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Antifungal 3,5-disubstituted furanones: From 5-acyloxymethyl to 5-alkylidene derivatives

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ABSTRACT

5-Acetoxymethyl-3-(4-bromophenyl)-2,5-dihydrofuran-2-one previously described as highly antifungally active was found to provide the corresponding 5-methylene derivative via an unusual DMSO-promoted elimination of the ester group at C5 under antifungal assay conditions. Since the latter possessed nearly the same antifungal effect as that originally reported for the former, the 5-acetoxymethyl furanone just served as a precursor of the actual antifungally active species. A few series of compounds with alkyloxy, aryloxy and alkylidene substituents at C5 of the parent furanone structure were therefore prepared and evaluated. In line with the ease of elimination of the substituent from C5, low activities of the 5-alkoxy compounds were observed. On the other hand, their 5-aryloxymethyl congeners were found to be capable of liberating the antifungally active 5-methylene furanone into the testing medium. The antifungal effect of the 5-alkylidene derivatives was highly sensitive to substitution of the alkylidene moiety; a substituent in the allylic position was necessary for a compound to retain high activity. Parallel evaluation of cytostatic activity showed moderate activities of the antifungally active derivatives against HeLa S3 and CCRF-CEM lines. Cell cycle analysis of CCRF-CEM cells following the treatment with 5-methylene-3-(4-bromophenyl)-2,5-dihydrofuran-2-one revealed that this compound is a necrotic agent.

1. Introduction

The past three decades have witnessed a significant increase of incidence of invasive fungal infections, especially among hospitalized patients. An increased morbidity and mortality of mycotic infections is associated with a growing number of vulnerable patients to opportunistic fungi as a consequence of progress in medicine, improved diagnostics and awareness of fungi as etiology of these infections.[1] Patients treated with immunosuppressive agents, broad-spectrum antibiotics, antineoplastics and anti-HIV agents as well as those undergoing extensive surgery or other invasive procedures are at higher risk of contracting a systemic mycosis.[2–4] The majority of invasive mycotic diseases are caused by *Candida* and *Aspergillus* species, even though other emerging fungi can play an important role in etiology[5] (*Mucorales*, *Scedosporium*, *Fusarium*). While there are several classes of antifungal drugs on the market, not all of them can be considered for a systemic use. In fact, amphotericin B of the polyene antibiotics and antifungal azoles remain the most often employed drugs for the treatment of systemic infections. While the former suffers from a poor pharmacokinetics, potential toxicity (conventional amphotericin B) or cost (lipid forms of amphotericin B), classical azole antifungals are continuously being developed[6,7] since a number of fungi can easily acquire resistance to them. Moreover, they usually have a limited effect on some new, not very common fungal species. In this regard, the shift of the spectrum in etiology resulting from the change in epidemiology of nosocomial and opportunistic mycoses is important to mention. For example, the proportion of non-*albicans Candida* yeasts with variable or no susceptibility to fluconazole such as *Candida krusei* and *Candida glabrata*, respectively, is on the rise.[8] For this reason, the development of structurally novel antifungal drugs is badly needed, with echinocandins being a notable example of an emerging group of novel antifungals based on a cyclic peptide structure.[9]

Some time ago, we reported the identification of 3,5-disubstituted furanones derived from the structure of a natural butenolide, (−)incrustoporin (Fig. 1), as potential antifungal agents.[10] Biological screening of several small libraries of (−)incrustoporin analogues has revealed[10,11] that the endocyclic double bond is necessary for antifungal effect of the compounds and that the effect may be further boosted by the substitution of the C3-aryl ring with halogens. Further elaboration of C5 substitution led to

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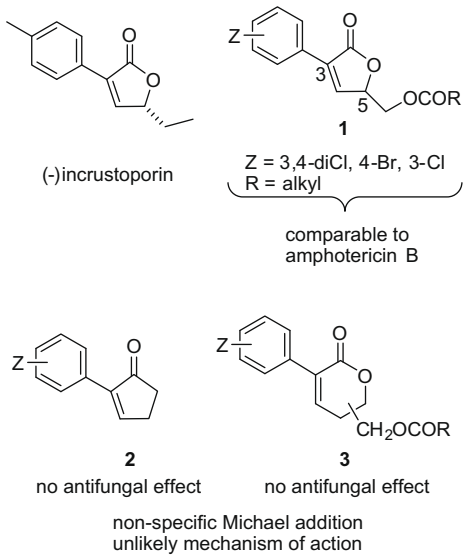
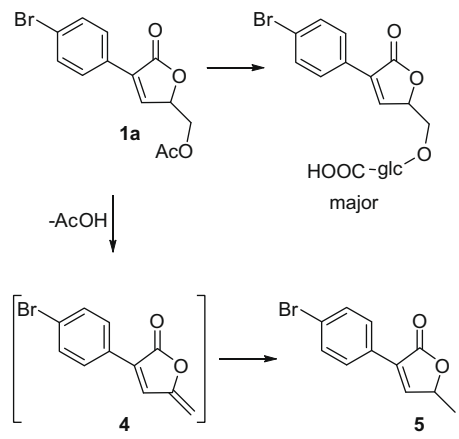


Figure 1. Overview of structure–antifungal activity relationships on (−)incrustoporin analogues **1**–**3**.

5-acyloxymethyl-3-(halogenated phenyl)-2,5-dihydrofuran-2-ones (**1**).[12] The activities of these compounds did not depend either on the nature of the acyl group or the sense of chirality at C5, and were comparable to amphotericin B in antifungal potency in vitro[13] (in terms of μg/mL). Even though the structure of the 3,5-disubstituted furanones seemed to indicate that the compounds could be non-specific inhibitors of fungal growth due to an easy Michael addition, ketone[11] (**2**) and pentenolide[14] (**3**) analogues of these butenolides were later found to possess no antifungal activity at all. Since ketones **2** in particular should be far better Michael acceptors than lactones **1**, a simple Michael addition to the β-carbon of the double bond is an unlikely mechanism of action. Unfortunately though, an in vivo evaluation in a mouse model of experimental candidiasis or aspergillosis has so far been hampered by the relatively high lipophilicity of the compounds, which did not enable intravenous administration.

A recent flow cytometric study[15] carried out with one of the most effective compounds **1** (**1a**: Z = *p*-Br, R = CH_3, Scheme 1) revealed that this substance interacted with an as yet unknown target in the fungal cell membrane. A preliminary evaluation[16] of metabolic conversions of **1a** after intraperitoneal administration in mice revealed that most of the compound underwent fast ester hydrolysis and conjugation to glucuronic acid. Somewhat unexpectedly, the 5-methylene and 5-methyl derivatives **4** and **5** were detected as well. In the process of further development of lactone esters of type **1** following our first report,[12] we also observed the formation of impurities, which were tentatively assigned as the corresponding 5-methylene derivatives based on the NMR spectra, upon long term storage of the compounds at room temperature. Thus, we assumed that in the biotransformation study, compound **1a** afforded the 5-methylene furanone **4** first, the enzymatic reduction of which subsequently furnished the 5-methyl metabolite **5**.



Scheme 1. Proposed metabolic conversion of **1a** to **5**.

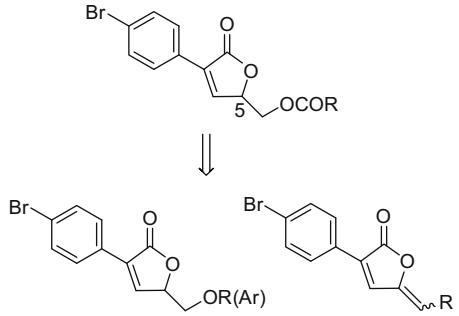


Figure 2. Proposed elaboration of substitution at C5.

Given the results of the biotransformation study and the stability of compounds **1** having arisen as an issue, the possibility that furanones of type **4** were the actual active species attacking the fungal cell membrane and esters of type **1** just served as their precursors was apparent. In addition, if these 5-methylene lactones were indeed the antifungally active species, our as yet unexplained observation[12] that the antifungal effect of lactones **1** did not depend on the sense of chirality at C5 would thus be logically clarified.

Importantly, these results meant a significant shift from our original assumption[12] that compounds **1** are just more lipophilic transport forms of their parent 5-hydroxymethyl analogues, which easily penetrate fungal cell walls and are hydrolyzed inside the cells. In order to shed more light on these issues, we focused on the issue of stability of **1a** under the conditions of in vitro studies first.

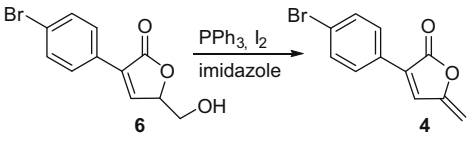
Second, replacement of the C5 acyloxy group with substituted aryloxy and alkyloxy functions was studied (Fig. 2). The replacement of the ester function with an ether group would prevent in vivo enzymatic hydrolysis with subsequent conjugation to glucuronic acid observed[16] for **1a**, and the attachment of a hydrophilic group (COOH) to the aryl or alkyl moiety would also help to improve the lipophilicity profile of the compounds. Moreover, since these groups eliminate less easily than the acyloxy function, derivatives with higher stability would be obtained. Third, the influence of the replacement of the C5 acyloxymethyl group with an alkylidene side chain was evaluated. In order to obtain more diverse SARs, cytostatic activity on a panel of cancer cell lines was also evaluated in addition to antifungal.

2. Chemistry

2.1. Stability of compound 1a

Synthetic standard of the elimination product **4** was prepared by the reaction of the previously described alcohol **6**[12] with PPh_3/I_2/imidazole. The initially formed 5-iodomethyl derivative underwent in situ elimination to produce the 5-methylene furanone **4** (Scheme 2).

Subsequently, stability of **1a** under antifungal assay conditions was explored. First, furanone **1a** was dissolved in DMSO-d_6. ^{1}H NMR monitoring of the solution showed the presence of a mixture



Scheme 2. Synthesis of elimination product **4**.

of **1a** and **4** in an approximate 1:1 ratio formed within minutes, with the ratio of both compounds having remained the same even after 24 h. Noteworthy, a solution of **1a** in THF-d_8 was stable after one day at rt. Second, following the procedure for antifungal activity assays, the cultivation medium was added to a freshly prepared stock solution of **1a** in DMSO. HPLC analysis revealed that the medium further accelerated the elimination, since only furanone **4** was detectable in the mixture in less than 3 h.

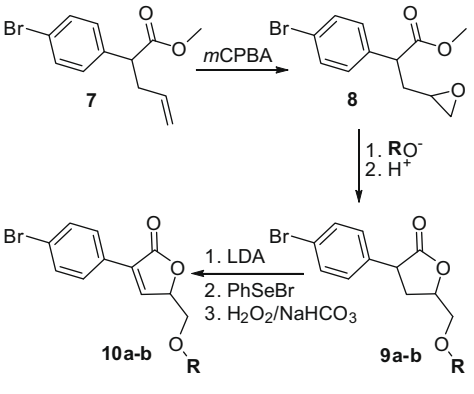
2.2. Synthesis and stability of 5-alkoxy- and phenoxy derivatives

Ester **7**[10] was subjected to epoxidation, and the resultant epoxide **8** was cleaved with sodium methanolate or ethanolate. Upon acidic workup, saturated lactones **9a** and **9b** were obtained. The double bond was then introduced via phenylselenenylation/selenoxide elimination[10] to yield the 5-alkoxymethyl compounds **10a**,**b** (Scheme 3).

A series of 5-(subst. phenoxy)methyl derivatives was prepared next, including two derivatives with a carboxyl on the phenyl ring, which were deliberately prepared in order to have more hydrophilic compounds. Hence, methoxymethylesters of 3- and 4-hydroxybenzoic acid **11a**,**b** were prepared first by partial protection of the acids with methoxymethyl chloride (Scheme 4). Even though the protection seems trivial, the compounds have only been mentioned in patent literature.[17]

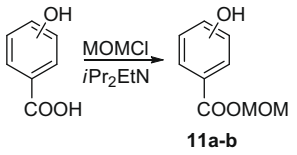
Since the softer phenolate anions were unable to cleave the epoxide ring in **8**, OH group in lactone **12**[12] was esterified with *p*-fluorobenzenesulfonyl chloride (FBSCl) to afford the sulfonyl ester **13**. Upon nucleophilic substitution with substituted phenolates **11a–g**, compound **13** furnished a series of 5-(subst. phenoxy)methyl derivatives **14a–g**, which were converted into the desired 2,5-dihydrofuranones **15a–g** in the same fashion as described above for **9a–b** (Scheme 5).

Compounds **15a** and **15b** were deprotected on Dowex 50 in MeOH to afford free acids **16a** and **16b** and compound **15c** was demethylated[16] to afford 4-hydroxy derivative **16c** (Scheme 6).



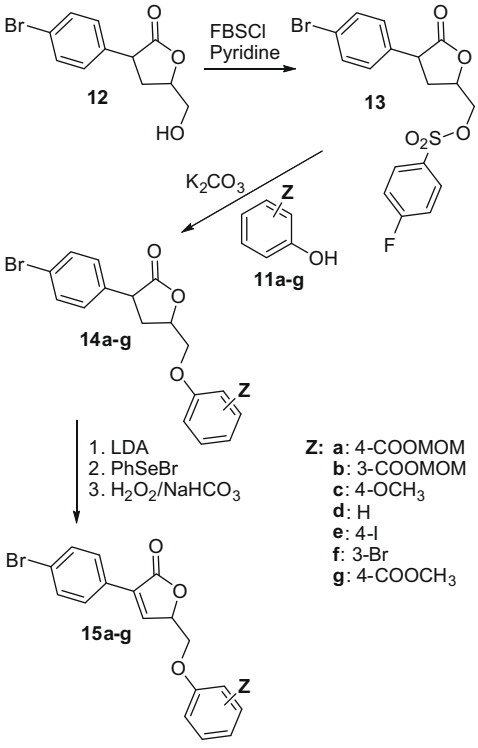
R = **a**: -CH_3, **b**: -CH_2CH_3

Scheme 3. Synthesis of 5-alkoxymethyl derivatives products **10a–b**.

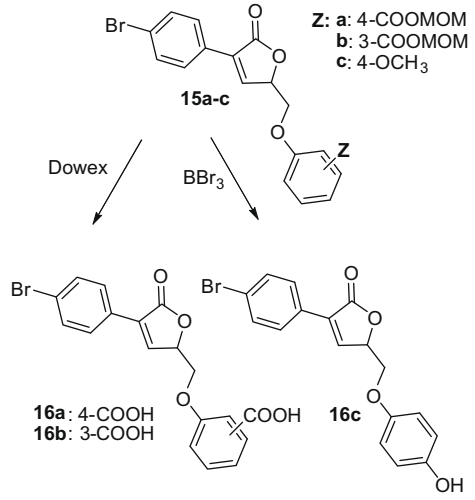


a: 4-OH, **b**: 3-OH

Scheme 4. Preparation of MOM-protected acids **11a–b**.



Scheme 5. Synthesis of phenoxymethyl derivatives **15a–g**.



Scheme 6. Synthesis of phenoxymethyl derivatives **16a–c**.

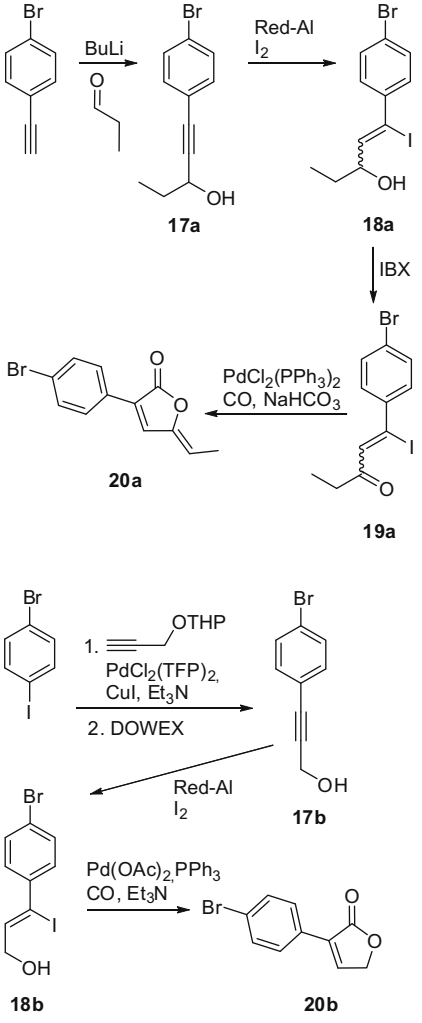
The stability of the target compounds was assessed using compounds **10a** and **16b** as the representatives of both groups as described above for **1a**. While the 5-methoxymethyl furanone **10a**

was found to be perfectly stable in both DMSO solution and the cultivation medium, elimination of the 5-(3-carboxyphenoxy)methyl derivative **16b** cleanly afforded the methylene lactone **4**, albeit more slowly than in the case of the 5-acetyloxymethyl furanone **1a**. As evidenced by HPLC, the elimination process under antifungal assay conditions was over in 5 h, and produced exclusively compound **4** and antifungally inactive (vide infra) *m*-hydroxybenzoic acid.

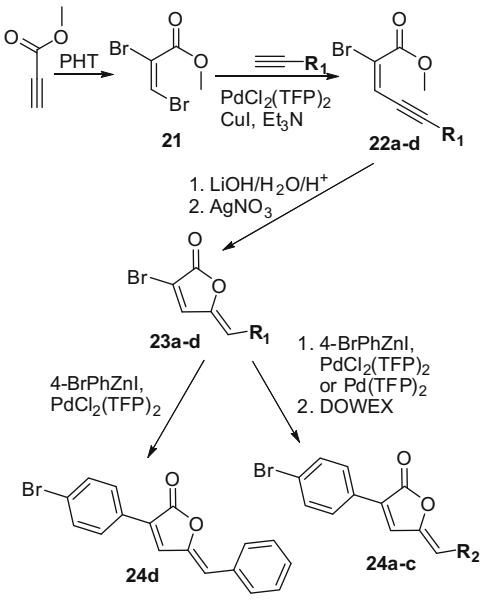
2.3. Syntheses of 5-alkylidene furanones and 5-unsubstituted furanone

Pd-catalyzed carbonylative lactonization[18] was used as the key step in the preparation of the 5-ethylidene compound **20a**. Propargylic alcohol **17a** was converted into iodo allylic alcohol **18a**. Subsequent oxidation[19] of **18a** afforded β-iodo enone **19a**, suitable for a cyclocarbonylation process. Even though the yield of the key step was low, no optimization attempts were made due to zero biological activity of furanone **20a**. Similarly, alcohol **17b**[20] was converted into furanone **20b**[21] (Scheme 7).

Most of the other 5-alkylidene furanones **24** were obtained via sequential functionalization of methyl (*E*)-dibromoacrylate **21**.[22] Thus, Sonogashira coupling into β-position gave a series of β-alkynyl esters **22a–d**, whose carboxyls were liberated by hydrolysis and subjected to cyclization onto the triple bond to yield the corresponding α-bromolactones **23a–d**. The 4-bromophenyl moiety

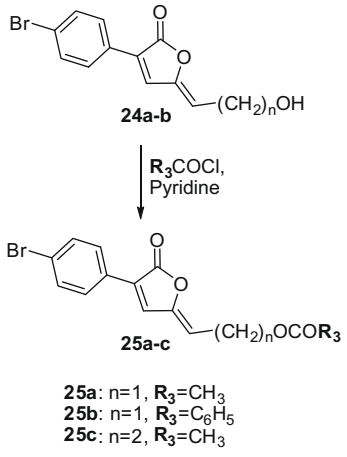


Scheme 7. Synthesis of lactones **20a**,**b**.



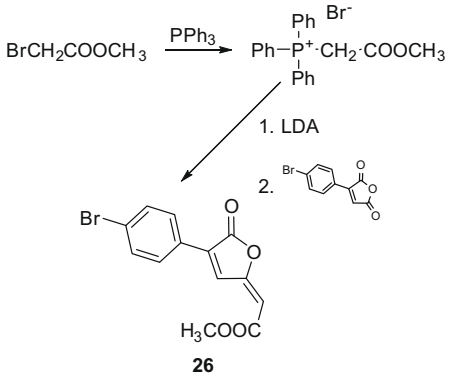
R$_1$ = **a**: -CH_2OTHP, **b**: -CH_2CH_2OTHP,
c: -$CH_2CH_2CH_2OTHP$, **d**: -C_6H_5
R$_2$ = **a**: -CH_2OH, **b**: -CH_2CH_2OH,
c: -$CH_2CH_2CH_2OH$

Scheme 8. Synthesis of alkylidene butenolides **24a**–**d**.

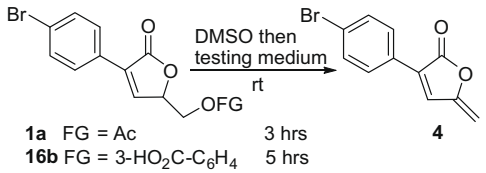


25a: n=1, **R**$_3$=CH_3
25b: n=1, **R**$_3$=C_6H_5
25c: n=2, **R**$_3$=CH_3

Scheme 9. Preparation of esters **25a**–**c**.



Scheme 10. Synthesis of lactone **26**.



Scheme 11. Production of active species **4**.

was then attached via another Pd-coupling process with 4-bromophenylzinc iodide. Noteworthy, $PdCl_2(TFP)_2$ was used[23,24] as the catalyst in this, somewhat difficult, coupling. Optimization of the conditions was done in case of bromolactone **23a**; replacement of THF with DMF and reduction of the catalyst to a more active $Pd(TFP)_2$ prior to the coupling increased the yield to 65% (Scheme 8).

Following the lead structure of active butenolides **1**, we also prepared esters of the 5-hydroxyalkylidene furanones **24a** and **24b**, resulting in 3 derivatives **25a–c** (Scheme 9).

Finally, the derivative bearing an ester group **26** was obtained by the Wittig condensation[25] of (4-bromophenyl)maleic anhydride[26] with (methoxycarbonylmethyl)triphenyl phosphonium ylide (Scheme 10).

3. Results and discussion

Evaluation of stability of the previously described furanone ester **1a** and furanone ether **16b** as a representative of the 5-aryloxymethyl derivatives has shown that both compounds smoothly produced the 5-methylene lactone **4** via elimination under antifungal assay conditions (Scheme 11). Notably, a couple of recent examples[27,28] of eliminations promoted by neat DMSO involve heating to higher temperatures and halogen as a leaving group. Elimination depicted in Scheme 11 is virtually unprecedented given the mild conditions and leaving groups as poor as an ester and even an ether (!). Hence, as the first MIC value reading is done in 24 h, the antifungal activity previously reported for **1a** can be ascribed to the elimination product **4** rather than lactone **1a**.

Antifungal activity (IC_{80}) evaluation (Table 1) of derivative **4** as well as the antifungal profile of the compounds then provided conclusive evidence for the 5-methylene furanone **4** being the antifungally active species. The synthetically prepared furanone **4** possessed excellent activity against *Candida albicans* ATCC44859 and *C. glabrata* 20/I strains, identical to that originally reported[12] for its 5-acetoxymethyl precursor **1a**. Of the filamentous strains, the effect on *Aspergillus fumigatus* is also noteworthy. As further shown in Table 1, the activities of the 5-aryloxymethyl and 5-alkoxymethyl compounds are apparently dependent on the ease of eliminating the substituent at C5. Clearly, the 5-phenoxy derivatives **15c**–**16c** with a surprisingly good ability of the C5-phenoxy group to leave under the assay conditions also served as precursors of furanone **4**. The screening of the compounds shows broad spectrum of activity, corresponding in all cases to that of **4**, with the exception of **15e** against *Candida parapsilosis*, *C. krusei 1* and *2*, and *Absidia corymbifera*. For the sake of excluding the contribution of the phenols liberated into the medium, a series of substituted phenols (*p*-COOH, 4-I, H) was evaluated under the same conditions, and the compounds showed no activity whatsoever, even at 500 μmol/L. On the other hand, 5-alkoxy compounds **10a** and **10b** displayed just a low effect on the growth of *C. albicans* ATCC44859, and a marginal effect of **10a** against *C. albicans* ATCC90028 and *A. fumigatus* was also observed. Furanone **20b** unsubstituted at C5 was also prepared, and found to be inactive. These results demonstrated the importance of the exocyclic double bond at C5, and further investigation was carried out in this direction.

Much surprisingly then, the 5-ethylidene derivative **20a** differing from the 5-methylene furanone **4** by an additional methyl group was completely inactive. Hence, another methyl group added to the exocyclic double bond in **20a** disabled the binding of the compound to its cell membrane target. However, the addition of a hydroxyl into an allylic position restored the antifungal effect to a significant degree suggesting the need for the presence of a binding group. The 5-(2-hydroxyethylidene) furanone **24a** possessed antifungal activity against *C. albicans* ATCC44859 comparable to furanone **4**, even though its efficiency against all other strains tested was somewhat reduced. Insertion of more carbons between the double bond and the OH group led to practically inactive compounds **24b–c**. Similarly, esterification of the OH group in **24a–c** had a detrimental effect, since ester **25a** had higher MICs than its precursor **24a**, and compounds **25b–c** were inactive.

The antifungal activity of furanone **4** and phenoxy derivatives **15c**–**16c** is superior to that of azole drug standard (fluconazole), especially against *C. krusei*, *C. glabrata* and filamentous strains. In addition, antifungal activity of amphotericin B is also presented.

Cytostatic activity of selected compounds expressed as IC_{50} was also evaluated (Table 2). Apparently, all antifungally active derivatives **4** (and hence its precursors **15d**–**16b**) as well as **24a** also possessed cytostatic activities in the micromolar range against HeLa S3 and CCRF-CEM cell lines. It is, however, interesting to note that cytostatic and antifungal action do not parallel each other. First, the 5-methylene furanone **4** itself has one of the highest antifungal activities shown in Table 1, but its cytostatic activity is moderate to low, and limited to CCRF-CEM and HeLa S3 cells. Similar data were obtained when another efficient precursor of **4**, compound **16a**, was subjected to cytostatic assays, with an even lower activity against CCRF-CEM cells (8.70 ± 0.43) than that of **4** (2.52 ± 0.11). Second, when the 5-phenoxy furanones **15d–16b** were subjected to screening, the results were somewhat different, even though all furanones **15d–16b** are prone to the elimination process leading to the same 5-methylene furanone **4** in DMSO. Unlike **16a** and **16b**, activities in the micromolar range against HL-60 cells were recorded upon screening of compounds **15d–g**. Also, the screening of furanone **16b** eliminating a molecule of *m*-hydroxybenzoic acid showed a higher effect on CCRF-CEM cells compared to the closely related **16a** releasing *p*-hydroxybenzoic acid on elimination. Thus, it appears that even though all compounds furnish the 5-methylene furanone **4**, cytostatic activities could be modulated by the phenolic side product. Again, a series of substituted phenols (*p*-COOH, 4-I, H) was evaluated under the same conditions, and the compounds displayed no activity at all at 10 μmol/L. In addition, antifungally inactive compounds **10a** and **10b** showed cytostatic activities against HeLa S3 and CCRF-CEM cells. The 5-methoxymethyl furanone **10a** exhibited micromolar activity against CCRF-CEM cells, and its 5-ethoxymethyl congener **10b** showed reasonable activity against HeLa S3 cells, while its effect on CCRF-CEM cells was reduced as compared to **10a**.

Since furanone **4** was the apparent active substance significantly reducing the growth of several fungal strains and displaying moderate cytostatic activity on HeLa S3 and CCRF-CEM lines, the compound was subjected to flow cytometric studies with a view to shedding more light on the nature of the cytostatic effect. The cell cycle analysis of CCRF-CEM cells (Fig. 3) following treatment with compound **4** at IC_{50} (2.5 μmol L^{-1}) showed G1 phase block accompanied by an accumulation of cells in S phase and depletion of G2/M phase. Such pronounced effect resulted in a high amount of cell debris (Fig. 3, blue area) and prompted that substance **4** induces cell death.

Annexin V binding method (combination of annexin V-FITC and propidium iodide) has shown that 72 h treatment of CCRF-CEM

Table 1
Antifungal activity of prepared compounds and drug standards (MIC [μmol L^{-1-1}])

No.	Time (h)	CA1[a]	CA2[b]	CP[c]	CK1[d]	CK2[e]	CT[f]	CG[g]	CL[h]	TB[i]	AF[j]	AC[k]	TM[l]
1a	24	0.49	3.90	3.90	3.90	7.81	7.81	1.95	3.90	7.81	0.98	7.81	1.95
	48	1.95	3.90	15.62	7.81	7.81	7.81	1.95	7.81	7.81	3.91	15.62	3.90
4	24	0.49	3.90	7.81	7.81	7.81	7.81	1.95	7.81	3.90	1.95	31.25	1.95
	48	1.95	7.81	15.62	7.81	7.81	15.62	1.95	15.62	15.62	7.81	125	3.90
10a	24	15.62	31.25	125	250	250	62.5	125	62.5	62.5	31.25	125	31.25
	48	31.25	31.25	250	250	500	62.5	125	125	125	62.5	250	31.25
10b	24	31.25	125	125	250	250	250	62.5	125	125	62.5	125	15.62
	48	62.5	125	250	250	250	250	62.5	250	250	125	125	15.62
15c	24	0.97	1.95	7.81	7.81	7.81	7.81	0.97	3.90	7.81	3.90	31.25	1.95
	48	1.95	7.81	15.62	15.62	15.62	7.81	1.95	7.81	15.62	7.81	62.5	1.95
15d	24	0.97	1.95	7.81	7.81	15.62	7.81	1.95	3.90	7.81	1.95	125	1.95
	48	1.95	7.81	31.25	31.25	31.25	15.62	1.95	7.81	31.25	15.62	500	3.90
15e	24	0.48	3.90	31.25	31.25	31.25	15.62	1.95	7.81	7.81	3.90	>500	1.95
	48	1.95	7.81	>500	>500	>500	31.25	3.90	31.25	500	7.81	>500	3.90
15f	24	0.97	3.90	7.81	7.81	7.81	7.81	1.95	7.81	7.81	3.90	31.25	1.95
	48	1.95	7.81	31.25	15.62	15.62	15.62	1.95	15.62	62.5	15.62	62.5	1.95
15g	24	0.48	3.90	15.62	15.62	15.62	15.62	3.90	7.81	7.81	3.90	62.5	3.90
	48	1.95	7.81	15.62	15.62	15.62	15.62	3.90	15.62	15.62	7.81	125	3.90
16a	24	0.48	0.97	7.81	7.81	7.81	3.90	0.97	3.90	7.81	1.95	15.62	0.97
	48	1.95	3.90	15.62	15.62	15.62	7.81	1.95	7.81	15.62	3.90	62.5	0.97
16b	24	0.97	3.90	15.62	15.62	15.62	7.81	1.95	7.81	15.62	1.95	62.5	0.97
	48	1.95	15.62	15.62	15.62	15.62	7.81	1.95	7.81	15.62	7.81	62.5	1.95
16c	24	0.97	1.95	7.81	7.81	7.81	7.81	1.95	3.90	7.81	1.95	15.62	1.95
	48	1.95	3.90	15.62	15.62	15.62	7.81	1.95	7.81	15.62	7.81	62.5	1.95
20a	24	250	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	31.25
	48	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	31.25
20b	24	250	500	250	250	250	250	500	500	250	250	500	250
	48	250	500	500	500	500	500	500	500	500	500	500	250
24a	24	1.95	7.81	31.25	15.62	31.25	15.62	15.62	7.81	31.25	7.81	15.62	7.81
	48	7.81	15.62	62.5	31.25	62.5	31.25	62.5	15.62	62.5	15.62	15.62	7.81
24b	24	62.5	125	125	125	250	125	62.5	125	125	125	62.5	31.25
	48	125	250	125	250	250	250	250	250	125	250	62.5	31.25
24c	24	62.5	>500	>500	>500	>500	>500	>500	>500	>500	>500	62.5	62.5
	48	125	>500	>500	>500	>500	>500	>500	>500	>500	>500	62.5	62.5
24d	24	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
	48	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
25a	24	7.81	31.25	31.25	7.81	62.5	31.25	31.25	62.5	31.25	15.62	31.25	7.81
	48	31.25	62.5	62.5	62.5	62.5	125	62.5	125	31.25	31.25	31.25	15.62
25b	24	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
	48	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
25c	24	15.62	>500	250	500	500	>500	250	>500	>500	>500	250	15.62
	48	500	>500	>500	>500	>500	>500	>500	>500	>500	>500	250	15.62
26	24	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
	48	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
AmB[m]	24	0.03	0.07	0.03	0.14	0.14	0.09	0.03	0.14	1.08	0.18	1.08	1.08
	48	0.06	0.14	0.14	0.14	0.18	0.11	0.09	0.27	1.80	0.23	2.16	1.08
FLU[n]	24	1	0.8	6.5	>50	>50	3	22	3.3	4	>50	>50	17
	48	2	3.3	13	>50	>50	5	>50	3.3	9	>50	>50	26

MIC were defined as 80% inhibition of the growth of control.
[a] *Candida albicans* ATCC44859.
[b] *C. albicans* ATCC90028.
[c] *C. parapsilosis* ATCC22019.
[d] *C. krusei* ATCC 6258.
[e] *C. krusei* E28.
[f] *C. tropicalis* 156.
[g] *C. glabrata* 20/I.
[h] *C. lusitaniae* 2446/I.
[i] *Trichosporon beigelii* 1188.
[j] *Aspergillus fumigatus* 231.
[k] *Absidia corymbifera* 272.
[l] *Trichophyton mentagrophytes* 445 (MIC values were determined after 72 and 120 h).
[m] Amphotericin B (MIC were defined as 95% inhibition of the growth of control).
[n] Fluconazole.

cells with furanone **4** (2.5 μmol L^{-1-1}) mostly induces necrosis (Fig. 4B, Q2) while the early apoptosis was not detected (Fig. 4B, Q1). At IC_{50} and higher concentrations, more than 80% of cells are in necrotic stage. These data indicate that the tested compound is a necrotic agent, which causes a concentration-dependent loss of plasma and nuclear membrane integrity. This result clearly falls in line with our previous finding that derivative **1a** destroys the fungal cell membrane in *C. albicans* and, given the above results, it does so via liberating the actual active compound **4**.

4. Conclusion

We have shown that 5-acyloxymethyl- and 5-aryloxymethyl-3-aryl-2,5-dihydrofuran-2-ones produce 5-methylene furanones such as **4** under standard antifungal assay conditions. The latter compounds then act as the active species that destroy fungal cell membranes. Notably, while the limited stability and fast biotransformation of the 5-acyloxymethyl furanones into the corresponding glucuronides precludes their further utilization in models

Table 2
Cytostatic activity (IC_{50} [μmol L^{-1}])

No.	L1210	HL60	HeLa S3	CCRF-CEM
4	21.00 ± 0.75	18.00 ± 1.66	2.09 ± 0.16	2.52 ± 0.11
10a	NT[a]	16.00 ± 0.63	NA[b]	2.21 ± 0.09
10b	NA	NA	1.94 ± 0.12	9.82 ± 0.68
15d	NT	4.37 ± 0.42	4.67 ± 0.30	1.09 ± 0.09
15e	NT	7.22 ± 0.19	4.00 ± 0.26	1.19 ± 0.19
15g	NT	4.05 ± 0.60	4.50 ± 0.30	1.25 ± 0.05
16a	12.31 ± 2.00	19.35 ± 1.02	2.69 ± 0.22	8.70 ± 0.43
16b	7.42 ± 0.22	NA	1.44 ± 0.08	1.64 ± 0.20
20a	NA	NA	NA	NA
20b	NA	NA	NA	NA
24a	7.71 ± 0.15	NA	3.06 ± 0.26	2.07 ± 0.07
24b	NA	NA	NA	NA
24c	NA	NA	NA	NA
24d	NA	NA	NA	NA
25a	NA	NA	4.18 ± 0.89	4.32 ± 0.52
25b	NA	NA	NA	NA
26	NA	NA	NA	NA

[a] Not tested.
[b] Not active at relevant concentrations.

in vivo, their 5-aryloxy counterparts could be interesting for in vivo experiments, since they (1) are stable upon storage, (2) can release the active 5-methylene furanones, and (3) the ether bond is not degradable by plasmatic esterases. The importance of the methylene group at C5 was further demonstrated by the screening of the 5-alkyloxymethyl derivatives, which were antifungally inactive. As regards further substitution of the 5-methylene moiety, its replacement of the 5-methylene group with more complex alkylidene substituents was unproductive, with a notable exception of 2-hydroxyethylidene (compound **24a**), which restored the antifungal activity close to the level of **4**. It is therefore likely that a substituent with a binding ability in the allylic position on the alkylidene side chain is necessary.

The antifungally active substances **4** (and hence its precursors **15d–16b**), and **24a** as well as inactive derivatives **10a** and **10b** also displayed cytostatic activities against HeLa S3 a CCRF-CEM cell lines, even though they can be generally referred to as moderate to low. Interestingly, the differences observed upon cytostatic activity screening of the 5-phenoxymethyl furanones **15d–16b** which are all subject to the elimination affording **4** seem to

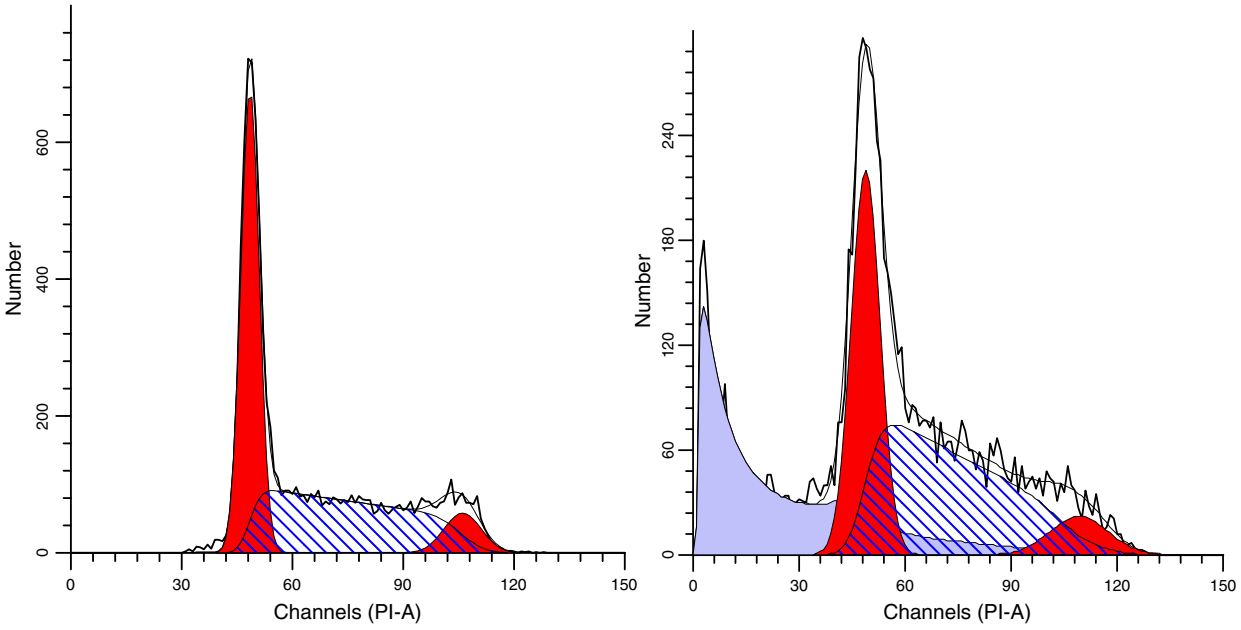


Figure 3. Cell cycle profile in CCRF-CEM cells treated with compound **4**. Control cells (left) and cells grown in the presence of tested compound **4** (2.5 μmol L^{-1-1}, right).

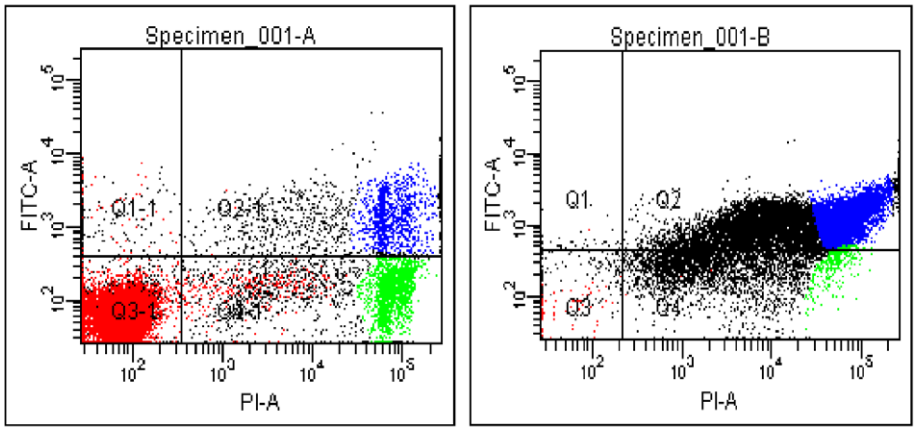


Figure 4. Annexin V binding—detection of apoptotic/necrotic cells. Control cells (A) and cells grown in the presence of tested compound **4** (2.5 μmol L^{-1-1}, B).

indicate an influence of the liberated phenolic derivative on the cytostatic effect of **4**. Furanone **4** itself was found to be a necrotic agent.

Finally, future work will be directed towards the introduction of a hydrophilic group onto the phenyl ring at C3 of the furanone core in compounds similar to **4**, and towards further elaboration of the allylic position in compounds similar to **24a**.

5. Experimental

5.1. General experimental procedures

All substances were purchased from Sigma–Aldrich and used as received. THF was freshly distilled from sodium benzophenone ketyl. DMF was dried over 3 Å molecular sieves. All anhydrous reactions were performed in flame-dried Schlenk tubes under Ar atmosphere. Analytical thin-layer chromatography (TLC) was conducted on E. Merck TLC plates (Silica Gel 60 F_{254}, aluminum back). Silica Gel 60 (230–400 mesh) for column chromatography was purchased from E. Merck. Melting points were determined on Kofler block or Büchi B-545 Melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded for $CDCl_3$, DMSO-d_6, CD_3OD and THF-d_8 solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts were recorded as δ values in parts per million (ppm), and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal. Coupling constants (J) are given in hertz. All assignments were made on the basis of DPFG-NOE, gHSQC and gHMBC experiments. In certain cases of mixtures of isomers, these were referred to as A and B. Infrared spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer. Low resolution mass spectra were measured on Finnigan LTQ XL apparatus. The purity of all target compounds was established by elemental analysis carried out on a CHNS-OCE FISONS EA 1110 instrument.

5.2. Stability evaluation (HPLC)

Routine chromatographic analyses were performed using a Thermo Electron chromatograph. The chromatographic system was composed of an SCM1000 solvent degasser, P4000 quaternary gradient pump, AS 3000 autosampler with a 100 μL sample loop, UV6000LP photodiode-array detector with Light Pipe Technology, SN4000 system controller and data station with the ChromQuest 4 analytical software (Thermo Electron, San Jose, CA, USA). The chromatographic conditions described in our previous paper[16] had to be modified in order to accelerate the acquisition of chromatographic data as a fast decomposition of the compounds **1a** and **16b** was expected.

A chromatographic column 250–4.6 mm containing LiChrospher 60 RP-selectB 5 μm with a 4 mm × 4 mm guard column (the same stationary phase, all from Merck) and an isocratic mobile phase containing only acetonitrile and UHQ water (6:4, v/v) with a flow rate of 1 mL min^{-1} were used for the separation of compounds 1a (t_R = 1.5 min) and 16b (t_R = 1.2 min) and their degradation products olefin 4 (t_R = 5.6 min) and 3-hydroxybenzoic acid (HBA, t_R = 0.86 min). A single wavelength mode at 263 nm was used to obtain chromatograms and a photodiode-array mode in the range 195–380 nm with a 1 nm scanning step was applied to record UV spectra of individual compounds.

Compounds **1a** (4.7 mg) and **16b** (5.8 mg) were dissolved in DMSO (280 μL) and 30 μL of the resultant mixture was added to 2.97 mL of RPMI 1640 medium (vide infra). The solution was kept at rt and samples were analyzed after period of 0.5, 1, 2, 3, 5, 10 and 24 h.

5.3. Antifungal activity

In vitro antifungal activities of the compounds were evaluated on a panel of four ATCC (*C. albicans* ATCC 44859, *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) and eight clinical isolates of yeasts (*C. krusei* E28, *C. tropicalis* 156, *C. glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon beigelii* 1188) and filamentous fungi (*A. fumigatus* 231, *A. corymbifera* 272, *Trichophyton mentagrophytes* 445) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three of the above ATCC strains (*C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258) also served as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested.

Minimum inhibitory concentrations (MICs) were determined by the microdilution format of the NCCLS M27-A guidelines.[29] Dimethyl sulfoxide (100%) served as a diluent for all compounds; the final concentration did not exceed 2%. RPMI 1640 (Sevapharma, Prague) medium supplemented with L-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 N NaOH was used as the test medium. The wells of the microdilution tray contained 100 μL of the RPMI 1640 medium with twofold serial dilutions of the compounds (500–0.48 μmol/L for the new compounds) and 100 μL of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu mL^{-1}. The trays were incubated at 35 °C and MICs were read visually for filamentous fungi and photometrically for yeasts as an absorbance at 540 nm after 24 and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h. The MICs were defined as 80% inhibition of the growth of control. MICs were determined twice and in duplicate. The deviations from the usually obtained values given in Tables 1 and 2 were no higher than the nearest concentration value up and down the dilution scale.

5.4. Cytostatic activity assays

Inhibition of the cell growth was estimated in *mouse lymphocytic leukemia L1210 cells* (ATCC CCL 219), *CCRF-CEM T lymphoblastoid cells* (human acute lymphoblastic leukemia, ATCC CCL 119), *human promyelocytic leukemia HL-60 cells* (ATCC CCL 240) and *human cervix carcinoma HeLa S3 cells* (ATCC CCL 2.2).[30] *L1210 cells*, *CCRF-CEM cells* and *HL-60* cells were cultivated in RPMI 1640 medium supplemented with calf fetal serum using 24-well tissue culture plates. The endpoint of the cell growth was 72 h following the drug addition. *HeLa S3 cells* were seeded to 24-well dishes in RPMI 1640 HEPES modification with fetal calf serum. 48 h following the drug addition the cultivation was stopped and the cell growth was evaluated. In parallel, the cell viability was quantified using XTT standard spectrophotometric assay.[31] The inhibitory potency of the compound tested was expressed as IC_{50} values.

5.5. Flow cytometry

The flow cytometry cell cycle analysis (BD FACSAria) was performed by using ethanol-fixed cells stained with propidium iodide in buffer containing RNase A at the endpoint of the cell growth (72 h). Tested compound was added to a culture medium at IC_{50} concentration. The cell cycle events were evaluated with the aid of BD FACSDiva™ Software v5.3. Apoptosis was evaluated by flow cytometry using Annexin V-FITC apoptosis kit (Clontech Laboratories, Inc.) according manufacturer's instructions.

5.6. Chemistry

5.6.1. 3-(4-Bromophenyl)-5-methylene-2,5-dihydrofuran-2-one (4)

Lactone **6**[12] (0.075 g, 0.28 mmol), triphenylphosphine (0.088 g, 0.34 mmol) and imidazole (0.023 g, 0.34 mmol) were dissolved in dry dichloromethane (3 mL), and the solution was stirred at room temperature for 30 min. The mixture was then cooled down to −10 °C, and a solution of iodine (0.085 mg, 0.34 mmol) in dry dichloromethane (3 mL) was added dropwise. The resultant mixture was allowed to warm up to room temperature, diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium thiosulphate solution (30 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 9:1) to afford lactone **4**.[16] Yield: 64%. White crystals, mp 85–87 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.83–7.76 (2H, m, AA′, BB′, H2′, H6′), 7.60–7.54 (2H, m, AA′, BB′, H3′, H5′), 7.53 (1H, s, H4), 5.29 (1H, d, J = 2.6 Hz, CH_2), 4.99 (1H, d, J = 2.6 Hz, CH_2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 168.2, 153.3, 134.5, 132.1, 131.0, 128.8, 127.8, 124.4, 98.1; IR: (ATR) ν_{max} 1293, 1404, 1486, 1584, 1642, 1765, 2852, 2922, 3098 cm^{-1}; LRMS: m/z (relative intensity) 251.1 $[M+H]^+$ (2), 233.2 (2), 217.2 (1), 201.1 (1), 183.1 (1), 102.2 (1). Anal. Calcd for $C_{11}H_7BrO_2$: C, 52.62; H, 2.81. Found: C, 52.42; H, 2.93.

5.6.2. General procedure for preparation of compounds 10a–10b

A mixture of LDA (1.5 M THF complex in cyclohexane, 8.42 mmol) in dry THF (40 mL) under argon atmosphere was cooled down to −70 °C, then corresponding lactone **9a–b** (7.17 mmol) in 20 mL of dry THF was added dropwise. The resultant mixture was stirred at −70 °C for 1 h and a solution of phenylselenenyl bromide (9.32 mmol) in 10 mL of dry THF was added. Reaction mixture was allowed to warm to rt in period of 2 h and solvents were removed under reduced pressure. The crude product was dissolved in ethyl acetate (200 mL), washed with saturated aqueous ammonium chloride solution (200 mL), organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. Product was immediately purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 9:1) to afford phenylselenenyl lactones, which were dissolved in mixture of ethyl acetate–THF (2 mL:1.25 mL). Solution was cooled down to 0 °C and sodium hydrogen carbonate (8.06 mmol) and 30% aqueous hydrogen peroxide solution (0.61 mL) were added. Reaction mixture was stirred at rt for 1 h, then it was diluted with ethyl acetate (100 mL), washed with brine (150 mL), organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 7:3) to afford lactones **10a–b**.

5.6.2.1. 3-(4-Bromophenyl)-5-methoxymethyl-2,5-dihydrofuran-2-one (10a). Yield: 65%. White crystals, mp 56–58 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.78–7.70 (2H, m, AA′, BB′, H2′, H6′), 7.60 (1H, d, J = 1.7 Hz, H4), 7.56–7.49 (2H, m, AA′, BB′, H3′, H5′), 5.14 (1H, td, J_1 = 5.1 Hz, J_2 = 1.9 Hz, H5), 3.70–3.63 (2H, t, J = 4.8 Hz, OCH_2), 3.40 (3H, s, CH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.0, 145.5, 131.9, 131.6, 128.6, 128.2, 123.8, 79.3, 72.2, 59.7; IR: (KBr) ν_{max} 1123, 1198, 1292, 1332, 1475, 1490, 1588, 1738, 2832, 2866, 2897, 2930, 2992, 3076 cm^{-1}; LRMS: m/z (relative intensity) 283.0 $[M+H]^+$ (21), 251.0 (9), 225.1 (28), 212.1 (11), 196.0 (8), 184.1 (28), 129.9 (20), 117.1 (40), 105.0 (52), 76.1 (30). Anal. Calcd for $C_{12}H_{11}BrO_3$: C, 50.91; H, 3.92. Found: C, 51.01; H, 3.88.

5.6.2.2. 3-(4-Bromophenyl)-5-ethoxymethyl-2,5-dihydrofuran-2-one (10b). Yield: 58%. Colorless crystals, mp 43–45 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.79–7.72 (2H, m, AA′, BB′, H2′, H6′), 7.63 (1H, d, J = 1.9 Hz, H4), 7.59–7.51 (2H, m, AA′, BB′, H3′, H5′), 5.15 (1H, td, J_1 = 5.5 Hz, J_2 = 1.9 Hz, H5), 3.77 (1H, dd, J_1 = 10.4 Hz, J_2 = 5.5 Hz, OCH_2), 3.66 (1H, dd, J_1 = 10.4 Hz, J_2 = 5.5 Hz, OCH_2), 3.63–3.50 (2H, m, OCH_2), 1.20 (3H, t, J = 7.0 Hz, CH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.0, 145.9, 131.9, 131.7, 128.6, 128.3, 123.8, 79.5, 70.4, 67.5, 15.0; IR: (ATR) ν_{max} 1110, 1294, 1333, 1487, 1588, 1748, 2866, 2928, 2975, 3097 cm^{-1}; LRMS: m/z (relative intensity) 297.0 $[M+H]^+$ (100), 291.5 (28), 265.1 (34), 251.2 (92), 225.2 (8), 207.2 (5). Anal. Calcd for $C_{13}H_{13}BrO_3$: C, 52.55; H, 4.41. Found: C, 52.73; H, 4.61.

5.6.3. General procedure for preparation of compounds 15a–15g

A mixture of LDA (1.5 M THF complex in cyclohexane, 2.25 mmol) in dry THF (10 mL) under argon atmosphere was cooled down to −70 °C, then corresponding lactone **14a–g** (1.88 mmol) in 3 mL of dry THF was added dropwise. The resultant mixture was stirred at −70 °C for 1 h and a solution of phenylselenenyl bromide (2.63 mmol) in 3 mL of dry THF was added. Reaction mixture was allowed to warm to rt in period of 2 h and solvents were removed under reduced pressure. The crude product was dissolved in ethyl acetate (20 mL), washed with saturated aqueous ammonium chloride solution (50 mL), organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. Product was immediately purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 9:1) to afford phenylselenenyl lactones, which were dissolved in mixture of ethyl acetate–THF (0.4 mL:0.25 mL). Solution was cooled down to 0 °C and sodium hydrogen carbonate (2.12 mmol) and 30% aqueous hydrogen peroxide solution (0.16 mL) were added. Reaction mixture was stirred at rt for 1 h, then it was diluted with ethyl acetate (20 mL), washed with brine (50 mL), organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. Product was recrystallized in ethyl acetate–hexane mixture to afford lactones **15a–g**.

5.6.3.1. Methoxymethyl 4-{[4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-2-yl]methoxy}benzoate (15a). Yield: 41%. White crystals, mp 90–92 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.07–8.01 (2H, m, AA′, BB′, H2, H6), 7.80–7.74 (2H, m, AA′, BB′, H2″, H6″), 7.70 (1H, d, J = 1.9 Hz, H4′) 7.60–7.52 (2H, m, AA′, BB′, H3″, H5″), 6.97–6.90 (2H, m, AA′, BB′, H3, H5), 5.46 (2H, s, OCH_2), 5.39 (1H, td, J_1 = 5.2 Hz, J_2 = 1.9 Hz, H5′), 4.37 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.2 Hz, OCH_2), 4.28 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.2 Hz, OCH_2), 3.54 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.6, 165.5, 161.7, 144.4, 132.2, 132.1, 132.0, 128.6, 127.9, 124.2, 123.3, 114.2, 90.8, 78.0, 67.5, 57.7; IR: (KBr) ν_{max} 1161, 1252, 1288, 1490, 1510, 1606, 1720, 1757, 2946, 3073 cm^{-1}; LRMS: m/z (relative intensity) 433.1 $[M+H]^+$ (100), 422.4 (18), 402.5 (12), 343.3 (6), 205.0 (4), 177.0 (4), 144.4 (8), 130.1 (42).

5.6.3.2. Methoxymethyl 3-{[4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-2-yl]methoxy}benzoate (15b). Yield: 40%. White crystals, mp 89–92 °C; ^{1}H NMR: (300 MHz, DMSO-d_6) δ 8.22 (1H, d, J = 1.9 Hz, H4′), 7.91–7.84 (2H, m, AA′, BB′, H2″, H6″), 7.71–7.64 (2H, m, AA′, BB′, H3″, H5″), 7.64–7.59 (1H, m, H4), 7.50–7.43 (2H, m, H2, H6), 7.35–7.24 (1H, m, H5), 5.63–5.57 (1H, m, H5′), 5.42 (2H, s, OCH_2), 4.53 (1H, dd, J_1 = 11.1 Hz, J_2 = 4.4 Hz, OCH_2), 4.34 (1H, dd, J_1 = 11.1 Hz, J_2 = 4.4 Hz, OCH_2), 3.44 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, DMSO-d_6) δ 171.2, 165.1, 158.3, 148.3, 131.9, 131.1, 130.4, 129.9, 129.0, 128.9, 122.9, 122.5, 120.6, 115.1, 91.0, 79.4, 67.6, 57.3; IR: (ATR) ν_{max} 1157, 1229, 1276, 1295, 1340, 1403, 1440, 1485, 1584, 1719, 1756, 2950, 3071 cm^{-1}; LRMS: m/z (relative

intensity) 433.1 $[M+H]^+$ (100), 402.9 (14), 388.9 (12), 339.2 (19), 294.3 (10), 251.0 (3), 219.3 (3), 184.3 (2), 139.1 (3), 102.2 (7).

5.6.3.3. 3-(4-Bromophenyl)-5-[(4-methoxyphenoxy)methyl]-2,5-dihydrofuran-2-one (15c). Yield: 42%. Brownish crystals, mp 89–91 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.78–7.75 (2H, m, AA′, BB′, H2′, H6′), 7.71 (1H, d, J = 1.9 Hz, H4) 7.57–7.54 (2H, m, AA′, BB′, H3′, H5′), 6.86–6.82 (4H, m, H2″, H3″, H5″, H6″), 5.33 (1H, td, J_1 = 5.2 Hz, J_2 = 1.9 Hz, H5), 4.28 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.4 Hz, OCH_2), 4.14 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.4 Hz, OCH_2), 3.77 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.9, 154.5, 152.0, 145.2, 131.9, 131.8, 128.6, 128.1, 124.0, 115.9, 114.7, 78.4, 68.5, 55.7; IR: (KBr) ν_{max} 1235, 1490, 1508, 1756, 2926, 3076 cm^{-1}; LRMS: m/z (relative intensity) 375.1 $[M+H]^+$ (100), 356.7 (53), 304.5 (38), 251.4 (28), 235.9 (31), 217.3 (14), 149.3 (22), 102.3 (29). Anal. Calcd for $C_{18}H_{15}BrO_4$: C, 57.62; H, 4.03. Found: C, 57.96; H, 4.33.

5.6.3.4. 3-(4-Bromophenyl)-5-phenoxymethyl-2,5-dihydrofuran-2-one (15d). Yield: 43%. White crystals, mp 113 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.80–7.74 (2H, m, AA′, BB′, H2′, H6′), 7.72 (1H, d, J = 1.9 Hz, H4), 7.59–7.53 (2H, m, AA′, BB′, H3′, H5′), 7.34–7.27 (2H, m, H3″, H5″), 7.04–6.97 (1H, m, H4″), 6.93–6.86 (2H, m, H2″, H6″), 5.37 (1H, td, J_1 = 5.5 Hz, J_2 = 1.9 Hz, H5), 4.35 (1H, dd, J_1 = 9.9 Hz, J_2 = 5.5 Hz, OCH_2), 4.18 (1H, dd, J_1 = 9.9 Hz, J_2 = 5.5 Hz, OCH_2). ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.8, 157.9, 145.2, 132.0, 131.9, 129.7, 128.6, 128.1, 124.0, 121.8, 114.7, 78.3, 67.6; IR: (KBr) ν_{max} 1127, 1244, 1491, 1497, 1589, 1599, 1745, 2926, 2953, 3065 cm^{-1}; LRMS: m/z (relative intensity) 345.0 $[M+H]^+$ (1), 282.0 (3), 250.1 (52), 194.0 (8), 180.2 (42), 171.1 (66), 115.0 (100), 101.1 (54), 75.2 (43). Anal. Calcd for $C_{17}H_{13}BrO_4$: C, 59.15; H, 3.80. Found: C, 59.49; H, 3.95.

5.6.3.5. 3-(4-Bromophenyl)-5-[(4-iodophenoxy)methyl]-2,5-dihydrofuran-2-one (15e). Yield: 43%. White crystals, mp 149–151 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.79–7.73 (2H, m, AA′, BB′, H2′, H6′), 7.68 (1H, d, J = 1.9 Hz, H4), 7.60–7.53 (4H, m, H3′, H5′, H3″, H5″), 6.71–6.64 (2H, m, AA′, BB′, H2″, H6″), 5.35 (1H, td, J_1 = 5.2 Hz, J_2 = 1.9 Hz, H5), 4.28 (1H, dd, J_1 = 10.1 Hz, J_2 = 5.2 Hz, OCH_2), 4.17 (1H, dd, J_1 = 10.1 Hz, J_2 = 5.2 Hz, OCH_2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.7, 157.8, 144.6, 138.4, 132.1, 131.9, 128.6, 127.9, 124.1, 117.0, 84.1, 78.1, 67.6; IR: (KBr) ν_{max} 1126, 1245, 1284, 1301, 1485, 1584, 1749, 2920, 2949, 3088 cm^{-1}; LRMS: m/z (relative intensity) 470.9 $[M+H]^+$ (1), 407.0 (1), 329.1 (1), 251.0 (49), 180.9 (40), 172.0 (65), 128.1 (24), 115.9 (100), 101.9 (53), 76.0 (40). Anal. Calcd for $C_{17}H_{12}BrIO_3$: C, 43.34; H, 2.57. Found: C, 43.69; H, 2.86.

5.6.3.6. 3-(4-Bromophenyl)-5-[(3-bromophenoxy)methyl]-2,5-dihydrofuran-2-one (15f). Yield: 38%. Brownish crystals, mp 115–117 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.80–7.74 (2H, m, AA′, BB′, H2′, H6′), 7.68 (1H, d, J = 1.9 Hz, H4), 7.59–7.53 (2H, m, AA′, BB′, H3′, H5′), 7.17–7.12 (2H, m, H4″, H5″), 7.07–7.04 (1H, m, H2″), 6.86–6.80 (1H, m, H6″), 5.36 (1H, td, J_1 = 5.2 Hz, J_2 = 1.9 Hz, H5), 4.30 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.2 Hz, OCH_2), 4.19 (1H, dd, J_1 = 10.0 Hz, J_2 = 5.2 Hz, OCH_2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.6, 158.6, 144.6, 132.1, 132.0, 130.7, 128.6, 127.9, 125.0, 124.1, 122.9, 118.1, 113.5, 78.0, 67.7; IR: (KBr) ν_{max} 1126, 1230, 1287, 1474, 1490, 1575, 1587, 1750, 2926, 3088 cm^{-1}; LRMS: m/z (relative intensity) 422.9 $[M+H]^+$ (1), 403.9 (1), 329.0 (1), 281.1 (1), 251.0 (53), 180.9 (41), 172.1 (67), 116.0 (100), 101.9 (55), 75.9 (45). Anal. Calcd for $C_{17}H_{12}Br_2O_3$: C, 48.15; H, 2.85. Found: C, 47.92; H, 3.03.

5.6.3.7. Methyl 4-{[4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-2-yl]methoxy}benzoate (15g). Yield: 41%. White crystals, mp 148–151 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.03–7.96 (2H, m, AA′, BB′, H2, H6), 7.80–7.74 (2H, m, AA′, BB′, H2″, H6″), 7.70 (1H, d, J = 1.9 Hz, H4′), 7.60–7.52 (2H, m, AA′, BB′, H3″, H5″), 6.96–6.87 (2H, m, AA′, BB′, H3, H5), 5.39 (1H, td, J_1 = 5.3 Hz, J_2 = 1.9 Hz, H5′), 4.37 (1H, dd, J_1 = 9.9 Hz, J_2 = 5.3 Hz, OCH_2), 4.26 (1H, dd, J_1 = 9.9 Hz, J_2 = 5.3 Hz, OCH_2), 3.89 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.6, 166.6, 161.4, 144.5, 132.2, 132.0, 131.8, 128.6, 127.9, 124.2, 123.7, 114.2, 78.0, 67.5, 52.0; IR: (KBr) ν_{max} 1170, 1254, 1283, 1317, 1436, 1490, 1511, 1589, 1606, 1717, 1759, 2952, 3076 cm^{-1}; LRMS: m/z (relative intensity) 403.0 $[M+H]^+$ (2), 372.0 (1), 283.5 (1), 252.1 (1), 177.9 (14), 131.1 (2), 122.2 (3), 90.3 (100) 76.8 (14). Anal. Calcd for $C_{19}H_{15}BrO_5$: C, 56.59; H, 3.75. Found: C, 56.91; H, 4.02.

5.6.4. General procedure for preparation of compounds 16a–16b

A mixture of corresponding methoxymethyl derivative **15a–b** (0.27 mmol) and DOWEX® 50 W (0.034 g) in 6 mL of methanol was stirred at 40 °C for 1 h. The precipitated pure product and resin were filtered off, product was dissolved in ethyl acetate (50 mL) and solvent was removed under reduced pressure.

5.6.4.1. 4-{[4-(4-Bromophenyl)-5-oxo-2,5-dihydrofuran-2-yl]-methoxy}benzoic acid (16a). Yield: 92%. White crystals, mp 224 °C; ^{1}H NMR: (300 MHz, DMSO-d_6) δ 12.68 (1H, bs, OH), 8.22 (1H, d, J = 1.9 Hz, H4′), 7.91–7.84 (4H, m, H2″, H6″, H2, H6), 7.70–7.63 (2H, m, H3″, H5″), 7.06–6.99 (2H, m, H3, H5), 5.64–5.58 (1H, m, H5′), 4.53 (1H, dd, J_1 = 11.2 Hz, J_2 = 4.4 Hz, OCH_2), 4.33 (1H, dd, J_1 = 11.2 Hz, J_2 = 4.4 Hz, OCH_2); ^{13}C NMR: (75 MHz, DMSO-d_6) δ 171.2, 167.1, 161.7, 148.2, 132.1, 131.9, 131.6, 129.9, 129.3, 123.8, 122.9, 114.7, 79.3, 67.4; IR: (KBr) ν_{max} 1170, 1256, 1425, 1490, 1513, 1607, 1685, 1717, 1751, 2954, 3079 cm^{-1}; LRMS: m/z (relative intensity) 389.9 $[M+H]^+$ (56), 371.2 (12), 282.3 (100), 277.2 (39), 266.2 (58), 251.0 (6), 194.9 (7), 171.0 (16), 163.0 (6), 149.1 (6), 133.0 (7), 102.2 (7). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.72; H, 3.14.

5.6.4.2. 3-{[4-(4-Bromophenyl)-5-oxo-2,5-dihydrofuran-2-yl]-methoxy}benzoic acid (16b). Yield: 95%. White crystals, mp 206 °C; ^{1}H NMR: (300 MHz, DMSO-d_6) δ 13.01 (1H, bbs, OH), 8.22 (1H, d, J = 1.9 Hz, H4′), 7.93–7.83 (2H, m, AA′, BB′, H2″, H6″), 7.72–7.63 (2H, m, AA′, BB′, H3″, H5″), 7.58–7.52 (1H, m, H2), 7.45–7.37 (2H, m, H4, H6), 7.24–7.16 (1H, m, H5), 5.64–5.55 (1H, m, H5′), 4.51 (1H, dd, J_1 = 11.1 Hz, J_2 = 4.4 Hz, OCH_2), 4.31 (1H, dd, J_1 = 11.1 Hz, J_2 = 4.4 Hz, OCH_2); ^{13}C NMR: (75 MHz, DMSO-d_6) δ 171.3, 167.2, 158.1, 148.3, 132.5, 131.9, 130.1, 129.9, 129.0, 128.9, 122.9, 122.4, 119.8, 115.1, 79.4, 67.5; IR: (ATR) ν_{max} 1243, 1257, 1308, 1385, 1421, 1458, 1489, 1587, 1677, 1707, 1757, 2951, 3069 cm^{-1}; LRMS: m/z (relative intensity) 388.9 $[M+H]^+$ (100), 369. (18), 329.1 (7), 301.1 (9), 291.4 (17), 269.3 (8), 251.1 (30), 227.3 (5), 213.5 (6). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.88; H, 3.28.

5.6.4.3. 3-(4-Bromophenyl)-5-[(4-hydroxyphenoxy)methyl]-2,5-dihydrofuran-2-one (16c). Methoxy derivative **15c** (0.111 g, 0.29 mmol) was dissolved in dry dichloromethane (2 mL), solution was cooled down to −50 °C, then solution of BBr_3 (1.0 M solution in dichloromethane, 1.20 mL) was added dropwise. Reaction mixture was allowed to warm to 5 °C in period of 1 h and water (2 mL) was added. After stirring at rt for further 30 min, reaction mixture was diluted with ethyl acetate (20 mL), washed with saturated aqueous sodium chloride solution (50 mL), organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. Product was recrystallized in ethyl acetate–hexane mixture to afford lactone **16c**. Yield: 75%. Brownish crystals, mp 118–120 °C; ^{1}H NMR: (300 MHz, CD_3OD) δ 7.95 (1H, d, J = 1.9, H4), 7.84–7.81 (2H, m, AA′, BB′, H3′, H5′), 7.60–7.57 (2H,

m, AA′, BB′, H2′, H6′), 6.80–6.76 (2H, m, AA′, BB′, H3″, H5″), 6.71–6.68 (2H, m, AA′, BB′, H2″, H6″), 5.42–5.39 (1H, m, H5), 4.31 (1H, dd, J_1 = 10.9 Hz, J_2 = 4.4 Hz, OCH_2), 4.19 (1H, dd, J_1 = 10.9 Hz, J_2 = 4.4 Hz, OCH_2); ^{13}C NMR: (75 MHz, CD_3OD) δ 172.0, 153.1, 153.1, 148.4, 132.9, 132.2, 130.2, 129.9, 124.5, 117.2, 116.9, 81.2, 69.5; IR: (KBr) ν_{max} 1215, 1234, 1489, 1511, 1758, 2872, 2923, 3077 cm^{-1}; LRMS: *m*/*z* (relative intensity) 369.1 $[M+H]^+$ (22), 360.9 (100), 304.7 (18), 271.9 (11), 251.2 (48), 238.1 (21), 225.1 (9), 194.9 (12), 164.9 (16), 149.0 (9), 133.0 (6), 102.1 (6). Anal. Calcd for $C_{17}H_{13}BrO_4$: C, 56.53; H, 3.63. Found: C, 56.83; H, 3.38.

5.6.4.4. (*Z*)-3-(4-Bromophenyl)-5-ethylidene-2,5-dihydrofuran-2-one (20a). Iodoenone **19a** (0.610 g, 1.67 mmol) was dissolved in dry DMF (10 mL), and $PdCl_2(PPh_3)_2$ (0.061 g, 0.0835 mmol) and $NaHCO_3$ (0.280 g, 3.34 mmol) were added. The reaction flask was evacuated and vacuum was replaced by carbon monooxide atmosphere. The reaction mixture was stirred at 60 °C for 24 h, then it was diluted with ethyl acetate (50 mL), washed with 5% aqueous sodium chloride solution (50 mL). The organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 8:2) and further recrystallized in mixture of hexane–ethyl acetate to afford lactone **20a** as (*Z*) isomer. Yield: 5%. White crystals, mp 122–124 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.82–7.75 (2H, m, AA′, BB′, H2′, H6′), 7.59–7.52 (2H, m, AA′, BB′, H3′, H5′), 7.47 (1H, s, H4), 5.43 (1H, q, J_1 = 7.5 Hz, CH), 2.02 (3H, d, J = 7.5 Hz, CH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 168.4, 148.8, 135.0, 132.0, 128.7, 128.5, 128.4, 123.7, 112.6, 12.2; IR: (KBr) ν_{max} 1295, 1323, 1405, 1489, 1582, 1667, 1744, 2854, 2939, 3072, 3086 cm^{-1}; LRMS: *m*/*z* (relative intensity) 265.0 $[M+H]^+$ (58), 225.0 (16), 186.1 (42), 181.0 (30), 129.9 (100), 102.1 (55), 75.4 (35). Anal. Calcd for $C_{12}H_9BrO_2$: C, 54.37; H, 3.42. Found: C, 54.09; H, 3.22.

5.6.4.5. 3-(4-Bromophenyl)-2,5-dihydrofuran-2-one (20b). Iodoalcohol **18b**[32] (1.140 g, 3.36 mmol) was dissolved in dry DMF (20 mL) and $Pd(OAc)_2$ (0.039 g, 0.168 mmol), triphenylphosphine (0.093 g, 0.355 mmol) and triethylamine (1.20 mL, 8.61 mmol) were added. The reaction flask was evacuated and vacuum was replaced by carbon monooxide atmosphere. The reaction mixture was stirred at 60 °C for 24 h, then it was diluted with ethyl acetate (50 mL), washed with 5% aqueous sodium chloride solution (50 mL). The organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 9:1) and further recrystallized in mixture of hexane–ethyl acetate to afford lactone **20b**.[21] Yield: 41%. Yellowish crystals, mp 117 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.78–7.72 (2H, m, AA′, BB′, H2′, H6′), 7.67 (1H, t, J = 2.1 Hz, H4), 7.58–7.52 (2H, m, AA′, BB′, H3′, H5′), 4.93 (2H, d, J = 2.1 Hz, H5); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.8, 144.5, 131.9, 130.7, 128.5, 128.3, 123.7, 69.5; IR: (ATR) ν_{max} 1116, 1296, 1315, 1343, 1403, 1442, 1485, 1586, 1737, 2852, 2943, 3085 cm^{-1}; LRMS: *m*/*z* (relative intensity) 239.1 $[M+H]^+$ (100), 221.1 (5), 211.1 (6), 193.1 (4), 180.9 (3), 160.1 (13), 149.1 (3), 116.2 (4). Anal. Calcd for $C_{10}H_7BrO_2$: C, 50.24; H, 2.95. Found: C, 50.74; H, 3.24.

5.6.4.6. (*Z*)-3-(4-Bromophenyl)-5-(2-hydroxyethylidene)-2,5-dihydrofuran-2-one (24a). *Solution A:* Dry THF (2 mL) was added to $PdCl_2(TFP)_2$ (84.0 mg, 0.13 mmol) at −80 °C under argon atmosphere; to the resultant stirred suspension butyllithium (1.6 M solution in hexanes, 0.26 mmol) was added dropwise. The reaction mixture was allowed to warm up to −30 °C in period of 1 h.

Solution B: Solution of **23a** (0.753 g, 2.60 mmol) in dry DMF (2 mL).

Solution C: The solvent from 4-bromophenylzinciodide (0.5 M solution in THF, 3.7 mmol) was almost completely removed under reduced pressure, then dry DMF (3 mL) was added under argon atmosphere.

Solution B was added via *cannula* to a stirred solution A at −30 °C under argon atmosphere. The reaction mixture was allowed to warm up to rt in period of 1 h, then solution C was added via *cannula.* The resultant mixture was stirred at rt for further 3 h, it was then diluted with ethyl acetate (25 mL) and washed with saturated aqueous ammonium chloride solution (50 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 94:6). The resultant THP-protected alcohol was dissolved in methanol (22 mL), 220 mg of DOWEX® 50 W was added and the reaction mixture was stirred at rt for 1 h. The resin was filtered off, solvent was removed under reduced pressure, the resultant solid was diluted with ethyl acetate (50 mL), washed with saturated aqueous sodium chloride solution (50 mL), the organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 6:4) and further recrystallized in mixture of hexane–ethyl acetate to afford lactone **24a** as (*Z*) isomer. Yield: 65%. White crystals, mp 131 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.82–7.76 (2H, m, AA′, BB′, H2′, H6′), 7.59–7.54 (2H, m, AA′, BB′, H3′, H5′), 7.51 (1H, s, H4), 5.55 (1H, t, J = 6.9 Hz, CH), 4.56 (2H, d, J = 6.9 Hz, OCH_2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 167.6, 134.8, 132.1, 131.8, 128.8, 128.6, 127.9, 124.4, 114.1, 57.4; IR: (KBr) ν_{max} 1176, 1234, 1296, 1311, 1404, 1490, 1584, 1601, 1672, 1762, 2862, 2923, 2951, 3090, 3281 cm^{-1}; LRMS: *m*/*z* (relative intensity) 280.9 $[M+H]^+$ (50), 263.1 (16), 237.1 (10), 203.2 (5), 187.2 (6), 158.0 (10), 130.0 (100), 113.3 (12). Anal. Calcd for $C_{12}H_9BrO_3$: C, 51.27; H, 3.23. Found: C, 51.55; H, 3.50.

5.6.4.7. (*Z*)-3-(4-Bromophenyl)-5-(3-hydroxypropylidene)-2,5-dihydrofuran-2-one (24b). $PdCl_2(TFP)_2$ (25.0 mg, 0.039 mmol) and 4-bromophenylzinciodide (0.5 M solution in THF, 1.0 mmol) were added to a stirred solution **23b** (0.235 g, 0.78 mmol) in dry DMF (1 mL) at rt under argon atmosphere. The reaction mixture was stirred at rt for 2 h, it was then diluted with ethyl acetate (15 mL) and washed with saturated aqueous ammonium chloride solution (20 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 95:5). The resultant THP-protected alcohol was dissolved in methanol (6 mL), 75 mg of DOWEX® 50 W was added and the reaction mixture was stirred at rt for 1 h. The resin was filtered off, solvent was removed under reduced pressure, the resultant solid was diluted with ethyl acetate (20 mL), washed with saturated aqueous sodium chloride solution (20 mL), the organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 6:4) and further recrystallized in mixture of hexane–ethyl acetate to afford lactone **24b** as (*Z*) isomer. Yield: 59%. White crystals, mp 98–100 °C; ^{1}H NMR: (300 MHz, CD_3OD) δ 7.89 (1H, s, H4), 7.88–7.84 (2H, m, AA′, BB′, H2′, H6′), 7.60–7.54 (2H, m, AA′, BB′, H3′, H5′), 5.58 (1H, t, J = 8.0 Hz, CH), 3.70 (2H, t, J = 6.3 Hz, OCH_2), 2.68–2.59 (2H, m, CH_2); ^{13}C NMR: (75 MHz, CD_3OD) δ 169.9, 150.7, 137.6, 133.0, 130.1, 129.8, 129.4, 124.5, 115.5, 61.7, 31.1; IR: (KBr) ν_{max} 1296, 1384, 1404, 1489, 1583, 1758, 2884, 2954, 3089, 3423 cm^{-1}; LRMS: *m*/*z* (relative intensity) 295.1 $[M+H]^+$ (11), 277.1 (18), 263.1 (12), 251.2 (7), 237.1 (6), 214.3 (9), 204.2 (15), 177.1 (100). Anal. Calcd for $C_{13}H_{11}BrO_3$: C, 52.91; H, 3.76. Found: C, 53.21; H, 4.01.

5.6.4.8. (*Z*)-3-(4-Bromophenyl)-5-(4-hydroxybutylidene)-2,5-dihydrofuran-2-one (24c). $PdCl_2(TFP)_2$ (12.0 mg, 0.019 mmol) and 4-bromophenylzinciodide (0.5 M solution in THF, 0.5 mmol) were added to a stirred solution **23c** (0.110 g, 0.35 mmol) in dry DMF (1 mL) at rt under argon atmosphere. The reaction was stirred at rt for 2 h, it was then diluted with ethyl acetate (10 mL) and washed with saturated aqueous ammonium chloride solution (15 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 95:5). The resultant THP-protected alcohol was dissolved in methanol (3 mL), 30 mg of DOWEX® 50 W was added and the reaction mixture was stirred at rt for 1 h. The resin was filtered off, solvent was removed under reduced pressure, the resultant solid was diluted with ethyl acetate (10 mL), washed with saturated aqueous sodium chloride solution (10 mL), the organic layer was dried (Na_2SO_4) and solvents were removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 6:4) and further recrystallized in mixture of hexane–ethyl acetate to afford lactone **24c** as (*Z*) isomer. Yield: 56%. White crystals, mp 99–102 °C; ^{1}H NMR: (300 MHz, CD_3OD) δ 7.89–7.80 (3H, m, H4, H2′, H6′), 7.61–7.52 (2H, m, AA′, BB′, H3′, H5′), 5.56 (1H, t, J = 8.0 Hz, CH), 3.61 (2H, t, J = 6.3 Hz, OCH_2), 2.56–2.42 (2H, m, CH_2), 1.80–1.66 (2H, m, CH_2); ^{13}C NMR: (75 MHz, CD_3OD) δ 169.9, 149.9, 137.6, 132.9, 129.8, 129.1, 128.0, 124.4, 118.5, 62.3, 32.9, 24.2; IR: (KBr) ν_{max} 1295, 1405, 1489, 1582, 1668, 1755, 2876, 2939, 3093, 3421 cm^{-1}; LRMS: m/z (relative intensity) 309.5 $[M+H]^+$ (97), 291.8 (23), 263.7 (6), 251.1 (2), 231.9 (2), 213.7 (2), 177.7 (100). Anal. Calcd for $C_{14}H_{13}BrO_3$: C, 54.39; H, 4.24. Found: C, 54.68; H, 4.04.

5.6.4.9. (*Z*)-3-(4-Bromophenyl)-5-benzylidene-2,5-dihydrofuran-2-one (24d). $PdCl_2(TFP)_2$ (15.0 mg, 0.023 mmol) and 4-bromophenylzinciodide (0.5 M solution in THF, 0.6 mmol) were added to a stirred solution **23d** (0.115 g, 0.46 mmol) in dry DMF (1 mL) at rt under argon atmosphere. The reaction was stirred at rt for 3 h, it was then diluted with ethyl acetate (10 mL) and washed with saturated aqueous sodium chloride solution (15 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 95:5) to afford lactone **24d** as (*Z*) isomer. Yield: 30%. Yellow crystals, mp 206–207 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.86–7.79 (4H, m, H2′, H6′, H2″, H6″), 7.62 (1H, s, H4), 7.60–7.54 (2H, m, H3′, H5′), 7.46–7.31 (3H, m, H3″, H4″, H5″), 6.11 (1H, s, CH); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 168.5, 147.0, 136.6, 133.1, 132.1, 130.7, 129.3, 128.9, 128.5, 128.3, 127.6, 123.9, 111.5; IR: (KBr) ν_{max} 1103, 1179, 1293, 1359, 1449, 1485, 1580, 1643, 1746, 2926, 3024, 3068, 3103 cm^{-1}; LRMS: m/z (relative intensity) 327.0 $[M+H]^+$ (54), 299.1 (5), 282.0 (7), 247.9 (13), 219.9 (38), 208.0 (18), 192.1 (100), 181.1 (20), 119.0 (62). Anal. Calcd for $C_{17}H_{11}BrO_2$: C, 62.41; H, 3.39. Found: C, 62.79; H, 3.26.

5.6.5. General procedure for preparation of compounds 25a–c

A solution of corresponding alcohol **24** (1.00 mmol), dry pyridine (1.10 mmol) and acyl chloride (1.10 mmol) in dry dichloromethane (15 mL) was stirred at rt for 1.5 h. Mixture was diluted with ethyl acetate (50 mL), washed with saturated aqueous sodium chloride solution (50 mL), the organic layer was dried (Na_2SO_4) and solvent was removed under reduced pressure. The crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 8:2) to afford esters **25a–c**.

5.6.5.1. (*Z*)-4-[(4-Bromophenyl)-5-oxo-2,5-dihydrofuran-2-ylidene]methyl acetate (25a). Yield: 87%. White crystals, mp 99.6 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.80–7.75 (2H, m, AA′, BB′, H2′, H6′), 7.59–7.54 (2H, m, AA′, BB′, H3′, H5′), 7.49 (1H, s, H4), 5.48 (1H, t, J = 7.2 Hz, CH), 4.93 (2H, d, J = 7.2 Hz, OCH_2), 2.10 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.7, 167.3, 149.4, 134.4, 132.1, 130.7, 128.7, 127.7, 124.5, 108.7, 58.5, 20.8; IR: (KBr) ν_{max} 1110, 1225, 1308, 1365, 1382, 1406, 1450, 1490, 1582, 1676, 1744, 1761, 2872, 2924, 3088 cm^{-1}; LRMS: m/z (relative intensity) 309.0 $[M+H]^+$ (1), 295.1 (42), 263.3 (100), 251.4 (2), 237.3 (7), 184.4 (10). Anal. Calcd for $C_{14}H_{11}BrO_4$: C, 52.04; H, 3.43. Found: C, 51.88; H, 3.09.

5.6.5.2. (*Z*)-4-[(4-Bromophenyl)-5-oxo-2,5-dihydrofuran-2-ylidene]methyl benzoate (25b). Yield: 70%. White crystals, mp 106 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.10–8.02 (2H, m, H2, H6), 7.86–7.74 (2H, m, H2″, H6″), 7.64–7.39 (5H, m, H3″, H5″, H3, H4, H5), 7.52 (1H, s, H4′), 5.62 (1H, t, J = 7.2 Hz, CH), 5.20 (2H, d, J = 7.2 Hz, OCH_2); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 167.4, 166.3, 149.6, 134.4, 133.3, 132.1, 130.7, 129.7, 128.9, 128.7, 128.4, 127.8, 124.6, 108.8, 58.9; IR: (KBr) ν_{max} 1117, 1176, 1268, 1319, 1378, 1407, 1451, 1491, 1585, 1602, 1678, 1731, 1750, 3071, 3106 cm^{-1}; LRMS: m/z (relative intensity) 385.0 $[M+H]^+$ (100), 329.1 (1), 295.0 (11), 282.3 (57), 263.1 (99), 254.3 (2), 239.1 (4), 184.1 (6), 149.2 (3). Anal. Calcd for $C_{19}H_{13}BrO_4$: C, 59.24; H, 3.40. Found: C, 59.51; H, 3.66.

5.6.5.3. (*Z*)-4-[(4-Bromophenyl)-5-oxo-2,5-dihydrofuran-2-ylidene]ethyl acetate (25c). Yield: 80%. White crystals, mp 89–92 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.82–7.75 (2H, m, AA′, BB′, H2′, H6′), 7.59–7.52 (2H, m, AA′, BB′, H3′, H5′), 7.48 (1H, s, H4), 5.38 (1H, t, J = 7.8 Hz, CH), 4.21 (2H, t, J = 6.3 Hz, OCH_2), 2.78 (2H, m, CH_2), 2.07 (3H, s, CH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.0, 168.0, 149.2, 134.7, 132.0, 129.3, 128.6, 128.1, 124.1, 112.1, 62.7, 26.2, 20.9; IR: (KBr) ν_{max} 1101, 1251, 1312, 1368, 1393, 1404, 1473, 1489, 1585, 1673, 1739, 1750, 2854, 2880, 2923, 2966, 3089 cm^{-1}; LRMS: m/z (relative intensity) 323.0 $[M+H]^+$ (1), 295.1 (16), 277.1 (100), 268.7 (6), 258.7 (4), 185.0 (1). Anal. Calcd for $C_{15}H_{13}BrO_4$: C, 53.43; H, 3.89. Found: C, 53.29; H, 4.29.

5.6.5.4. (*E*)-Methyl 2-[4-(4-bromophenyl)-5-oxo-2,5-dihydrofuran-2-ylidene]acetate (26). Methyl bromoacetate (1.523 g, 10.00 mmol) and triphenylphosphine (3.150 g, 12.00 mmol) were dissolved in toluene (100 mL) and the resultant mixture was heated under reflux for 5 h. Precipitated product was filtered and washed with toluene (50 mL). Dry THF (20 mL) was added to the prepared phosphonium salt (1.530 g, 3.68 mmol), the suspension was cooled down to −80 °C and LDA (1.5 M THF complex in cyclohexane, 4.05 mmol) was added dropwise at argon atmosphere. The reaction mixture was heated up to −40 °C in period of 1 h and a solution of 4-bromophenylmaleic anhydride[26] (0.911 g, 3.60 mmol) in dry THF (20 mL) was slowly added. The resultant mixture was allowed to heat up to rt in period of 2 h, it was then diluted with ethyl acetate (50 mL) and washed with saturated aqueous ammonium chloride solution (50 mL). The organic layer was dried (Na_2SO_4), solvents were removed under reduced pressure and the crude product was purified by column chromatography (gradient elution, hexane/hexane–ethyl acetate 95:5) to afford lactone **26** as (*E*) isomer. Yield: 15%. White crystals, mp 146 °C; ^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.50 (1H, s, H4), 7.92–7.83 (2H, m, AA′, BB′, H2′, H6′), 7.64–7.56 (2H, m, AA′, BB′, H3′, H5′) 5.96 (1H, s, CH), 3.83 (3H, s, OCH_3); ^{13}C NMR: (75 MHz, $CDCl_3$) δ 166.6, 165.7, 159.1, 133.7, 132.4, 132.3, 129.2, 127.3, 125.7, 101.8, 52.1; IR: (KBr) ν_{max} 1142, 1215, 1254, 1316, 1379, 1405, 1434, 1487, 1581, 1594, 1648, 1721, 1776, 2951, 3087 cm^{-1}; LRMS: m/z (relative intensity) 309.0 $[M+H]^+$ (62), 278.1 (27), 251.4 (44), 221.0 (5), 193.9 (8), 181.1 (27), 101.0 (63). Anal. Calcd for $C_{13}H_9BrO_2$: C, 50.51; H, 2.93. Found: C, 50.27; H, 3.22.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.030.

References and notes

1. Richardson, M. D. *J. Antimicrob. Chemother.* **2005**, *56*, i5.
2. Kale, P.; Johnson, L. B. *Drugs Today* **2005**, *41*, 91.
3. Spellberg, B. J.; Filler, S. G.; Edwards, J. E., Jr. *Clin. Pract.* **2006**, *42*, 244.
4. Oppenheim, B. A.; Herbrecht, R. *Clin. Infect. Dis.* **1995**, *21*, 1145.
5. Bhatti, Z.; Shaukat, A.; Almyroudis, N. G.; Segal, B. H. *Mycopathologia* **2006**, *162*, 1.
6. De Sarro, A.; La Camera, E.; Fera, M. T. *J. Chemother.* **2008**, *20*, 661.
7. La Regina, G.; D'Auria, F. D.; Tafi, A.; Piscitelli, F.; Olla, S.; Caporuscio, F.; Nencioni, L.; Cirilli, R.; La Torre, F.; De Melo, N. R.; Kelly, S. L.; Lamb, D. C.; Artico, M.; Botta, M.; Palamara, A. T.; Silvestri, R. *J. Med. Chem.* **2008**, *51*, 3841.
8. Pfaller, M. A.; Diekema, D. J. *Clin. Microbiol. Rev.* **2007**, *20*, 133.
9. Playford, E. G.; Eggimann, P.; Calandra, T. *Curr. Opin. Infect. Dis.* **2008**, *21*, 610.
10. Pour, M.; Špulák, M.; Balšánek, V.; Kuneš, J.; Buchta, V.; Waisser, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1893.
11. Pour, M.; Špulák, M.; Balšánek, V.; Kuneš, J.; Buchta, V. *Bioorg. Med. Chem.* **2003**, *11*, 2843.
12. Pour, M.; Špulák, M.; Buchta, V.; Kubanová, P.; Vopršalová, M.; Wsól, V.; Fáková, H.; Koudelka, P.; Pourová, H.; Schiller, R. *J. Med. Chem.* **2001**, *44*, 2701.
13. Buchta, V.; Pour, M.; Kubanová, P.; Silva, L.; Votruba, I.; Vopršalová, M.; Schiller, R.; Fáková, H.; Špulák, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 873.
14. Šnajdr, I.; Pavlík, J.; Schiller, R.; Kuneš, J.; Pour, M. *Collect. Czech. Chem. Commun.* **2007**, *72*, 1472.
15. Vale-Silva, L. A.; Buchta, V.; Vokurková, D.; Pour, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2492.
16. Nobilis, M.; Pour, M.; Šenel, P.; Pavlík, J.; Kuneš, J.; Vopršalová, M.; Kolářová, L.; Holčapek, M. *J. Chromatogr., B* **2007**, *853*, 10.
17. Horimoto, A. Jpn. Kokai Tokkyo Koho 2007277137, 2007.
18. Fáková, H.; Pour, M.; Kuneš, J.; Šenel, P. *Tetrahedron Lett.* **2005**, *46*, 8137.
19. More, J. D.; Finney, N. S. *Org. Lett.* **2002**, *4*, 3001.
20. Tretyakov, E. V.; Tkachev, A. V.; Rybalova, T. V.; Gatilov, Y. V.; Knight, D. W.; Vasilevsky, S. F. *Tetrahedron* **2000**, *56*, 10075.
21. Gabriele, B.; Salerno, G.; Costa, M.; Chiusoli, G. P. *Tetrahedron Lett.* **1999**, *40*, 989.
22. (a) Rossi, R.; Bellina, F.; Mannina, L. *Tetrahedron Lett.* **1998**, *39*, 3017; (b) Rossi, R.; Bellina, F.; Biagetti, M.; Mannina, L. *Tetrahedron Lett.* **1998**, *39*, 7799; (c) Biagetti, M.; Bellina, F.; Carpita, A.; Viel, S.; Mannina, L.; Rossi, R. *Eur. J. Org. Chem.* **2002**, 1063.
23. Pavlík, J.; Šnajdr, I.; Kuneš, J.; Špulák, M.; Pour, M. *J. Org. Chem.* **2009**, *74*, 703.
24. Pour, M.; Negishi, E.-I. *Tetrahedron Lett.* **1997**, *38*, 525.
25. Li, X.-C.; Ferreira, D.; Jacob, M. R.; Zhang, Q.; Khan, S. I.; ElSohly, H. N.; Nagle, D. G.; Smillie, T. J.; Khan, I. A.; Walker, L. A.; Clark, A. M. *J. Am. Chem. Soc.* **2004**, *126*, 6872.
26. Dean, W. D.; Blum, D. M. *J. Org. Chem.* **1993**, *58*, 7916.
27. Li, W.; Li, J.; Lin, M.; Wacharasindhu, S.; Tabei, K.; Mansour, T. S. *J. Org. Chem.* **2007**, *72*, 6016.
28. Li, W.; Li, J.; Wan, Z.-K.; Wu, J.; Massefski, W. *Org. Lett.* **2007**, *9*, 4607.
29. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved Standard, NCCLS document M27-A, 771 E. Lancaster Avenue, Villanova, PA, 19085, 1997.
30. Hocek, M.; Holý, A.; Votruba, I.; Dvořáková, H. *J. Med. Chem.* **2000**, *43*, 1817.
31. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
32. Havranek, M.; Sauerberg, P.; Mogensen, J. P.; Kratina, P.; Jeppesen, C. B.; Pettersson, I.; Pihera, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4144.