77–6.78 (m, 1H, H-2″), 6.83–6.88 (m, 2H, H-4″ and H-6″), 6.96 (d, 1H, J = 9.0 Hz, H-3′), 7.23 (d, 1H, J = 3.0 Hz, H-6′), 7.27 (dd, 1H, J = 8.0, 8.0 Hz, H-5″), 7.40 (dd, 1H, J = 9.0, 3.0 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.7 (C-9), 19.7 (C-10), 28.0 (C-8), 49.7 (C-7), 55.6 ($-OCH_3$), 56.3 ($-OCH_3$), 113.0 (C-3′), 114.0 (C-4″), 115.0 (C-2″), 115.3 (C-6), 116.6 (C-3), 121.6 (C-1′), 122.1 (C-6″), 125.6 (C-5′), 129.4 (C-5″), 130.8 (C-4′), 130.8 (C-6′), 135.9 (C-1″), 138.7 (C-5), 145.4 (C-4), 155.9 (C-2′), 159.5 (C-3″), 166.9 (C-2). *These assignments could be reversed; MS, *m/z* (%) 479 ($[M+4]^{+\bullet}$, 26), 477 ($[M+2]^{+\bullet}$, 100), 475 ($[M]^{+\bullet}$, $C_{23}H_{23}BrClNO_3$, 78), 398 (16), 396 (43), 356 (17), 355 (16), 354 (37), 353 (36), 352 (50), 342 (24), 341 (21), 340 (88), 338 (24), 309 (25), 149 (14), 148 (15), 121 (23), 102 (18), 57 (24), 55 (21). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_3]^+$: 476.0623, found 476.0547.

Data for **13g**: green solid, purified by column chromatography, eluent hexane/dichloromethane (1:6 v/v). m.p. 92.4–94.7 °C. R_f = 0.15 (hexane:dichloromethane, 1:6, v/v). IR (ATR) ν_{max} 3002, 2958, 2933, 2872, 2835, 1698, 1576, 1486, 1462, 1399, 1325, 1268, 1158, 1088, 863, 733 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.00 (d, 3H, J = 6.6 Hz, H-9), 1.01 (d, 3H, J = 6.6 Hz, H-10), 2.08–2.22 (m, 1H, H-8), 3.56 (s, 3H, $-OCH_3$), 3.60 (dd, 1H, J = 14.0, 7.0 Hz, H-7a), 3.61 (s, 3H, $-OCH_3$), 3.68 (dd, 1H, J = 14.0, 7.0 Hz, H-7b), 6.38 (m, 1H, H-2″), 6.44 (d, 1H, J = 8.7 Hz, H-3′), 6.50–6.56 (m, 2H, H-4″ and H-6″), 6.56 (s, 1H, H-6), 6.90 (d, 1H, J = 2.7 Hz, H-6′), 6.91 (dd, 1H, J = 8.0, 8.0 Hz, H-5″), 7.03 (dd, 1H, J = 8.7, 2.7 Hz, H-4′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.4 (C-9), 20.5 (C-10), 28.5 (C-8), 47.8 (C-7), 55.2 ($-OCH_3$), 55.7 ($-OCH_3$), 111.6 (C-3′), 113.6 (C-4″)*, 114.9 (C-2″), 115.7 (C-6), 121.0 (C-3), 121.9 (C-6″)*, 123.4 (C-5′), 125.1 (C-1′), 128.4 (C-6′)**, 129.8 (C-4′), 130.1 (C-5″)**, 135.0 (C-1″), 138.4 (C-5), 141.2 (C-4), 155.0 (C-2′), 158.8 (C-3″), 164.4 (C-2). *, **These assignments could be reversed; MS, *m/z* (%) 479 ($[M+4]^{+\bullet}$, 26), 477 ($[M+2]^{+\bullet}$, 100), 475 ($[M]^{+\bullet}$, $C_{23}H_{23}BrClNO_3$, 76), 434 (16), 421 (16), 398 (15), 396 (40), 354 (35), 353 (34), 352 (49), 341 (20), 340 (85), 338 (25), 309 (26), 57 (23), 43 (25), 41 (88). HRMS (ESI TOF-MS) calcd. for $[C_{23}H_{24}BrClNO_3]^+$: 476.0623, found 476.0583.

4.2.5. Bacterial growth biofilm inhibition assays

Biofilm production and quantifications were performed as described previously [45]. In brief, polystyrene 96-well microtiter plates were inoculated with bacterial suspension prepared at 5×10^8 UFC/mL in TSB supplemented with 4% sucrose (w/v) and 3.5% (v/v) DMSO, to ensure that the concentration of this solvent remained constant as the tested molecules were sequentially diluted for the inhibition assays. Previous results indicated that at this concentration, DMSO is innocuous both to bacterial growth and biofilm formation (data not shown). Compounds to be tested for antibiofilm activity were added at an initial concentration of 87.5 μg/mL, and serially diluted at 1:2 ratio until a final concentration of 0.17 μg/mL was reached. Microtiter plates were incubated for 20 h at 37 °C in a humidified chamber, and bacterial growth was quantified by absorbance at 630 nm readings using a microtiter plate reader device, to assess effects on bacterial growth prior to biofilm quantifications. Bacterial suspensions were then discarded, and after washing, remaining biofilms were stained with a 0.1% (w/v) crystal violet solution, washed and solubilized in 1% sodium dodecyl sulphate (SDS). Biofilm quantification was assessed by measuring the absorbance at 595 nm using a microtiter plate reader device. Each assay was performed in triplicate. Data were transferred into a graphics program (OriginPro8), sigmoidal dose–response curves determined, and IC_{50} values calculated.

Acknowledgements

We are grateful to the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research fellowships (AJD, CRAM, LCAB, ALP), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG-APQ-00276-13), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and FINEP for financial support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.035.

References

[1] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Bacterial biofilms: from the natural environment to infectious diseases, Nat. Rev. Microbiol. 2 (2004) 95–108.
[2] K. Lewis, Persister cells, dormancy and infectious disease, Nat. Rev. Microbiol. 5 (2007) 48–56.
[3] D. Davies, Understanding biofilm resistance to antibacterial agents, Nat. Rev. Drug Discov. 2 (2003) 114–122.
[4] N. Høiby, T. Bjarnsholt, M. Givskov, S. Molin, O. Ciofu, Antibiotic resistance of bacterial biofilms, Int. J. Antimicrob. Agents 35 (2010) 322–332.
[5] D.J. Musk Jr., P.J. Hergenrother, Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets, Curr. Med. Chem. 13 (2006) 2163–2177.
[6] R. Kolter, Biofilms in lab and nature: a molecular geneticist's voyage to microbial ecology, Int. Microbiol. 13 (2010) 1–7.
[7] J. Davies, D. Davies, Origins and evolution of antibiotic resistance, Microbiol. Mol. Biol. Rev. 74 (2010) 417–433.
[8] L.C.A. Barbosa, U.A. Pereira, R.R. Teixeira, C.R.A. Maltha, S.A. Fernandes, G. Forlani, Synthesis and phytotoxic activity of ozonides, J. Agric. Food. Chem. 56 (2008) 9434–9440.
[9] L.C.A. Barbosa, U.A. Pereira, C.R.A. Maltha, R.R. Teixeira, V.M.M. Valente, J.R.O. Ferreira, L.V. Costa-Lotufo, M.O. Moraes, C. Pessoa, Synthesis and biological evaluation of 2,5-bis(alkylamino)-1,4-benzoquinones, Molecules 15 (2010) 5629–5643.
[10] L.C.A. Barbosa, C.R. Maltha, M.R. Lage, R.C. Barcelos, A. Donà, J.W.M. Carneiro, G. Forlani, Synthesis of rubrolide analogues as new inhibitors of the photosynthetic electron transport chain, J. Agric. Food. Chem. 60 (2012) 10555–10563.
[11] L.C.A. Barbosa, J.O.S. Varejão, D. Petrollino, P.F. Pinheiro, A.J. Demuner, C.R.A. Maltha, G. Forlani, Tailoring nostoclide structure to target the chloroplastic electron transport chain, Arkivoc 4 (2012) 15–32.
[12] S. Miao, R.J. Andersen, Rubrolides A-H, metabolites of the colonial tunicate *Riterrella rubra*, J. Org. Chem. 56 (1991) 6275–6280.
[13] M.J. Ortega, E. Zubía, J.M. Ocaña, S. Naranjo, J. Salvá, New rubrolides from the Ascidian *Synoicum blochmanni*, Tetrahedron 56 (2000) 3963–3967.
[14] A.N. Pearce, E.W. Chia, M.V. Berridge, E.W. Maas, M.J. Page, V.L. Webb, J.L. Harper, B.R. Copp, *E*/*Z*-Rubrolide O, an anti-inflammatory halogenated furanone from the New Zealand ascidian *Synoicum* n. sp. J. Nat. Prod. 70 (2007) 111–113.
[15] J. Boukouvalas, N. Lachance, M. Ouellet, M. Trudeau, Facile access to 4-aryl-2(5*H*)-furanones by Suzuki cross coupling: efficient synthesis of rubrolides C and E, Tetrahedron Lett. 39 (1998) 7665–7668.
[16] F. Bellina, C. Anselmi, S. Viel, L. Mannina, R. Rossi, Selective synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones, Tetrahedron 57 (2001) 9997–10007.
[17] A. Kar, N.P. Argade, A facile synthesis of rubrolide E, Synthesis 14 (2005) 2284–2286.
[18] S.P. Chavan, A.B. Pathak, A. Pandey, U.R. Kalkote, Short and efficient synthesis of rubrolide E, Synth. Commun. 37 (2007) 4253–4263.
[19] R. Nys, A.D. Wright, G.M. König, O. Sticher, New halogenated furanones from the marine alga *Delisea pulchra* (cf. *fimbriata*), Tetrahedron 49 (1993) 11213–11220.
[20] D. Ren, J.J. Sims, T.K. Wood, Inhibition of biofilm formation and swarming of *Escherichia coli* by (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone, Environ. Microbiol. 3 (2001) 731–736.
[21] D. Ren, J.J. Sims, T.K. Wood, Inhibition of biofilm formation and swarming of *Bacillus subtilis* by (5*Z*)-4-bromo-5-bromomethylene)-3-butyl-2(5*H*)-furanone, Lett. Appl. Microbiol. 34 (2002) 293–299.
[22] Y. Han, S. Hou, K.A. Simon, D. Ren, Y.-Y. Luk, Identifying the important structural elements of brominated furanones for inhibiting biofilm formation by *Escherichia coli*, Bioorg. Med. Chem. Lett. 18 (2008) 1006–1010.
[23] B. Nay, N. Riache, L. Evanno, Chemistry and biology of non-tetramic γ-hydroxy-γ-lactams and γ-alkylidene-γ-lactams from natural sources, Nat. Prod. Rep. 26 (2009) 1044–1062.
[24] S. Suzuki, T. Hosoe, K. Nozawa, K. Kawai, T. Yaguchi, S. Udagawa, Antifungal substances against pathogenic fungi, talaroconvolutins, from *Talaromyces convolutes*, J. Nat. Prod. 63 (2000) 768–772.
[25] T. Yamada, E. Imai, K. Nakatuji, A. Numada, R. Tanaka, Cephalimysin A, a potent cytotoxic metabolite from an Aspergillus species separated from a marine fish, Tetrahedron Lett. 48 (2007) 6294–6296.
[26] Y. Asami, H. Kakeyda, R. Onose, A. Yoshida, H. Matsuzaki, H. Odasa, Azaspirene: a novel angiogenesis inhibitor containing a 1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione skeleton produced by the fungus *Neosartorya* sp. Org. Lett. 4 (2002) 2845–2848.
[27] W.K. Goh, G. Iskander, D.S. Black, N. Kumar, An efficient lactamization of fimbrolides to novel 1,5-dihydropyrrol-2-ones, Tetrahedron Lett. 48 (2007) 2287–2290.
[28] B.E. Murray, The life and times of the Enterococcus, Clin. Microbiol. Rev. 3 (1990) 46–65.
[29] T.E. Barder, S.D. Walker, J.R. Martinelli, S.L. Buchwald, Catalysts for Suzuki-Miyaura coupling processes: scope and studies of the effect ligand structure, J. Am. Chem. Soc. 127 (2005) 4685–4696.
[30] K.E. Koenig, G.M. Lein, P. Stuckler, T. Kaneda, D.J. Cram, Host-guest complexation. 16. Synthesis and cation binding characteristics of macrocyclic polyethers containing convergent methoxyaryl groups, J. Am. Chem. Soc. 101 (1979) 3553–3566.
[31] W. Chen, P. Li, L. Wang, Silica supported palladium-phosphine complex: recyclable catalyst for Suzuki-Miyaura cross-coupling reactions at ambient temperature, Tetrahedron 67 (2011) 318–325.
[32] A. Kar, N. Mangu, H.M. Kaiser, M.K. Tse, Gold-catalyzed direct oxidative coupling reactions of non-activated arenes, J. Organomet. Chem. 694 (2009) 524–537.
[33] R.R. Teixeira, L.C.A. Barbosa, G. Forlani, D. Piló-Veloso, J.W.M. Carneiro, Synthesis of photosynthesis-inhibiting nostoclide analogues, J. Agric. Food. Chem. 56 (2008) 2321–2329.
[34] J.T. Gupton, N. Telang, E.J. Banner, E.J. Kluball, K.E. Hall, K.L. Finzel, X. Jia, S.R. Bates, R.S. Welden, B.C. Giglio, J.E. Eaton, P.J. Barelli, L.T. Firich, J.A. Stafford, M.B. Coppock, E.F. Worrall, R.P.F. Kanters, K. Keertikar, R. Osterman, The application of (*Z*)-3-aryl-3-haloenoic acids to the synthesis of (*Z*)-5-benzylidene-4-arylpyrrol-2(5*H*)-ones, Tetrahedron 66 (2010) 9113–9122.
[35] C.M. Lewis, M.J. Zervos, Clinical manifestations of enterococcal infection, Eur. J. Clin. Microbiol. Infect. Dis. 9 (1990) 111–117.
[36] M.P. Edwards, D.A. Price, Role of physicochemical properties and ligand lipophilicity efficiency in addressing drug safety risks, Annu. Rep. Med. Chem. 45 (2010) 381–389.
[37] D.F. Veber, S.R. Johnson, H.-Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615–2623.
[38] J. Kelder, P.D.J. Grootenhuis, D.M. Bayada, L.P.C. Delbressine, J.-P. Ploemen, Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs, Pharm. Res. 16 (1999) 1514–1519.
[39] J. Nakayama, Y. Cao, T. Horii, S. Sakuda, H. Nagasawa, Chemical synthesis and biological activity of the gelatinase biosynthesis-activating pheromone of *Enterococcus faecalis* and its analogues, Biosci. Biotechnol. Biochem. 65 (2001) 2322–2325.
[40] J. Nakayama, R. Yokohata, M. Sato, T. Suzuki, T. Matsufuji, K. Nishiguchi, T. Kawai, Y. Yamanaka, K. Nagata, M. Tanokura, K. Sonomoto, Development of a peptide antagonist against *fsr* quorum sensing of *Enterococcus faecalis*, ACS Chem. Biol. 8 (2013) 804–811.
[41] W.R.J.D. Galloway, J.T. Hodgkinson, S.D. Bowden, M. Welch, D.R. Spring, Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways, Chem. Rev. 111 (2011) 28–67.
[42] D.D. Perrin, W.L.F. Armarego, Purification of Laboratory Chemicals, third ed., Pergamon, Oxford, 1988.
[43] F. Bellina, R. Rossi, An efficient and inexpensive multigram synthesis of 3,4-dibromo- and 3,4-dichlorofuran-2(5*H*)-one, Synthesis 12 (2007) 1887–1889.
[44] G.A. Taylor, Mucobromic acid, Org. Synth. Coll. 4 (1956) 688.
[45] A.L. Pimenta, P. Martino, E. Bouder, C. Hulen, M.A. Blight, In vitro identification of two adherence factors required for in vivo virulence of *Pseudomonas fluorescens*, Microbes Infect. 5 (2003) 1177–1187.