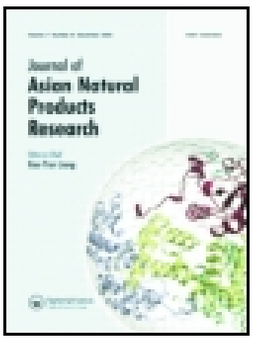

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# Halogenated benzoate derivatives of altholactone with improved anti-fungal activity

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**ABSTRACT**

Altholactone exhibited the anti-fungal activity with a high MIC value of 128 μg ml$^{-1}$ against *Cryptococcus neoformans* and *Saccharomyces cerevisiae*. Fifteen ester derivatives of altholactone **1**–**15** were modified by esterification and their structures were confirmed by spectroscopic methods. Most of the ester derivatives exhibited stronger anti-fungal activities than that of the precursor altholactone. 3-Bromo- and 2,4-dichlorobenzoates (**7** and **15**) exhibited the lowest minimal inhibitory concentration (MIC) values against *C. neoformans* at 16 μg ml$^{-1}$ while the 4-bromo-, 4-iodo-, and 1-bromo-3-chlorobenzoates (**11**–**13**) displayed potent activity against *S. cerevisiae* with MIC values of 1 μg ml$^{-1}$. In conclusion, this analysis indicates that the anti-fungal activity of altholactone is enhanced by addition of halogenated benzoyl group to the 3-OH group.

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**KEYWORDS**
Altholactone; anti-fungal activity; ester derivative; halogenated benzoate; *Polyalthia crassa*



## 1. Introduction

Currently, the impact of fungal infections on human health is under-appreciated, even though these infections have a high mortality rate (>50%) resulting in the death of over 1.5 million people annually [1]. Fungi in the genera of *Aspergillus*, *Candida*, *Cryptococcus*, and *Pneumocystis* are responsible for nearly 90% of all deaths due to fungal infections [1]. *Candida albicans* and *Cryptococcus neoformans* are opportunistic pathogenic yeasts, causing candidiasis and cryptococcal meningitis in patients that are immunocompromised as a result of cancer or AIDS [2,3]. Approximately, 46,000 cases of health care-associated invasive candidiasis are recorded each year in the US [4]. In addition, more than 1 million cases of cryptococcal meningitis among people with AIDS were reported worldwide in 2006, resulting in 625,000 deaths, predominantly in sub-Saharan Africa [5]. *Saccharomyces cerevisiae* is

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**Table 1.** MIC values of altholactone and ester derivatives (**1**–**15**) against three indicator yeasts.

| | MIC (μg ml$^{-1}$) | | |
|---|---|---|---|
| Compound | *C. albicans* MT 2014/1 | *C. neoformans* MT 2014/2 | *S. cerevisiae* IFO 10217 |
| **Altholactone** | >128 | 128 | 128 |
| **1** | >128 | >128 | 32 |
| **2** | >128 | >128 | 16 |
| **3** | >128 | 128 | 8 |
| **4** | >128 | >128 | 16 |
| **5** | >128 | 32 | 8 |
| **6** | >128 | 32 | 8 |
| **7** | >128 | 16 | 8 |
| **8** | >128 | 64 | 4 |
| **9** | >128 | 32 | 2 |
| **10** | >128 | 32 | 4 |
| **11** | >128 | 32 | 1 |
| **12** | >128 | >128 | 1 |
| **13** | >128 | 32 | 1 |
| **14** | >128 | 32 | 2 |
| **15** | >128 | 16 | 2 |
| Amphotericin B | 0.25 | 0.25 | 0.25 |



a well-known yeast used in baking and brewing industry and not typically associated with fungal infections. However, *S. cerevisiae*-induced vaginitis has been recently identified in bone marrow transplant patients and other immunocompromised patients [6].

A possible anti-fungal agent is altholactone (goniothalenol), a well-known styryl-lactone bioactive natural product found in *Goniothalamus* and *Polyalthia* (Annonaceae) species. This furano-pyrone was initially isolated from the bark of an unknown species of *Polyalthia* [7] and later from the bark of *G. giganteus* (Panan chaang) [8]. It was also identified as a cytotoxic compound present in leaves and twigs of *Polyalthia crassa* Parker by our research group [9]. Several studies have characterized the anti-proliferative activity of altholactone against various human tumor cell lines [10,11]. The cytotoxic mechanism of this styryl-lactone against mammalian cancer cells has been postulated to be mediated through apoptosis, following inhibition of the mitochondrial respiratory chain [12,13]; however, the precise mechanism remains unclear. In addition to its anti-proliferative effects, altholactone displays anti-inflammatory [14] and anti-plasmodial [15] activities.

Structure modification of altholactone has the potential to enhance its bioactive properties as was observed with 3-acetylaltholactone. Inhibition of NADH: oxidase activity by 3-acetylaltholactone, from beef-heart submitochondrial particles, was greater compared to that of altholactone [12]. Preparation of new altholactone derivatives is possible utilizing Steglich esterification [16]. Therefore, a procedure for the incorporation of various alkyl and benzoyl groups at the 3-hydroxyl group of altholactone through esterification was designed. The resulting altholactone ester derivatives were analyzed and many of these compounds displayed enhanced anti-fungal activity toward *C. neoformans* and *S. cerevisiae*.

## 2. Results and discussion

Altholactone exhibits a high MIC value of 128 μg ml$^{-1}$ or more toward *C. albicans*, *C. neoformans,* and *S. cerevisiae* as shown in Table 1. The anti-fungal activity of altholactone and its modified derivatives against these indicator yeasts has not been reported before. Therefore, modification of the hydroxyl group of altholactone to the 15 derivatives by Steglich esterification was designed. Under mild conditions, altholactone was reacted with carboxylic acid

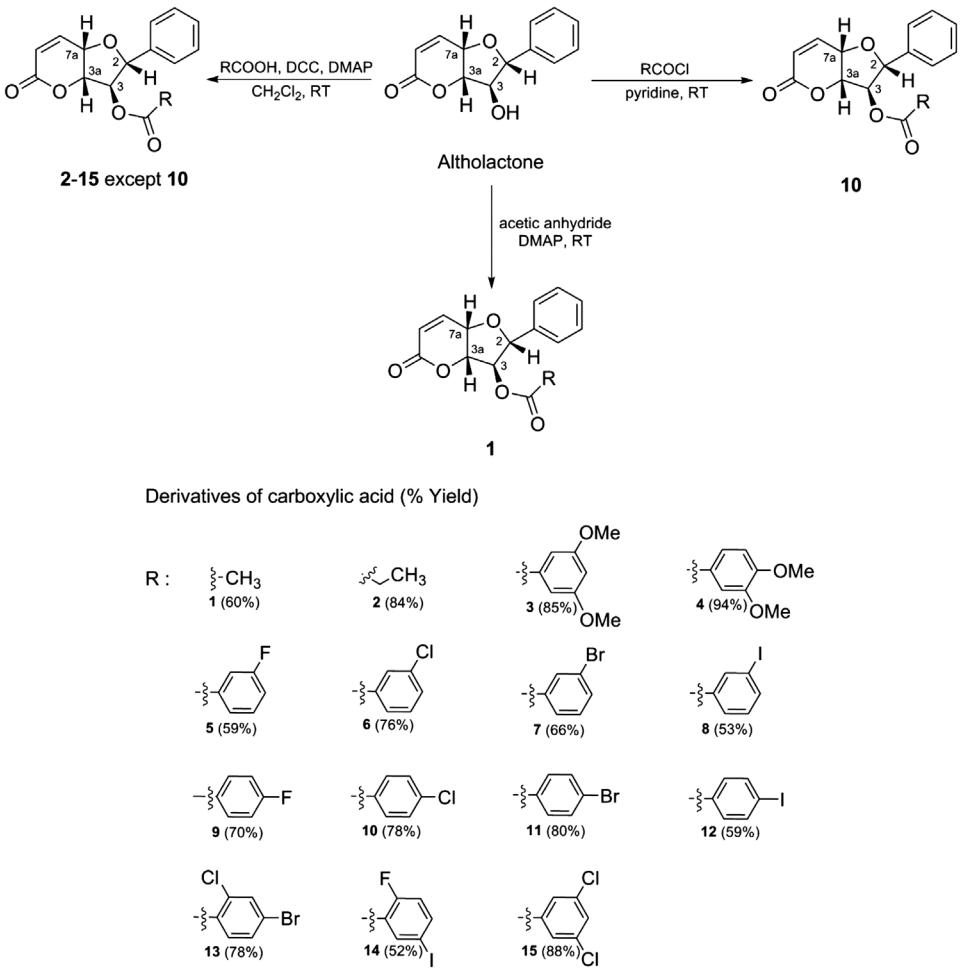


**Figure 1.** Synthesis of ester derivatives of altholactone. Reagents and conditions: (a) RCOOH, DCC, DMAP(cat.), $CH_2Cl_2$, RT; (b) acetic anhydride, DMAP (cat.), RT; (c) RCOCl, pyridine, RT.

derivatives in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as catalyst (cat) in dry dichloromethane to give the desired products (**2**–**9** and **11**–**15**) in 52–94% yield (Figure 1). Compound **1** was prepared through acetylation of altholactone with acetic anhydride in the presence of DMAP, while esterification of altholactone with 4-chlorobenzoyl chloride in the presence of pyridine gave **10**. The structure of each product was characterized using spectroscopic methods (FTIR, UV, $^1$H NMR, $^{13}$C NMR, EI-MS, and HR-TOF-MS) (see Experimental). The $^1$H NMR and $^{13}$C NMR spectra of **1**–**15** were provided as shown in Figures S1–S30 of the supporting information.

All modified compounds and the reference drug, amphotericin B, were tested for antifungal activity. The results are shown in Table 1. The majority of the ester derivatives displayed enhanced anti-fungal activity compared to the parent compound. When the R was alkyl group [methyl (Me) or ethyl (Et) in **1** and **2)** or dimethoxybenzoyl group (**3** and **4**), the MIC values against *S. cerevisiae* decreased to 8–32 μg ml$^{-1}$. Most of the halogenated benzoates **5**–**15** exhibited MIC values against both *C. neoformans* (16–32 μg ml$^{-1}$) and *S.*

*cerevisiae* (1–8 μg ml$^{-1}$), which were dramatically lower than that of altholactone. An exception to this was observed with the iodobenzoyl derivative. The 3-iodo benzoate derivative **8** was less active against *C. neoformans* (≥64 μg ml$^{-1}$) compared to *S. cerevisiae* (≥4 μg ml$^{-1}$), while the 4-iodobenzoate derivative only displayed activity against *S. cerevisiae* (≥1 μg ml$^{-1}$). In contrast, dihalogenated benzoates (**13**–**15**) significantly decreased MIC values to 16–32 and 1–2 μg ml$^{-1}$ against *C. neoformans* and *S. cerevisiae*, respectively. Among these new 15 derivatives, 3-bromo- and 3,5-dichlorobenzoates (**7** and **15**) were the most potent against *C. neoformans* (MIC = 16 μg ml$^{-1}$) and displayed an eightfold lower MIC value compared to the parent altholactone. Many of the derivatives were very toxic toward *S. cerevisiae*, among these 4-bromo-, 4-iodo-, and 4-bromo-2-chlorobenzoates (**11**–**13**, respectively) were 128-fold more potent (MIC = 1 μg ml$^{-1}$). These findings demonstrated that the anti-fungal activities of altholactone can be significantly improved by modification of the 3-OH group to the halogenated benzoate derivatives. To our knowledge, there are no previous reports on the detailed structure–activity relationship of altholactone derivatives. The current study demonstrated that several of the new ester derivatives of altholactone possessed enhanced anti-fungal activity compared to the parent compound. Improved anti-fungal activity against yeasts and molds has been documented for synthesized halogenated or ester derivatives of other compounds [17,18]. However, the reason for higher anti-fungal activity of halogenated derivatives is not well understood but could be caused by better surfactant properties or through other anti-fungal mechanism(s) [18,19]. Further study on mechanism and cytotoxicity are necessarily to understand the potential clinical utility of these compounds. In our analysis, altholactone and the derivatives at a concentration of 128 μg ml$^{-1}$ could not inhibit growth of *C. albicans* MT2014/1. This result is inconsistent with one previous report that demonstrated anti-fungal activity of altholactone against *C. albicans* ATCC 10231, with a MIC value of 2.5 μg ml$^{-1}$ [20]. However, reduced susceptibility of *C. albicans* clinical isolates, similar to the strain used in this study, to azole drugs has been reported [21]. *C. albicans* strain MT2014/1 may also exhibit enhanced resistance to antibiotic(s) similar to other clinical isolates, although this strain did not display resistance to amphotericin B (Table 1). Drug resistance in yeast is due to mutations in genes encoding drug targets and enzymes in metabolic pathways as well as activation of stress response pathways including drug-efflux pump proteins [22,23]. It is possible that the failure of altholactone to inhibit growth of the *C. albicans* clinical isolate used in our analysis could be due to genetic changes from prior drug exposure.

In conclusion, this study is the first report on the anti-fungal activity of altholactone against *C. neoformans* and *S. cerevisiae*. Ester derivatives of altholactone with modification at 3-hydroxyl group were prepared and tested against two pathogenic fungi and one strain of Baker's yeast. The results demonstrate that most altholactone derivatives, especially the halogenated benzoates, possess significantly increased anti-fungal activity, with lower MIC values than that of altholactone.

## 3. Experimental

### 3.1. General experimental procedures

Melting points were measured using a digital electrothermal melting point apparatus (BUCHI, Flawil, Switzerland). Optical rotations were determined on JASCO DIP 370 digital polarimeter (JASCO, Tokyo, Japan), using a 50-mm microcell (1 ml). UV (in acetonitrile)

and IR (KBr) spectra were recorded on a JASCO 530 (JASCO, Tokyo, Japan) and a Perkin-Elmer 2000 FT-IR spectrometers (Perkin-Elmer, Massachusetts, USA), respectively. The $^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectra were recorded on Bruker Ascend 400 spectrometer (Bruker, Fallenden, Germany) in $CDCl_3$, using TMS as internal standard. EIMS were recorded at 70 eV (probe) on a Thermo Finnigan Polaris Q mass spectrometer (Thermo scientific, Texas, USA). The HR-TOF-MS were recorded on a Micromass model VQ-ToF-2 spectrometer (Bruker, Bremen, Germany). Silica gel 60H (Merck, 70–230 mesh ASTM, Darmstadtt, Germany) was used for column chromatography, while preparative TLC was carried out with silica gel 60 $PF_{254}$ (Merck, Darmstadtt, Germany) plates. Altholactone was isolated from leaves and twigs of *P. crassa* Parker using the previously described procedure (Tuchinda et al. [9]). All solvents used for extraction and isolation were distilled prior to use at their boiling point ranges.

### 3.2. General method for preparation of compound 1

A mixture of altholactone (106 mg, 0.457 mmol), acetic anhydride (0.99 ml, 10.578 mmol), and 4-dimethylaminopyridine (cat.) was stirred at room temperature for 4 h. The reaction was quenched with a saturated sodium hydrogen carbonate solution (25 ml). The aqueous layer was extracted with $CH_2Cl_2$ (3 × 25 ml). The combined organic layer was washed with $H_2O$, brine and dried over anhydrous magnesium sulfate. After filtration and removal of solvent under reduced pressure, a crude yellow liquid (1.81 g) was obtained. This material was purified by preparative TLC (50% ethyl acetate-hexanes), followed by recrystallization from EtOH to give the desired product **1** (75.2 mg, 60% yield) as colorless needles.

#### 3.2.1. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl ethanoate (1)

mp 140.8–141.5 C. $[\alpha]_D^{28}$ +126.4 (*c* 0.088, $CHCl_3$); UV ($CH_3CN$) $\lambda_{max}$ nm (log ε): 202 (5.11). IR (KBr) $\upsilon_{max}$ $cm^{-1}$: 1745, 1722, 1632, 1495, 1457, 1378, 1363, 1253, 1223, 1147, 1100, 1056, 1022, 918, 884, 764, 702. $^{1}H$ NMR (400 MHz, $CDCl_3$) *δ*: 7.30–7.36 (5H, *m*, Ar–H), 7.04 (1H, *dd*, *J* = 9.8, 5.1 Hz), 6.28 (1H, *d*, *J* = 9.8 Hz), 5.39 (1H, *brd*, *J* = 3.3 Hz), 4.98 (1H, *d*, *J* = 3.3 Hz), 4.96 (1H, *brd*, *J* = 3.6 Hz), 4.63 (1H, *dd*, *J* = 5.1, 4.2 Hz), 2.16 (C*H*$_3$, *s*). $^{13}C$ NMR (100 MHz, $CDCl_3$): 169.4 (C), 160.4 (C), 139.2 (CH), 137.5 (C), 128.7 (2 × CH), 128.5 (CH), 126.2 (2 × CH), 124.7 (CH), 86.1 (CH), 83.6 (CH), 83.6 (CH), 69.1 (CH), 20.9 ($CH_3$). EIMS *m/z* (relative intensity): 275 $[M + H]^+$ (19), 214 (3), 197 (4), 170 (17), 146 (10), 145 (100), 115 (9), 106 (14), 97 (5), 78 (5). HR-TOF-MS (ESI): *m/z* 297.0748 $[M + Na]^+$ (calcd for $C_{15}H_{14}O_5Na$, 297.0739)

### 3.3. General method to prepare compounds 2–15 (except 10)

A mixture of altholactone (1.0 eq), acid derivatives (1.2 eq), *N*,*N*′-dicyclohexylcarbodiimide (1.2 eq), and 4-dimethylaminopyridine (cat.) in dry dichloromethane (2 ml) was stirred at room temperature for 2 h to overnight. The reaction mixture was monitored by TLC (ethyl acetate-hexane). Removal of the solvent was performed under reduced pressure and the residue was further purified by column chromatography on preparative TLC (silica gel) to give the ester derivatives.

### 3.3.1. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl propionate (2)

Compound **2** was prepared by reacting altholactone (50.0 mg, 0.216 mmol) with propanoic acid (19.2 mg, 0.259 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (53.4 mg, 0.259 mmol) and 4-dimethylaminopyridine (cat.) at room temperature overnight. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane), followed by recrystallization from MeOH to give **2** (52.1 mg, 84% yield) as colorless needles; mp 182.4–183.2 °C. $[\alpha]_D^{28}$ +70.6 (*c* 0.088, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 206 (4.28). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1744, 1724, 1632, 1495, 1459, 1382, 1368, 1355, 1249, 1177, 1146, 1100, 1085, 1070, 1054, 1025, 884, 821, 764, 702, 666. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.28–7.37 (5H, *m*, Ar–H), 7.04 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.28 (1H, *d*, *J* = 9.8 Hz), 5.40 (1H, *brd*, *J* = 3.1 Hz), 4.98 (1H, *d*, *J* = 3.1 Hz), 4.95 (1H, *brd*, *J* = 3.3 Hz), 4.64 (1H, *dd*, *J* = 5.3, 4.1 Hz), 2.43 ($CH_2$, *q*, *J* = 7.5 Hz), 1.18 ($CH_3$, *t*, *J* = 7.5 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 172.9 (C), 160.4 (C), 139.2 (CH), 137.5 (C), 128.7 (2 × CH), 128.4 (CH), 126.2 (2 × CH), 124.7 (CH), 86.2 (CH), 83.6 (CH), 83.5 (CH), 69.1 (CH), 27.4 ($CH_2$), 8.9 ($CH_3$). EIMS *m/z* (relative intensity): 289 $[M + H]^+$ (3), 214 (5), 197 (<1), 170 (20), 146 (5), 145 (100), 116 (7), 106 (6), 78 (10). HR-TOF-MS (ESI): *m/z* 311.0893 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_5Na$, 311.0895)

### 3.3.2. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3,5-dimethoxybenzoate (3)

Compound **3** was prepared by reacting altholactone (30.6 mg, 0.132 mmol) with 3,5-dimethoxybenzoic acid (28.8 mg, 0.158 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (32.6 mg, 0.158 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane) to yield **3** (44.2 mg, 85% yield) as a colorless oil; $[\alpha]_D^{28}$ +20.3 (*c* 0.127, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 308 (3.47), 253 (3.86), 207 (4.67). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1736, 1596, 1496, 1459, 1429, 1353, 1325, 1306, 1224, 1206, 1158, 1105, 1064, 1049, 924, 850, 820, 763, 699. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.31–7.42 (5H, *m*, Ar–H), 7.19 (2H, *d*, *J* = 2.2 Hz), 7.08 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.69 (1H, *t*, *J* = 2.2 Hz), 6.31 (1H, *d*, *J* = 9.8 Hz), 5.61 (1H, *brd*, *J* = 3.2 Hz), 5.14 (1H, *d*, *J* = 3.2 Hz), 5.10 (1H, *brd*, *J* = 3.3 Hz), 4.73 (1H, *dd*, *J* = 5.3, 4.1 Hz), 3.84 (2xO$CH_3$, *s*). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 164.9 (C), 160.8 (2 × C), 160.4 (C), 139.1 (CH), 137.5 (C), 130.7 (C), 128.7 (2 × CH), 128.5 (CH), 126.2 (2 × CH), 124.8 (CH), 107.6 (2 × CH), 106.0 (CH), 86.2 (CH), 84.3 (CH), 83.7 (CH), 69.3 (CH), 55.7 (2 × $OCH_3$). EIMS *m/z* (relative intensity): 397 $[M + H]^+$ (1), 214 (2), 184 (11), 182 (100), 166 (28), 138 (14), 122 (8), 108 (5), 78 (9). HR-TOF-MS (ESI): *m/z* 419.1116 $[M + Na]^+$ (calcd for $C_{22}H_{20}O_7Na$, 419.1107)

### 3.3.3. (2R, 3R, 3aR, 7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3,4-dimethoxybenzoate (4)

Compound **4** was prepared by reacting altholactone (30.6 mg, 0.132 mmol) with 3,4-dimethoxybenzoic acid (28.8 mg, 0.158 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (32.6 mg, 0.158 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 6 h. The reaction mixture was purified by column chromatography (35% ethyl acetate-hexane) to yield **4** (49.3 mg, 94% yield) as a yellow oil; $[\alpha]_D^{28}$ +75.1 (*c* 0.006, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 293 (3.62), 264 (3.88), 219 (4.19). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1735, 1719, 1600, 1516, 1465, 1459, 1453, 1420, 1349, 1295, 1273, 1246, 1220, 1176, 1104, 1022, 876, 821, 760, 700. $^1$H NMR

(400 MHz, $CDCl_3$) $\delta$: 7.71 (1H, *dd*, *J* = 8.5, 1.9 Hz), 7.55 (1H, *d*, *J* = 1.9 Hz), 7.31–7.43 (5H, *m*, Ar–H), 7.08 (1H, *dd*, *J* = 9.8, 5.4 Hz), 6.92 (1H, *d*, *J* = 8.5 Hz), 6.31 (1H, *d*, *J* = 9.8 Hz), 5.61 (1H, *brd*, *J* = 3.4 Hz), 5.16 (1H, *d*, *J* = 3.4 Hz), 5.12 (1H, *dd*, *J* = 4.1, 0.8 Hz), 4.75 (1H, *dd*, *J* = 5.4, 4.1 Hz), 3.96 ($OCH_3$, *s*), 3.94 ($OCH_3$, *s*). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.9 (C), 160.5 (C), 153.7 (C), 148.9 (C), 139.2 (CH), 137.6 (C), 128.7 (2 × CH), 128.5 (CH), 126.2 (2 × CH), 124.8 (CH), 123.9 (CH), 121.3 (C), 112.2 (CH), 110.3 (CH), 86.2 (CH), 84.0 (CH), 83.7 (CH), 69.3 (CH), 56.1 (2 × $OCH_3$). EIMS *m/z* (relative intensity): *m/z* 397 $[M + H]^+$ (<1), 214 (1), 184 (10), 183 (100), 165 (22), 145 (6), 122 (4), 96 (4), 78 (13). HR-TOF-MS (ESI): *m/z* 419.1106 $[M + Na]^+$ (calcd for $C_{22}H_{20}O_7Na$, 419.1107)

### 3.3.4. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3-fluorobenzoate (5)

Compound **5** was prepared by reacting altholactone (32.6 mg, 0.141 mmol) with 3-fluorobenzoic acid (26.3 mg, 0.169 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (34.8 mg, 0.169 mmol) and 4-dimethylaminopyridine (cat.) at room temperature overnight. The reaction mixture was purified by column chromatography (25% ethyl acetate-hexane) to yield **5** (29.4 mg, 59% yield) as a yellow oil; $[\alpha]_D^{28}$ + 109.2 (*c* 0.012, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 279 (2.94), 227 (3.82). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1736, 1638, 1593, 1450, 1291, 1271, 1245, 1200, 1105, 1068, 821, 754, 699. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.89 (1H, *brd*, *J* = 7.8 Hz), 7.76 (1H, *brd*, *J* = 8.5 Hz), 7.49 (1H, *m*), 7.32–7.44 (6H, *m*), 7.10 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.25 (1H, *d*, *J* = 9.8 Hz), 5.65 (1H, *brd*, *J* = 3.3 Hz), 5.16 (1H, *d*, *J* = 3.3 Hz), 5.14 (1H, *brd*, *J* = 4.1 Hz), 4.67 (1H, *dd*, *J* = 5.3, 4.1 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 160.3 (C), 139.1 (CH), 137.3 (C), 130.4 (C), 130.3 (C), 128.8 (2 × CH), 128.6 (CH), 126.2 (2 × CH), 125.6 (C), 124.8 (CH), 121.0 (CH), 120.8 (CH), 116.8 (CH), 116.6 (CH), 86.1 (CH), 84.4 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 355 $[M]^+$ (22), 214 (6), 197 (11), 170 (24), 145 (43), 144 (100), 123 (33), 105 (28), 95 (16), 77 (11). HR-TOF-MS (ESI): *m/z* 377.0804 $[M + Na]^+$ (calcd for $C_{20}H_{15}FO_5Na$, 377.0801)

### 3.3.5. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3-chlorobenzoate (6)

Compound **6** was prepared by reacting altholactone (29.7 mg, 0.128 mmol) with 3-chlorobenzoic acid (24.0 mg, 0.154 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (31.7 mg, 0.154 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (15% ethyl acetate-hexane) to yield **6** (35.9 mg, 76% yield) as a yellow oil; $[\alpha]_D^{28}$ +95.5 (*c* 0.031, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 283 (2.95), 231 (3.84). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1735, 1638, 1575, 1496, 1452, 1428, 1384, 1293, 1243, 1129, 1104, 1070, 1025, 909, 881, 820, 747, 699. $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 7.94 (1H, *brs*), 7.87 (1H, *brd*, *J* = 7.8 Hz), 7.51 (1H, *dd*, *J* = 8.0, 1.0 Hz), 7.21–7.37 (6H, *m*, Ar–H), 6.99 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.23 (1H, *d*, *J* = 9.8 Hz), 5.54 (1H, *brd*, *J* = 3.3 Hz), 5.06 (1H, *d*, *J* = 3.3 Hz), 5.02 (1H, *dd*, *J* = 4.2, 0.8 Hz), 4.65 (1H, *dd*, *J* = 5.3, 4.2 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 163.9 (C), 160.4 (C), 139.1 (CH), 137.4 (C), 134.8 (C) 133.8 (CH), 130.7 (C), 130.0 (CH), 129.8 (CH), 128.7 (2 × CH), 128.6 (CH), 128.0 (CH), 126.2 (2 × CH), 124.8 (CH), 86.0 (CH), 84.4 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 371 $[M + H]^+$ (<1), 214 (5), 170 (19), 145 (100), 139 (15), 116 (10), 105 (10), 76 (16). HR-TOF-MS (ESI): *m/z* 393.0502 $[M + Na]^+$ (calcd for $C_{20}H_{15}ClO_5Na$, 393.0506)

### 3.3.6. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3-bromobenzoate (7)

Compound **7** was prepared by reacting altholactone (31.3 mg, 0.135 mmol) with 3-bromobenzoic acid (32.5 mg, 0.162 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (33.4 mg, 0.162 mmol) and 4-dimethylaminopyridine (cat.) at room temperature overnight. The reaction mixture was purified by column chromatography (10% ethyl acetate-hexane) to yield **7** (37.2 mg, 66% yield) as a yellow oil; $[\alpha]_D^{28}$ +108.4 (*c* 0.0096, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 280 (3.70), 228 (4.59), 206 (5.13). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1735, 1719, 1638, 1571, 1560, 1459, 1291, 1243, 1123, 1103, 1067, 820, 746, 700. $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 8.18 (1H, *dd*, *J* = 1.9, 1.5 Hz), 8.00 (1H, *ddd, J* = 8.1, 1.5, 1.2 Hz), 7.75 (1H, *ddd, J* = 8.0, 1.9, 1.2 Hz), 7.29–7.42 (6H, *m*), 7.08 (1H, *dd*, *J* = 9.8, 5.4 Hz), 6.32 (1H, *d*, *J* = 9.8 Hz), 5.63 (1H, *dd,* *J* = 3.6, 1.1 Hz), 5.14 (1H, *d*, *J* = 3.6 Hz), 5.11 (1H, *dd*, *J* = 4.1, 1.1 Hz), 4.74 (1H, *dd*, *J* = 5.4, 4.1 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 163.8 (C), 160.3 (C), 139.1 (CH), 137.3 (C), 136.7 (CH), 132.7 (CH), 130.8 (C), 130.3 (CH), 128.8 (2 × CH), 128.6 (CH), 128.5 (CH), 126.2 (2 × CH), 124.8 (CH), 122.7 (C), 86.1 (CH), 84.4 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 414 $[M]^+$ (1), 214 (2), 186 (3), 185 (43), 183 (44), 170 (7), 146 (13), 144 (100), 143 (16), 115 (15), 76 (10). HR-TOF-MS (ESI): *m/z* 437.0000/438.9978 $[M + Na]^+$ ($^{79}$Br/$^{81}$Br) (calcd for $C_{20}H_{15}BrO_5Na$, 437.0001/438.9980)

### 3.3.7. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3-iodobenzoate (8)

Compound **8** was prepared by reacting altholactone (31.6 mg, 0.136 mmol) with 3-iodobenzoic acid (40.43 mg, 0.163 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (33.6 mg, 0.163 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 2.5 h. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane) to yield **8** (33.6 mg, 53% yield) as a yellow oil; $[\alpha]_D^{28}$ +90.4 (*c* 0.023, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 287 (2.66), 218 (4.08). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1734, 1565, 1561, 1419, 1289, 1242, 1102, 819, 743, 699. $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 8.37 (1H, *brt*, *J* = 1.6 Hz), 8.03 (1H, *brd, J* = 7.8 Hz), 7.95 (1H, *brd*, *J* = 8.1 Hz), 7.29–7.42 (5H, *m*, Ar–H), 7.24 (1H, *t, J* = 7.9 Hz), 7.08 (1H, *dd*, *J* = 9.8, 5.4 Hz), 6.32 (1H, *d*, *J* = 9.8 Hz), 5.62 (1H, *dd, J* = 3.5, 1.0 Hz), 5.14 (1H, *d, J* = 3.5 Hz), 5.11 (1H, *dd, J* = 4.1, 1.0 Hz), 4.74 (1H, *dd*, *J* = 5.4, 4.1 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 163.6 (C), 160.4 (C), 142.6 (CH), 139.1 (CH), 138.5 (CH), 137.3 (C), 130.8 (C), 130.3 (CH), 129.1 (CH), 128.7 (2 × CH), 128.6 (CH), 126.2 (2 × CH), 124.8 (CH), 94.0 (C), 86.1 (CH), 84.4 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 463 $[M]^+$ (<1), 232 (7), 231 (82), 214 (10), 203 (16), 171 (46), 145 (40), 144 (100), 105 (22), 77 (16). HR-TOF-MS (ESI): *m/z* 484.9862 $[M + Na]^+$ (calcd for $C_{20}H_{15}IO_5Na$, 484.9862)

### 3.3.8. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 4-fluorobenzoate (9)

Compound **9** was prepared by reacting altholactone (29.8 mg, 0.128 mmol) with 4-fluorobenzoic acid (21.6 mg, 0.154 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (31.8 mg, 0.154 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane), followed by recrystallization from MeOH to yield **9** (31.5 mg, 70% yield) as white needles; mp 112.9–113.8 °C. $[\alpha]_D^{28}$ +189.9 (*c* 0.015, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 230 (4.66). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1729, 1602, 1508, 1453, 1412, 1375, 1366, 1351, 1267, 1248, 1153, 1112, 1092, 1067, 858, 819, 764,

701, 607. $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 8.10 (1H, *d*, *J* = 8.7 Hz), 8.08 (1H, *d*, *J* = 8.6 Hz), 7.29–7.42 (5H, *m*, Ar–H), 7.17 (1H, *d*, *J* = 8.6 Hz), 7.15 (1H, *d*, *J* = 8.7 Hz), 7.08 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.32 (1H, *d*, *J* = 9.8 Hz), 5.62 (1H, *brd*, *J* = 3.3 Hz), 5.15 (1H, *d*, *J* = 3.3 Hz), 5.11 (1H, *brd*, *J* = 4.0 Hz), 4.74 (1H, *dd*, *J* = 5.3, 4.0 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 167.5 (C), 164.9 (C), 164.1 (C), 160.4 (C), 139.1 (CH), 137.4 (C), 132.5 (CH), 132.4 (CH), 128.7 (2 × CH), 128.5 (CH), 126.2 (2 × CH), 124.8 (CH), 116.0 (CH), 115.8 (CH), 86.1 (CH), 84.2 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 355 $[M]^+$ (<1), 214 (5), 170 (22), 145 (15), 144 (100), 123 (28), 105 (10), 96 (24), 75 (14). HR-TOF-MS (ESI): *m/z* 377.0807 $[M + Na]^+$ (calcd for $C_{20}H_{15}FO_5Na$, 377.0801)

### 3.3.9. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 4-bromobenzoate (11)

Compound **11** was prepared by reacting altholactone (22.3 mg, 0.096 mmol) with 4-bromobenzoic acid (23.2 mg, 0.115 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (23.8 mg, 0.115 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane) to yield **11** (31.6 mg, 80% yield) as a colorless oil; $[\alpha]_D^{28}$ +123.1 (*c* 0.011, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 246 (4.23), 205 (4.32). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1726, 1591, 1483, 1457, 1398, 1376, 1319, 1272, 1252, 1115, 1101, 1062, 1029, 845, 817, 768, 752, 704, 681. $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 7.92 (2H, *d*, *J* = 8.6 Hz), 7.63 (2H, *d*, *J* = 8.6 Hz), 7.30–7.42 (5H, *m*, Ar–H), 7.07 (1H, *dd*, *J* = 9.8, 5.4 Hz), 6.31 (1H, *d*, *J* = 9.8 Hz), 5.62 (1H, *dd*, *J* = 3.6, 1.0 Hz), 5.14 (1H, *d*, *J* = 3.6 Hz), 5.11 (1H, *dd*, *J* = 4.2, 1.0 Hz), 4.73 (1H, *dd*, *J* = 5.4, 4.2 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.4 (C), 160.3 (C), 139.1 (CH), 137.3 (C), 132.0 (2 × CH), 131.3 (2 × CH), 129.1 (C), 128.7 (2 × CH), 128.6 (CH), 127.8 (C), 126.2 (2 × CH), 124.8 (CH), 86.0 (CH), 84.3 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 415 $[M]^+$ (<1), 214 (6), 186 (6), 185 (54), 184 (52), 171 (33), 145 (100), 144 (51), 105 (16), 97 (2), 78 (13). HR-TOF-MS (ESI): *m/z* 437.0007/448.9983 $[M + Na]^+$ ($^{79}$Br/$^{81}$Br) (calcd for $C_{20}H_{15}BrO_5Na$, 437.0001/438.9980)

### 3.3.10. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 4-iodobenzoate (12)

Compound **12** was prepared from altholactone (32.6 mg, 0.141 mmol) reacted with 4-iodobenzoic acid (41.8 mg, 0.169 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (34.7 mg, 0.169 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (20% ethyl acetate-hexane), followed by recrystallization from MeOH to yield **12** (38.4 mg, 59% yield) as colorless needles; mp 186.4–187.2 °C. $[\alpha]_D^{28}$ +36.6 (c 0.093, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 257 (4.56), 209 (4.58). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1724, 1587, 1481, 1457, 1395, 1376, 1272, 1253, 1115, 1101, 1063, 1010, 817, 767, 749, 703. $^{1}$H NMR (400 MHz, $CDCl_3$), $\delta$: 7.85 (2H, d, J = 8.5 Hz), 7.76 (2H, d, J = 8.5 Hz), 7.29–7.41 (5H, m, Ar–H), 7.07 (1H, dd, J = 9.8, 5.3 Hz), 6.31 (1H, d, J = 9.8 Hz), 5.61 (1H, brd, J = 3.3 Hz), 5.13 (1H, d, J = 3.3 Hz), 5.11 (1H, brd, J = 4.1 Hz), 4.73 (1H, dd, J = 5.3, 4.1 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 164.7 (C), 160.3 (C), 139.0 (CH), 138.0 (2 × CH), 137.3 (C) 131.1 (2 × CH), 128.7 (2 × CH), 128.6 (CH), 128.4 (C), 126.2 (2 × CH), 124.8 (CH), 101.8 (C), 86.0 (CH), 84.3 (CH), 83.6 (CH), 69.3 (CH). EIMS m/z (relative intensity): 464 $[M + H]^+$ (<1), 233 (4), 231 (74), 215 (6), 203 (2), 171 (28), 146 (34), 145 (100), 105 (14), 77 (14). HR-TOF-MS (ESI): *m/z* 484.9863 $[M + Na]^+$ (calcd for $C_{20}H_{15}IO_5Na$, 484.9862)

### 3.3.11. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 4-bromo-2-chlorobenzoate (13)

Compound **13** was prepared by reacting altholactone (29.1 mg, 0.125 mmol) with 4-bromo-2-chlorobenzoic acid (35.2 mg, 0.150 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (30.9 mg, 0.150 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (15% ethyl acetate-hexane) to yield **13** (43.7 mg, 78% yield) as a yellow oil; $[\alpha]_D^{28}$ +72.9 (*c* 0.017, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 282 (2.97), 246 (4.02), 209 (4.46). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1736, 1580, 1496, 1469, 1451, 1371, 1289, 1240, 1124, 1103, 1048, 877, 819, 766, 699. $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 7.68 (1H, *d, J* = 8.4 Hz), 7.6 (1H, *d*, *J* = 1.7 Hz), 7.43 (1H, *dd, J* = 8.4, 1.7 Hz), 7.22–7.34 (5H, *m*, Ar–H), 6.99 (1H, *dd*, *J* = 9.8, 5.3 Hz), 6.23 (1H, *d*, *J* = 9.8 Hz), 5.54 (1H, *brd, J* = 3.2 Hz), 5.08 (1H, *d*, *J* = 3.2 Hz), 5.02 (1H, *brd*, *J* = 4.0 Hz), 4.63 (1H, *dd*, *J* = 5.3, 4.0 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 163.7 (C), 160.3 (C), 139.1 (CH), 137.3 (C), 135.0 (C), 134.1 (CH), 132.9 (CH), 130.3 (CH), 128.7 (2 × CH), 128.6 (CH), 127.6 (C), 127.5 (C), 126.2 (2 × CH), 124.8 (CH), 86.1 (CH), 84.8 (CH), 83.4 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 451 $[M + H]^+$ (<1), 221 (12), 220 (49), 218 (36), 171 (11), 146 (22), 145 (100), 143 (6), 106 (10), 78 (7). HR-TOF-MS (ESI): *m/z* 470.9617 $[M + Na]^+$ (calcd for $C_{20}H_{14}BrClO_5Na$, 470.9611)

### 3.3.12. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 2-fluoro-5-iodobenzoate (14)

Compound **14** was prepared by reacting altholactone (29.8 mg, 0.128 mmol) with 2-fluoro-5-iodobenzoic acid (40.9 mg, 0.154 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (31.7 mg, 0.154 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 2 h. The reaction mixture was purified by column chromatography (15% ethyl acetate-hexane), followed by recrystallization from MeOH to yield **14** (32.1 mg, 52% yield) as colorless needles; mp 105.3–106.0 °C. $[\alpha]_D^{28}$ +98.1 (*c* 0.018, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 216 (4.04). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1741, 1732, 1600, 1484, 1449, 1386, 1365, 1293, 1275, 1235, 1140, 1108, 1083, 1068, 1059, 915, 880, 819, 736, 700, 610. $^1$H NMR (400 MHz, $CDCl_3$), *δ*: 8.25 (1H, *dd, J* = 5.2, 2.4 Hz), 7.86 (1H, *ddd, J* = 8.8, 5.2, 2.4 Hz), 7.30–7.42 (5H, *m*, Ar–H), 7.07 (1H, *dd*, *J* = 9.8, 5.4 Hz), 6.96 (1H, *dd, J* = 10.3, 8.8 Hz), 6.31 (1H, *d*, *J* = 9.8 Hz), 5.62 (1H, *brd, J* = 3.3 Hz), 5.13 (1H, *d*, *J* = 3.3 Hz), 5.10 (1H, *dd*, *J* = 4.1, 0.9 Hz), 4.72 (1H, *dd*, *J* = 5.4, 4.1 Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): *δ* 160.3 (2 × C), 144.1 (CH), 144.0 (CH), 140.8 (CH), 139.1 (CH), 137.3 (C), 128.7 (2 × CH), 128.6 (CH), 126.2 (2 × CH), 124.8 (CH), 119.6 (C), 119.5 (CH), 119.3 (CH), 86.1 (CH), 84.8 (CH), 83.4 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 481 $[M + H]^+$ (<1), 250 (46), 249 (25), 215 (6), 171 (12), 170 (21), 146 (27), 145 (100), 123 (11), 106 (13), 95 (11), 94 (7). HR-TOF-MS (ESI): *m/z* 502.9767 $[M + Na]^+$ (calcd for $C_{20}H_{14}FIO_5Na$, 502.9768)

### 3.3.13. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 3,5-dichlorobenzoate (15)

Compound **15** was prepared by reacting altholactone (31.2 mg, 0.134 mmol) with 3,5-dichlorobenzoic acid (30.8 mg, 0.161 mmol) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (33.13 mg, 0.161 mmol) and 4-dimethylaminopyridine (cat.) at room temperature for 3 h. The reaction mixture was purified by column chromatography (15% ethyl acetate-hexane), followed by recrystallization from EtOH to yield **15** (47.9 mg,

88% yield) as colorless needles; mp 144.4–145.4 °C. $[\alpha]_D^{28}$ +143.9 (*c* 0.035, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 294 (2.69), 237 (3.72), 209 (4.46). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1739, 1571, 1497, 1450, 1435, 1400, 1372, 1351, 1265, 1244, 1152, 1100, 1032, 1022, 907, 878, 829, 804, 758, 737, 702. $^1H$ NMR (400 MHz, $CDCl_3$), **δ**: 7.84 (2H, *d*, *J* = 1.8 Hz), 7.53 (1H, *t*, *J* = 1.8 Hz), 7.22–7.33 (5H, *m*, Ar–H), 6.99 (1H, *dd*, *J* = 9.8, 5.0 Hz), 6.23 (1H, *d*, *J* = 9.8 Hz), 5.54 (1H, *brd*, *J* = 3.8 Hz), 5.02–5.04 (2H, *overlapping signal*), 4.66 (1H, *dd*, *J* = 5.1, 4.8 Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$): *δ* 162.8 (C), 160.2 (C), 139.0 (CH), 137.2 (C), 135.6 (2 × C), 133.6 (CH), 131.7 (C), 128.8 (2 × CH), 128.6 (CH), 128.2 (2 × CH), 126.2 (2 × CH), 124.8 (CH), 85.9 (CH), 84.7 (CH), 83.5 (CH), 69.2 (CH). EIMS *m/z* (relative intensity): 404.68 $[M]^+$ (<1), 214 (6), 175 (30), 173 (43), 170 (25), 144 (100), 109 (8), 105 (18), 77 (12). HR-TOF-MS (ESI): *m/z* 427.0113 $[M + Na]^+$ (calcd for $C_{20}H_{14}Cl_2O_5Na$, 427.0116)

### 3.4. General method for preparation of compound 10

A mixture of altholactone (50 mg, 0.216 mmol), and 4-chlorobenzoyl chloride (0.15 ml, 0.259 mmol) in pyridine (1 ml) was stirred at room temperature overnight. The reaction was quenched with aqueous ammonium chloride (10 ml). The aqueous layer was extracted with $CH_2Cl_2$ (3 × 25 ml). The combined organic layer was washed with $H_2O$, brine and dried over anhydrous magnesium sulfate. After filtration and removal of solvent under reduced pressure, a crude product (148.3 mg) was obtained and purified by preparative TLC (25% ethyl acetate-hexane), followed by recrystallization from MeOH to afford the desired product **10** (61.8 mg, 78% yield) as colorless needles.

#### 3.4.1. (2R,3R,3aR,7aS)-5-Oxo-2-phenyl-3,3a,5,7a-tetrahydro-2H-furo[3,2-b]pyran-3-yl 4-chlorobenzoate (10)

mp 134.8–135.6 °C. $[\alpha]_D^{28}$ +158.7 (*c* 0.049, $CHCl_3$). UV $\lambda_{max}^{ACN}$ nm (log ε): 241 (4.24). IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 1725, 1591, 1489, 1401, 1291, 1260, 1226, 1173, 1104, 1090, 1063, 1015, 956, 878, 853, 827, 819, 757, 743, 699. $^1H$ NMR (400 MHz, $CDCl_3$), **δ**: 8.0 (2H, *d*, *J* = 8.5 Hz), 7.47 (2H, *d*, *J* = 8.5 Hz), 7.30–7.41 (5H, *m*, Ar–H), 7.08 (1H, *dd*, *J* = 9.8, 5.2 Hz), 6.32 (1H, *d*, *J* = 9.8 Hz), 5.62 (1H, *brd*, *J* = 3.4 Hz), 5.14 (1H, *d*, *J* = 3.4 Hz), 5.11 (1H, *brd*, *J* = 4.4 Hz), 4.74 (1H, *dd*, *J* = 5.2, 4.4 Hz). $^{13}C$ NMR (100 MHz, $CDCl_3$): *δ* 164.3 (C), 160.4 (C), 140.4 (C), 139.1 (CH), 137.4 (C), 131.2 (2 × CH), 129.0 (2 × CH), 128.7 (2 × CH), 128.6 (CH), 127.4 (C), 126.2 (2 × CH), 124.8 (CH), 86.0 (CH), 84.3 (CH), 83.6 (CH), 69.3 (CH). EIMS *m/z* (relative intensity): 371 $[M + H]^+$ (4), 215 (4), 198 (4), 171 (19), 145 (100), 140 (23), 139 (10), 112 (10), 105 (6), 77 (5). HR-TOF-MS (ESI): *m/z* 393.0509 $[M + Na]^+$ (calcd for $C_{20}H_{15}BrO_5Na$, 393.0506)

### 3.5. Anti-fungal susceptibility testing

Altholactone and its derivatives were tested against three yeast species: *C. albicans* clinical isolate MT 2013/1, *Cryptococccus neoformans* clinical isolate MT 2013/2, and *Saccharomyces cerevisiae* IFO 10217. *C. albicans* and *C. neoformans* were from yeast stock cultures stored at the Faculty of Medical Technology, Mahidol University, Thailand. *C. albicans* and *C. neoformans* were cultivated and maintained on potato dextrose agar (PDA) (Merck, Darmstadt, Germany) at 37 °C, whereas *S. cerevisiae* was cultivated on the same media at 30 °C.

Minimal inhibition concentrations (MICs) were determined using the broth microdilution method, in 96-well microplates (ExtraGENE, California, USA), based on the guideline from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) definitive document EDef 7.2 [24]. The procedure was carried out by preparing overnight cultures of indicator yeast on PDA agar followed by colony suspension in sterile water to obtain 0.5 McFarland standard turbidity (1–5 × $10^6$ CFU $ml^{-1}$),and samples were further diluted 10-fold to obtain a cell density of 1–5 × $10^5$ CFU $ml^{-1}$. Compounds dissolved in 100% DMSO (Merck, Hohenbrunn, Germany) were prepared, using twofold serial dilutions in RPMI 1640 broth (Sigma-Aldrich, Missouri, USA) with 2% glucose to obtain a concentration ranging from 256 to 0.5 µg $ml^{-1}$. The highest concentration of DMSO in the test was 2.5%. Yeast cultures (100 µl) and compounds (100 µl) were added to each well of microtiter plates and incubated at 37 °C for 1 day for *C. albicans* and 3 days for *C. neoformans*. In case of *S. cerevisaie,* YPD (yeast peptone dextrose broth contained yeast extract 10 g, peptone 20 g, and dextrose 20 g in 1.0 liter distilled water) was used and incubation was performed at 30 °C for 1 day. Following incubation, the growth inhibition was monitored using a Wallac 1420 Victor2 microplate reader (Perkin Elmer, Turku, Finland). Blank medium was used as the sterility control. DMSO alone (under the same dilution condition) was also employed as a negative control. Amphotericin B (Biolab, Thailand) was included in the test as a positive control. All experiments were performed in triplicate and results are from two independent trials.



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No potential conflict of interest was reported by the authors.

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## References

[1] G.D. Brown, D.W. Denning, N.A.R. Gow, S.M. Levitz, M.G. Netea, and T.C. White, *Sci. Transl. Med*. **4**, 165rv13 (2012).
[2] T. Kourkoumpetis, D. Manolakaki, G. Velmahos, Y. Chang, H.B. Alam, M.M. De Moya, E.A. Sailhamer, and E. Mylonakis, *Virulence*. **1**, 359 (2010).

[3] M. Kuroki, C. Phichaichumpon, A. Yasuoka, P. Chiranairadul, T. Chosa, P. Sirinirund, T. Miyazaki, H. Kakeya, Y. Higashiyama, Y. Miyazaki, Y. Ishida, and S. Kohno, *Yeast*. **21**, 809 (2004).
[4] The Centers for Disease Control and Prevention (CDC), Antibiotic resistance threats in the United States Report, (2013).
[5] B.J. Park, K.A. Wannemuehler, B.J. Marston, N. Govender, P.G. Pappas, and T.M. Chiller, *AIDS*. **23**, 525 (2009).
[6] A. Murphy, and K. Kavanagh, *Enzyme. Microb. Tech*. **25**, 551 (1999).
[7] J.W. Loder, and R.H. Nearn, *Heterocycles* **7**, 113 (1977).
[8] A.A.E. El-Zayat, N.R. Ferrigni, T.G. McCloud, A.T. McKenzie, S.R. Byrn, J.M. Cassady, C. Chang, and J.L. McLaughlin, *Tetrahedron. Lett*. **26**, 955 (1985).
[9] P. Tuchinda, B. Munyoo, M. Pohmakotr, P. Thinapong, S. Sophasan, T. Santisuk, and V. Reutrakul, *J. Nat. Prod*. **69**, 1728 (2006).
[10] B. Zhao, and X. Li, *Oncol. Rep*. **31**, 2769 (2014).
[11] W. Uthaisang-Tanechpongtamb, P. Sriyabhaya, and P. Wilairat, *Cell. Biol. Int*. **37**, 471 (2013).
[12] E. Peris, E. Estornell, N. Cabedo, D. Cortes, and A. Bermejo, *Phytochemistry* **54**, 311 (2000).
[13] A. de Fatima, L.V. Modolo, L.S. Conegero, R.A. Pilli, C.V. Ferreira, L.K. Kohn, and J.E. de Carvalho, *Curr. Med. Chem*. **13**, 3371 (2006).
[14] T.A. Johnson, J. Sohn, A.E. Ward, T.L. Cohen, N.D. Lorig-Roach, H. Chen, R.A. Pilli, E.A. Widjaja, M. Hanafi, L.B.S. Kardono, P.D. Lotulung, K. Boundy-Mills, and L.F. Bjeldanesa, *Bioorg. Med. Chem*. **21**, 4358 (2013).
[15] R. Lekphrom, S. Kanokmedhakul, and K. Kanokmedhakul, *J. Ethnopharmacol*. **125**, 47 (2009).
[16] B. Neises, and W. Steglich, *Angew. Chem. Int. Ed. Engl*. **17**, 522 (1978).
[17] P.A. Castelo Branco, *Quim. Nova.* **35**, 948 (2012).
[18] D. Wang, S. Ren, H. Wang, H. Yan, J. Feng, and X. Zhang, *Chem. Biodivers*. **11**, 886 (2014).
[19] T.L. Lemke, V.F. Roche, and S.W. Zito, *Review of Organic Functional Groups: Introduction to Medicinal Organic Chemistry*, 5th ed. (Lippincott Williams & Wilkins, Philadelphia, 2012).
[20] F.A. Al Momani, A.S. Alkofahi, and N.M. Mhaidat, *Molecules* **16**, 4560 (2011).
[21] L. Zhang, H.F. Yang, Y.Y. Liu, X.H. Xu, Y. Ye, and J.B. Li, *Diagn. Microbiol. Infect. Dis*. **77**, 327 (2013).
[22] R.D. Cannon, E. Lamping, A.R. Holmes, K. Niimi, K. Tanabe, M. Niimi, and B.C. Monk, *Microbiology* **153**, 3211 (2007).
[23] S.L. Panwar, R. Pasrija, and R. Prasad, *Biosci. Rep*. **28**, 217 (2008).
[24] M.C. Arendrup, M. Cuenca-Estrella, C. Lass-Flörl, W. Hope, and EUCAST-AFST., *Clin. Microbiol. Infect.* **18**, E246 (2012).