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Cytotoxic and Antimicrobial Activity of Dehydrozingerone based Cyclopropyl Derivatives

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Abstract

A small series of 1-acetyl-2-(4-alkoxy-3-methoxyphenyl)cyclopropanes was prepared, starting from dehydrozingerone (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one) and its *O*-alkyl derivatives. Their microbiological activities toward some strains of bacteria and fungi were tested, as well as their *in vitro* cytotoxic activity against some cancer cell lines (HeLa, LS174 and A549). All synthesized compounds showed significant antimicrobial activity and expressed cytotoxic activity against tested carcinoma cell lines, but they showed no significant influence on normal cell line (MRC5). Butyl derivative is the most active on HeLa cells (IC_{50} = 8.63 μm), while benzyl one is active against LS174 and A549 cell lines (IC_{50} = 10.17 and 12.15 μm, respectively).

Keywords: Cytotoxic activities, Antimicrobal activities, Dehydrozingerone, Cyclopropyl moiety, Crystal structure elucidation

Introduction

Enone system of chalcones is almost planar with *trans*-double bond. This structure enables various transformations of enone system, which undergo cyclization reactions with urea, thiourea, hydroxylamine, hydrazine, guanidine,[12] forming heterocyclic unit between aromates, and active methylenic compounds (malononitrile, esters of cyanoacetic and acetoacetic acid, acetylacetone, nitromethane.[34] Sole double bond of enone system is reactive toward *Michael* initiated ring closure (MIRC) as well as the sulfoxonium salts (*Corey–Chaykovsky* reaction),[5] yielding cyclopropane derivatives.

A pungent constituent of ginger rhizome dehydrozingerone (**1**; 4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one), as a half analogue of curcumin, is present in different bioactive compounds, showing broad spectrum of biological activities, such as anti-inflammatory, antidepressant, antibacterial, antiviral, anticancer[6-12] and many others. Although conjugate enone system is presented in this phenolic compound, its structure differs from chalcones in possessing the methyl group connected to carbonyl instead of the aryl one. This unique chalcone-like structure, planar enone system and aromatic ring offer bifunctional site for various transformations.

As cyclopropane ring is present in a huge number of molecules isolated from nature,[13] such as terpenes, fatty acids, alkaloids and steroids so it is no surprise that many of them show pronounced biological activities, from enzyme inhibition of herpes roteases[14][15] to antibiotic, herbicidal, antitumor, and antiviral properties.[16-18] Also, well-known are chrysanthemic acid, pyrethrin, and pyrethroid derivatives, as compounds related to natural and synthetic insecticides, with good insecticidal activities.[19][20] This motif is attached to main frame of the molecules at different ways. Cyclopropyl group is connected to C-atom in heterocyclic fragment[21] or nitrogen atom in ciprofloxacin derivatives,[22] to carbon chain of C_{29} sterols,[23] as 1,1-dichloro derivatives of diaryl cyclopropanes.[24][25] It is also present in combretastatin derivatives as cyclopropyl-vinyl or a cyclopropyl-amide bridge,[26][27] in cyclopropyl indolequinones[28] or cinnamic acid derivatives.[29]

Based on data from different sources, nearly 50% of all modern scientific drugs on the market are natural products or natural-based materials and they play an important role in a drug design in pharmaceutical industries.[30] Although many drugs have been introduced into the market, their response to therapy is still poor. For this reasons more efficient drugs should be developed.

Radiotherapy and chemotherapy, as the most frequently used procedures in cancer treatment, are not specific to cancer cells and are known to cause severe and often adverse side effects, such as gastrointestinal reactions, immune suppression, bone marrow

suppression, hair loss, nerve injury, and even development of secondary malignancies. Therefore, we decided to modify dehydrozingerone, as a natural product easily accessible by simple synthetic procedure, into a new group of compounds. In this way attractive motif, cyclopropyl ring was introduced into the molecule. Microbiological and cytotoxic properties of synthesized compounds had been tested.

Results and Discussion

Chemistry

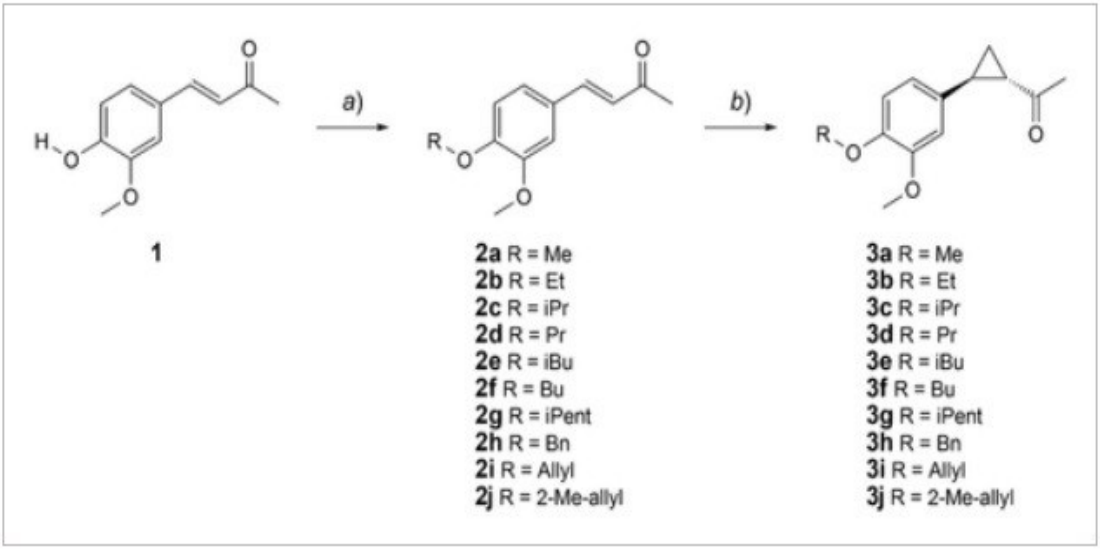
Due to our research interest for the synthesis of molecules exhibiting some biological activity,[1231-34] herein we described the synthesis and characterization, starting from dehydrozingerone (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one; **1**), of a series of compounds **2a** – **2j**. In these compounds, the *trans*-olefinic bond is replaced by a conformationally restricted and inherent ring strain cyclopropyl moiety, affording a series of novel cyclopropyl derivatives **3a** – **3j**.

Dehydrozingerone was synthesized following previously described procedure.[35] Alkylation of their free phenolic group was performed in the boiling acetone solution in the presence of corresponding alkyl halide and anh. K_2CO_3, yielding alkyl derivatives, 4-(4-alkoxy-3-methoxyphenyl)-3-buten-2-ones **2a** – **2j**. Isolated alkyl derivatives were used

as substrates in *Corey–Chaykovsky* reaction. Knowing that this reaction is stereospecific, the series of *trans*-cyclopropyl products were synthesized (*Scheme* 1). The prepared compounds, 1-acetyl-2-(4-alkoxy-3-methoxyphenyl)cyclopropanes **3**, were characterized by their spectral data (IR, MS, ^{1}H- and ^{13}C-NMR). All four protons from the cyclopropane ring have a different environment, and each of them is coupling with others and four groups of signals are presented. As result of it, each proton has three different coupling constants. The crystal structure of 1-acetyl-2-(4-isopropoxy-3-methoxyphenyl)-cyclopropane (**3c**) was also reported.

X-Ray Analysis

The crystal structure of molecule **3c** and the selected geometrical parameters are given in *Fig.* 1, while the selected geometrical parameters are given in *Table* 1. The C(3)–C(5) bond of the middle cyclopropane fragment (cp) is the longest bond in the structure. A slight elongation of this bond in comparison to the other two C–C bonds of the strained cp ring can be attributed to the presence of the acetyl and aryl substituents at the C(3) and C(5) atoms, respectively.



Sheme 1. Synthesis of cyclopropyl derivates **3-a – 3j** of dehydrozingerone a) acetone.K_2CO_3, RX, reflux overnight; *b)* dry DMSO, NaH, $Me_3SO^+|^-$

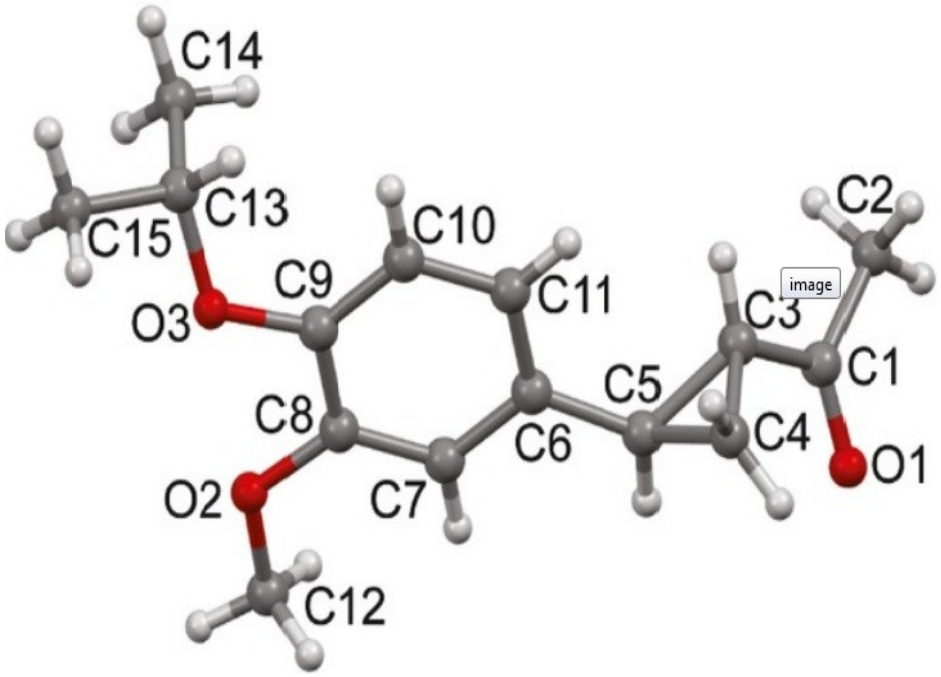


Figure 1 Crystal structure of compound **3c.**

Table 1. Selected bond lenghts[Å] and angles [°] of compound **3c**

	Bond length		Angle
C(1)–O(1)	1.212(2)	C(2)–C(1)–O(1)	121.5(2)
C(8)–O(2)	1.369(2)	C(3)–C(1)–O(1)	121.2(2)
C(9)–O(3)	1.372(2)	C(8)–O(2)–C(12)	117.6(1)
C(3)–C(4)	1.493(3)	C(9)–O(3)–C(13)	119.0(1)
C(4)–C(5)	1.485(2)	C(3)–C(4)–C(5)	61.6(1)
C(3)–C(5)	1.524(2)	C(4)–C(5)–C(3)	59.5(1)
		C(5)–C(3)–C(4)	59.0(1)

The angle opposite to the C(3)–C(5) bond also slightly expands at the expense of the remaining two angles (*Table* 1). Similar asymmetry within the disubstituted cp ring has been observed in related crystal structures such as phenylcyclopropanecarboxylic acids.[36-38] The angles outside the cp ring depend on the size of the attached substituents; thus, the angles between the C–C bonds of the cp ring and the corresponding bonds attaching the aryl and acetyl fragments have the average values of 121.3° and 117.6°, respectively.

The dihedral angle between the phenyl and cp rings of 87.8(1)° describes the nearly perpendicular arrangement of these two systems, as also observed in the case of the free

phenylcyclopropane (88.6°).39 In addition, the best plane of the phenyl ring in **3c** bisects the opposite cp C(3)–C(4) bond so the torsion angle C(11)–C(6)–C(5)–Ct1 (where Ct1 is the midpoint of C(3)–C(4)) equals to 6.1°. Acetyl fragment displays similar perpendicular position with respect to the middle cp ring with the dihedral angle between the best planes C(2)/C(1)/O(1) and C(3)/C(4)/C(5) of 88.9(1)° and the torsion angle O(1)–C(1)–C(3)–Ct2 of 1.52° (where Ct2 is the midpoint of C(4)–C(5)). The mutual orientation of the acetyl and phenyl systems is described by the torsion angle C(1)–C(3)–C(5)–C(6) which equals to −143.5(1)°. Similarly to the previously described methoxyphenyl derivatives,12 the methoxy fragment attached to the phenyl ring only slightly deviates from the phenyl mean plane (O(2): −0.019 (2); C(12): −0.077 (3) Å); this is in contrast to the i-propoxy moiety where the deviation of the corresponding atoms is more pronounced (O(3): 0.078 (2); C(13): −0.308 (3) Å).

In the crystal, the centrosymmetrically related molecules form dimers by a pair of weak C(7)–H…O(1) interactions (C…O 3.290(2), H…O 2.40 Å, C–H…O 160°) (Fig. S1, see *Supporting Information*). Further arrangement of the adjacent molecules is based on weak C(4)–H(4b)…π interaction (C…Cg1 3.464(2), H…π 2.78 Å, C–H…Cg1 129°, where Cg1 is the centroid of phenyl ring) (Fig. S2).

Antimicrobial Activity

The antimicrobial activities of the investigated compounds against the test microorganisms are shown in *Tables* 2 and 3. The tested compounds demonstrated relatively strong antimicrobial activity inhibiting all tested microorganisms. The minimum inhibitory concentration (*MIC*) for these compounds relative to the tested bacteria ranged from 0.009 to 1.25 mg/ml (*Table* 2). The strongest antibacterial activity was found in **3b** compound inhibiting all the species of bacteria in low concentrations, especially *Bacillus subtilis* where measured *MIC* value was 0.009 mg/ml (stronger than streptomycin). Among the bacteria, *Escherichia coli* showed the highest resistance while *B. subtilis* was the most sensitive. The tested compound also inhibited the growth of the all tested fungi (*Table* 3) but in slightly higher concentrations (*MIC* values were from 0.156 to 5 mg/ml). Among the fungi, *Candida albicans* appeared to be the most sensitive.

The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The experimental results showed that the tested compound had just slightly weaker effect than streptomycin (compound **3b** even better than streptomycin), while ketoconazole had stronger activity than the tested samples as shown in *Tables* 2 and 3. In a negative control, DMSO had no inhibitory effect on the tested organisms.

In these experiments, antibacterial effect was observed against the both *Gram*-positive and *Gram*-negative bacteria but it should be noted that the *Gram*-negative bacteria were more

resilient. It has been generally reported that the *Gram*-negative bacteria are more resistant than *Gram*-positive.[12][40][41] This resistance is likely, due to the fact that *Gram*-negative bacteria have a wall which itself is surrounded by an outer complex membrane, slowing down the passage of hydrophobic compounds.

Table 2. Antimicrobial activities of tested compounds

Microorganisms	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus cereus*	*Escherichia coli*	*Proteus mirabilis*
3a	0.312	0.019	0.078	0.312	0.312
3b	0.078	0.009	0.019	0.312	0.156
3c	0.312	0.019	0.078	1.25	0.625
3d	0.156	0.019	0.039	0.625	0.312
3e	0.156	0.078	0.078	0.625	0.312
3f	0.312	0.019	0.019	1.25	0.312
3g	0.156	0.078	0.078	0.312	0.156
3h	0.625	0.039	0.078	1.25	1.25
3i	0.156	0.039	0.078	0.312	0.156
3j	0.156	0.078	0.078	0.625	0.312
Streptomycin	0.031	0.016	0.016	0.062	0.062

Minimum inhibitory concentration given as mg/ml. The values are the mean of three replicate. In all cases the standard deviation is ±0.002.

Table 3 Antimicrobial activities of tested compounds.

Microorganisms	*Aspergillus flavus*	*Aspergillus fumigatus*	*Candida albicans*	*Penicillium purpurescens*	*Penicillium verucosum*
3a	1.25	0.312	0.625	0.625	0.625
3b	0.625	0.312	0.312	0.312	0.625
3c	1.25	1.25	0.625	2.5	2.5
3d	0.625	0.312	0.625	0.312	0.625
3e	5	1.25	0.156	2.5	2.5
3f	1.25	0.625	0.625	1.25	2.5
3g	2.5	0.625	0.312	1.25	2.5
3h	2.5	1.25	1.25	2.5	2.5
3i	2.5	0.625	0.312	1.25	2.5
3j	2.5	1.25	0.156	2.5	1.25
Ketoconazole	0.156	0.078	0.039	0.156	0.156

Minimum inhibitory concentration given as mg/ml. The values are the mean of three replicate. In all cases the standard deviation is ± 0.001.

Lacking outer membrane, *Gram*-positive bacteria are more susceptible to the antibiotic agents.[42] Compared to the bacteria, fungi were more resistant due to the more complex structure of the cell wall.[43]

Cytotoxic Activity

The cytotoxicity of cyclopropyl derivatives **3a** – **3j** against the human carcinoma cell lines HeLa, A549, and LS174 was assessed by the MTT test, using *cis*-DDP as the control. The cytotoxic activity of tested compounds is given in *Table* 4, as the IC_{50} values [μm]. IC_{50} values are expressed as the mean ± SD determined from the results of MTT assay in three independent experiments.

As noted in the table, all samples showed marked cytotoxic activity against tested carcinoma cell lines. Compound **3h** showed the highest effect against HeLa cells (IC_{50} = 8.63 μm), whereas compound **3f** showed best activity against LS174 and A549 cell lines (IC_{50} = 10.17 and 12.15 μm, respectively).

Moreover, compared to *cis*-DDP, all samples showed no cytotoxicity toward normal tissue cells, human fetal lung fibroblast cell line (MRC5) (IC_{50} > 200). Selectivity index, defined as IC_{50} (MRC5)/IC_{50} (cell line) ratio (*Table* 5), indicates that all tested compounds exhibit superior selectivity comparing to *cis*-DDP (up to ten times). According to the supreme cytotoxicity and selectivity (low IC_{50} and high selectivity index), compounds **3e**, **3f**, and **3h** demonstrate the most promising cytotoxic activity against the selected carcinoma cell lines.

Table 4. Cytotoxic activity (IC_{50} values) of compounds 3a – 3j against human cancer cell lines HeLa, LS174, A549, and human fetal lung fibroblast cell line (MRC5)

Compounds	IC_{50} [μM]			
	HeLa	LS174	A549	MRC5
3a	38.34 ± 1.95	40.91 ± 1.22	24.05 ± 2.43	> 200
3b	24.14 ± 0.76	29.76 ± 0.57	32.98 ± 0.72	> 200
3c	16.22 ± 1.48	36.36 ± 2.72	28.77 ± 3.41	> 200
3d	38.86 ± 2.59	41.57 ± 0.79	36.37 ± 2.55	> 200
3e	17.61 ± 1.87	18.73 ± 0.55	16.85 ± 1.32	> 200
3f	34.63 ± 2.81	**10.17 ± 1.23**	**12.15 ± 0.49**	> 200
3g	12.16 ± 1.84	31.11 ± 2.59	30.85 ± 1.20	> 200
3h	**8.63 ± 0.49**	17.53 ± 2.45	26.89 ± 0.77	> 200
3i	19.18 ± 0.62	23.74 ± 1.36	26.63 ± 3.48	> 200
3j	24.52 ± 1.14	32.52 ± 1.81	32.11 ± 0.31	> 200
cis-DDP	2.45 ± 0.21	5.03 ± 0.47	9.48 ± 0.73	15.22 ± 0.68

'Bold' type refers to the most active compound on corresponding cell line.

Tabele 5. Selectivity index

Compounds	HeLa	LS174	A549
	IC_{50}(MRC5)/IC_{50}(cell line)		
3a	5.2	4.9	8.3
3b	8.3	6.7	6.1
3c	12.3	5.5	7.0
3d	5.1	4.8	5.5
3e	11.4	10.7	11.9
3f	5.8	19.7	16.5
3g	16.4	6.4	6.5
3h	23.2	11.4	7.4
3i	10.4	8.4	7.5
3j	8.2	6.2	6.2
cis-DDP	6.2	3.0	1.6

Conclusions

Dehydrozingerone, as an easily accessible natural product, was modified by the simple synthetic procedure. In the present study, the results clearly demonstrate that, although simple by structure, the synthesized compounds show significant microbiological activities toward tested strains of microorganisms; the strongest activity was observed towards *Bacillus* strains of bacteria. Moreover, *in vitro* cytotoxic activity of the synthesized compounds against tumor cell lines, human epithelial carcinoma HeLa cells, human colon carcinoma LS174 cells and human lung carcinoma A549 cells was observed. Most importantly, compared to cis-DDP, the synthesized compounds showed very low cytotoxicity (IC_{50} > 200) toward normal human fetal lung fibroblast MRC5 cell line.

The good activity of synthesized compounds, fewer side effects, along with the lack of toxicity (very low cytotoxicity against MRC5 cells) and the feasible synthesis, underscore their value as promising novel scaffolds for the development of new antimicrobial and anticancer drugs.

Experimental Section

Chemistry

All starting chemicals were commercially available and used as received, except for the solvents being purified by distillation. (*E*)-4-(4-Hydroxy-3-methoxyphenyl)but-3-en-2-one,

dehydrozingerone (**1**), was prepared following the previously mentioned procedure.[35] *O*-Alkyl dehydrozingerones were synthesized following described procedures.[44-46] The chemical synthesis of compounds **2a** – **2d** and **2f** – **2i** was published earlier.[47] Isobutyl derivative **2e** and methallyl derivative **2j** are new compounds and their structure and spectral data are already given. Out of the products of type **3**, only compound **3a** was earlier described,[48] whereas **3b** – **3j** are new compounds, prepared according described procedure[5] (*Scheme* 1).

Column chromatography: silica gel *60* (*Merck*, 230 – 400 mesh ASTM). TLC: Silica gel *60 F254*-precoated plates (*Merck*). IR Spectra: *PerkinElmer Spectrum One* FT-IR spectrometer with a KBr disc, *v* in cm^{1}. NMR Spectra: *Varian Gemini 200* MHz spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C), using $CDCl_3$ as the solvent and TMS as the internal standard. ^{1}H and ^{13}C-NMR chemical shifts were reported in parts per million [ppm] and were referenced to the solvent peak; $CDCl_3$ (7.26 ppm for ^{1}H and 76.90 ppm for ^{13}C). Multiplicities are represented by *s* (*singlet*), *d* (*doublet*), *t* (*triplet*), *q* (*quartet*), *ddd* (*doublet* of *doublet* of *doublet*), and *m* (*multiplet*). Coupling constants (*J*) are in Hertz [Hz]. Mass spectrometry was performed by *Waters Micromass ZQ* mass spectrometer and MassLynx software for control and data processing. Electro spray ionization in the positive mode was used. The electro spray capillary was set at 4.3 kV and the cone at 40 V. The ion source temp. was set at 125 °C and the nitrogen flow rates were 400 and 50 l/h, for desolvation and cone gas flow, resp. The

collision energy was 40 eV. The melting point of products was determined by using *MelTemp1000* apparatus.

*General Procedure for the Synthesis of 4-(4-Alkoxy-3-methoxyphenyl)-but-3-en-2-ones (2a – 2j.)*A mixture of dehydrozingerone (1; 1.92 g, 10 mmol), appropriate alkyl halide (excess, 30 mmol) and K_2CO_3 (7 g, anh.) in acetone (60 ml) was heated to reflux overnight under argon. Acetone and the excess of alkyl halide were evaporated under reduced pressure and the solid residue was dissolved in water. The mixture was distilled with steam to remove excess of alkyl halide and their side products. After cooling, the water mixture was extracted with CH_2Cl_2 (3 × 50 ml). The combined extracts were washed with water and dried over anh. Na_2SO_4. After the removal of the main part of solvent, the residue was filtered over silica gel pad. Compounds 2e and 2j were isolated as oils, while others were isolated as white crystalline substances.

(3*E*)-4-[3-Methoxy-4-(2-methylpropoxy)phenyl]-but-3-en-2-one (**2e**). Yield: 66%. IR (KBr): 2956, 1665, 1583, 1518, 1422, 1255, 1138, 1022. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.06 (*d*, *J* = 6.6, 6 H); 2.05 – 2.25 (*m*, 1 H); 2.37 (*s*, 3 H); 3.81 (*d*, *J* = 6.8, 2 H); 3.89 (*s*, 3 H); 6.59 (*d*, *J* = 16.2, 1 H); 6.84 – 7.12 (*m*, 3 H); 7.46 (*d*, *J* = 16.2, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 19.2; 27.2; 28.1; 56.1; 75.4; 110.6; 112.7; 122.9; 125.1; 127.1; 143.6; 149.7; 151.3; 198.3 (CO).

(3*E*)-4-{3-Methoxy-4-[(2-methylprop-2-en-1-yl)oxy]phenyl}but-3-en-2-one (**2j**). Yield: 62%. IR (KBr): 2979, 1665, 1641, 1593, 1517, 1424, 1263, 1168, 1144, 1032, 980. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.83 (*s*, 3 H); 2.37 (*s*, 3 H); 3.91 (*s*, 3 H); 4.56 (*s*, 2 H); 5.00 – 5.09 (*m*, 2 H); 6.59 (*d*, *J* = 16.2, 1 H); 6.86 (*d*, *J* = 8.8, 1 H); 7.05 – 7.11 (*m*, 2 H); 7.45 (*d*, *J* = 16.4, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 19.2; 27.2; 55.9; 72.5; 110.3; 112.9; 113.1; 122.7; 125.2; 127.5; 140.2; 143.4; 149.7; 150.6; 198.1 (CO).

General Procedure for the Synthesis of 1-Acetyl-2-(4-alkoxy-3-methoxyphenyl)cyclopropanes ***(3a – 3j).*** The solution of the ylide was prepared under nitrogen from sodium hydride (3 mmol, 0.15 g of 50% oil suspension), trimethyloxosulfonium iodide (0.726 g, 3.3 mmol) and DMSO (3 ml). Enone solution in DMSO (3 mmol in 5 ml) was added to this mixture while being stirred and cooled in a water bath. The mixture was stirred at r.t. for 2 h and then at 50 °C for 1 h. The solvent mixture of toluene/AcOEt (95:5, 20 ml) was added to a reaction flask with stirring. The mixture was then poured into 50 ml of cold water and extracted with toluene. The extracts were washed twice with water, dried over anh. Na_2SO_4 and evaporated, yielding pale yellow oil. The crude mixture was chromatographed on silica gel column, using hexane/AcOEt mixture (7:3).

1-[2-(3,4-Dimethoxyphenyl)cyclopropyl]ethanone (**3a**). Yield: 68%. M.p. 63 – 64 °C. IR (KBr): 3001, 2835, 1695, 1608, 1590, 1519, 1397, 1258, 1236, 1141, 1028, 968. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.35 (*ddd*, *J* = 4.2, 6.8, 8.2, 1 H); 1.64 (*ddd*, *J* = 4.2, 5.2, 9.2, 1 H); 2.17

(*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.49 (*ddd*, *J* = 4.0, 6.8, 9.0, 1 H); 3.85 (*s*, 3 H); 3.87 (*s*, 3 H); 6.62 – 6.66 (*m*, 2 H); 6.79 (*d*, *J* = 8.6, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.7; 28.7; 30.6; 32.6; 55.8; 55.9; 109.9; 111.3; 117.8; 132.7; 147.8; 148.9; 206.6 (CO). ESI-MS (40 eV): 220 (14), 177 (42), 164 (10), 151 (15.5), 138 (8), 91 (61).

1-[2-(4-Ethoxy-3-methoxyphenyl)cyclopropyl]-ethanone (**3b**). Yield: 69%. M.p. 65 – 66 °C. IR (KBr): 3004, 2933, 1689, 1609, 1589, 1520, 1399, 1266, 1231, 1140, 1037, 965, 846. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.35 (*ddd*, *J* = 4.0, 6.8, 8.2, 1 H); 1.44 (*t*, *J* = 7.0, 3 H); 1.63 (*ddd*, *J* = 4.0, 5.0, 9.0, 1 H); 2.16 (*ddd*, *J* = 4.0, 5.0, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.8, 9.0, 1 H); 3.86 (*s*, 3 H); 4.07 (*q*, *J* = 7.0, 2 H); 6.58 – 6.63 (*m*, 2 H); 6.79 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 14.7; 18.8; 28.8; 30.7; 32.7; 55.8; 64.3; 110.1; 112.8; 117.8; 132.7; 147; 149.2; 206.8 (CO). ESI-MS (40 eV): 234 (17), 191 (33), 189 (26), 165 (28).

1-{2-[3-Methoxy-4-(propan-2-yloxy)phenyl]cyclopropyl}ethanone (**3c**). Yield: 70%. Oil. IR (KBr): 2976, 2935, 1697, 1607, 1586, 1515, 1397, 1385, 1259, 1230, 1139, 1112, 1038, 848. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.35 (*ddd*, *J* = 4.2, 6.8, 8.4, 1 H); 1.33 (*d*, *J* = 6.2, 6 H); 1.64 (*ddd*, *J* = 4.4, 5.4, 9.4, 1 H); 2.18. (*ddd*, *J* = 4.2, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.2, 6.8, 9.0, 1 H); 3.84 (*s*, 3 H); 4.41 – 4.53 (*m*, 1 H); 6.57 – 6.65 (*m*, 2 H); 6.81 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.8; 22; 28.8; 30.6; 32.7; 55.9; 71.6; 110.6; 116.4; 117.8; 133.3; 146; 150.5; 206.7 (CO). ESI-MS (40 eV): 248 (17), 206 (81), 189 (100), 163 (68), 157 (26), 137 (24).

1-[2-(3-Methoxy-4-propoxyphenyl)cyclopropyl]-ethanone (**3d**). Yield: 79%. M.p. 48 – 49 °C. IR (KBr): 2967, 2940, 1683, 1610, 1588, 1521, 1389, 1260, 1237, 1136, 1017, 975, 843. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.03 (*t*, *J* = 7.6, 3 H); 1.33 (*ddd*, *J* = 4.4, 6.6, 8.2, 1 H); 1.64 (*ddd*, *J* = 4.0, 5.2, 9.2, 1 H); 1.76 – 1.92 (*m*, 2 H); 2.17 (*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.6, 9.2, 1 H); 3.85 (*s*, 3 H); 3.95 (*t*, *J* = 6.8, 2 H); 6.58 – 6.64 (*m*, 2 H); 6.79 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 10.3; 18.7; 22.4; 28.8; 30.6; 32.7; 55.9; 70.6; 110.4; 113.2; 117.9; 132.7; 147.3; 149.4; 206.7 (CO). ESI-MS (40 eV): 248 (13), 205 (53), 189 (48), 163 (33).

1-{2-[3-Methoxy-4-(2-methylpropoxy)phenyl]cyclopropyl}ethanone (**3e**). Yield: 53%. M.p. 50 – 51 °C. IR (KBr): 2959, 2906, 1684, 1588, 1522, 1389, 1258, 1234, 1025, 976, 844. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.02 (*d*, *J* = 6.6, 6 H); 1.34 (*ddd*, *J* = 4.4, 6.8, 8.2, 1 H); 1.64 (*ddd*, *J* = 4.4, 5.2, 9.2, 1 H); 2.14 (*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.8, 9.2, 1 H); 3.74 (*d*, *J* = 7.0, 2 H); 3.85 (*s*, 3 H); 6.59 – 6.63 (*m*, 2 H); 6.79 (*d*, *J* = 8.0, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.8; 19.2; 28.1; 28.8; 30.7; 32.7; 56.2; 75.8; 110.9; 113.6; 118; 132.8; 147.8; 149.6; 206.7 (CO). ESI-MS (10 eV): 263 (100, $[M + 1]^+$).

1-[2-(4-Butoxy-3-methoxyphenyl)cyclopropyl]-ethanone (**3f**). Yield: 52%. M.p. 55 – 56 °C. IR (KBr): 2961, 2872, 1685, 1609, 1589, 1523, 1390, 1259, 1236, 1134, 1033, 1025, 979, 843. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.97 (*t*, *J* = 7.4, 3 H); 1.32 (*ddd*, *J* = 4.0, 6.8, 8.2, 1 H); 1.39 – 1.54 (*m*, 2 H); 1.63 (*ddd*, *J* = 4.2, 5.2, 9.2, 1 H); 1.74 – 1.88 (*m*, 2 H); 2.16 (*ddd*, *J* =

4.2, 5.4, 8.4, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.6, 9.2, 1 H); 3.85 (*s*, 3 H); 3.99 (*t*, *J* = 6.8, 2 H); 6.58 – 6.63 (*m*, 2 H); 6.79 (*d*, *J* = 8.2, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.8; 18.7; 19.1; 28.8; 30.7; 31.2; 32.7; 56; 68.9; 110.5; 113.2; 117.9; 132.7; 147.4; 148.4; 206.7 (CO). ESI-MS (40 eV): 263 (11, $[M + 1]^+$), 219 (16), 207 (19), 189 (72), 157 (24.5).

1-{2-[3-Methoxy-4-(3-methylbutoxy)phenyl]cyclopropyl}ethanone (**3g**). Yield: 78%. M.p. 74 – 75 °C. IR (KBr): 2954, 2868, 1682, 1587, 1521, 1387, 1255, 1237, 1136, 1025, 979, 850. ^{1}H-NMR (200 MHz, $CDCl_3$): 0.96 (*d*, *J* = 6.4, 6 H); 1.34 (*ddd*, *J* = 4.2, 6.8, 8.2, 1 H); 1.59 – 1.86 (*m*, 4 H), 2.16 (*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.6, 9.0, 1 H); 3.85 (*s*, 3 H); 4.01 (*t*, *J* = 6.8, 2 H); 6.59 – 6.63 (*m*, 2 H); 6.80 (*d*, *J* = 8.4, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.8; 22.6; 25.1; 28.8; 30.7; 32.7; 37.9; 56.1; 67.7; 110.5; 113.3; 117.9; 132.8; 147.5; 149.5; 206.8 (CO). ESI-MS (10 eV): 277 (100, $[M + 1]^+$).

1-{2-[4-(Benzyloxy)-3-methoxyphenyl]cyclopropyl}ethanone (**3h**). Yield: 82%. M.p. 101 – 102 °C. IR (KBr): 2935, 2860, 1689, 1605, 1588, 1518, 1388, 1378, 1254, 1230, 1003, 927, 846. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.31 (*ddd*, *J* = 4.2, 6.8, 8.2, 1 H); 1.63 (*ddd*, *J* = 4.4, 5.2, 9.4, 1 H); 2.15 (*ddd*, *J* = 4.2, 5.2, 8.2, 1 H); 2.29 (*s*, 3 H); 2.49 (*ddd*, *J* = 4.0, 6.6, 9.0, 1 H); 3.87 (*s*, 3 H); 5.12 (*s*, 2 H); 6.52 – 6.66 (*m*, 2 H); 6.78 (*d*, *J* = 8.2, 1 H); 7.25 – 7.44 (*m*, 5 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.9; 28.8; 30.7; 32.7; 56; 71.2; 110.6; 114.4; 117.8; 127.2; 127.7; 128.4; 133.5; 137.2; 146.9; 149.7; 206.7 (CO). ESI-MS (40 eV): 297 (7, $[M + 1]^+$), 253 (20.5), 219 (23.5), 205 (44.5), 189 (37), 158 (16.5), 91 (53).

1-{2-[3-Methoxy-4-(prop-2-en-1-yloxy)phenyl]cyclopropyl}ethanone (**3i**). Yield: 69%. Oil. IR (KBr): 2923, 1695, 1590, 1517, 1396, 1257, 1230, 1141, 1023, 929. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34 (*ddd*, *J* = 4.2, 6.6, 8.2, 1 H); 1.64 (*ddd*, *J* = 4.2, 5.2, 9.2, 1 H); 2.16 (*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.6, 9.2, 1 H); 3.86 (*s*, 3 H); 4.56 – 4.60 (*m*, 2 H); 5.24 – 5.44 (*m*, 2 H); 5.97 – 6.14 (*m*, 1 H); 6.58 – 6.65 (*m*, 2 H); 6.79 (*d*, *J* = 8.2, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.8; 28.7; 30.7; 32.7; 55.9; 70; 110.4; 113.7; 117.8; 133.2; 133.3; 146.8; 149.5; 206.7 (CO). ESI-MS (10 eV): 247 (100, $[M + 1]^+$).

1-(2-{3-Methoxy-4-[(2-methylprop-2-en-1-yl)oxy]-phenyl}cyclopropyl)ethanone (**3j**). Yield: 72%. Oil. IR (KBr): 2922, 1694, 1591, 1516, 1393, 1264, 1229, 1139, 1035, 903. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34 (*ddd*, *J* = 4.2, 6.8, 8.2, 1 H); 1.62 (*ddd*, *J* = 4.2, 5.0, 9.6, 1 H); 1.81 (*s*, 3 H); 2.16 (*ddd*, *J* = 4.0, 5.2, 8.2, 1 H); 2.30 (*s*, 3 H); 2.48 (*ddd*, *J* = 4.0, 6.6, 9.2, 1 H); 3.86 (*s*, 3 H); 4.48 (*s*, 2 H); 5.02 (*d*, *J* = 20.0, 2 H); 6.56 – 6.65 (*m*, 2 H); 6.79 (*d*, *J* = 8.2, 1 H). ^{13}C-NMR (50 MHz, $CDCl_3$): 18.7; 19.2; 28.7; 30.6; 32.6; 55.9; 72.8; 110.5; 112.5; 113.9; 117.8; 133.1; 140.8; 147; 149.5; 206.6 (CO). ESI-MS (10 eV): 261 (100, $[M + 1]^+$).

X-Ray Diffraction Experiment

The diffraction data for molecule **3c** were collected at r.t. on *Agilent Gemini S* diffractometer equipped with CuK_α radiation (λ = 1.54184 Å). Data reduction and empirical absorption correction were performed with CrysAlisPro.[49] The crystal structure was solved by direct methods, using Sir2002[50] and refined using SHELXL[51] by full-matrix least-squares on F^2.

All H-atoms were placed geometrically [C–H = 0.93 – 0.98 Å] and refined using the riding model with isotropic displacement parameters set to 1.2 or 1.5 times the U_{eq} values of the parent C-atoms. Crystallographic data and refinement parameters are listed in Table S1. The software used for the preparation of the materials for publication: WinGX,[52] Mercury,[53] PLATON,[54] PARST.[55]

Antimicrobial Activity

Antimicrobial activities of tested compounds were evaluated against ten microorganisms, including five strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 10987), *E. coli* (ATCC 25922), and *Proteus mirabilis* (ATCC 29906) and five species of fungi: *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (ATCC 1022), *C. albicans* (ATCC 10259), *Penicillium purpurescens* (ATCC 48987), and *Penicillium verucosum* (ATCC 48959) obtained from the American Type Culture Collection (ATCC).

The bacteria isolates were picked from overnight cultures in *Mueller–Hinton* agar and the suspensions were prepared in sterile distilled water by adjusting the turbidity to match 0.5 *McFarland* standards to approximately 10^8 CFU/ml. The fungal suspensions were prepared from 3- to 7-day-old cultures that grown on a potato dextrose agar except for *C. albicans* that was maintained on *Sabourad* dextrose (SD) agar. The spores were rinsed with sterile distilled

water, used to determine turbidity spectrophotometrically at 530 nm, and then further diluted to approximately 10^6 CFU/ml, following the procedure recommended by NCCLS.[56]

The 96-well microtiter assay using resazurin as the indicator of cell growth,[57] was employed for the determination of the *MIC* of the active components. The starting solutions of tested compounds were obtained by dissolving it in 5% DMSO. The twofold serial dilutions of tested compounds were made in a concentration range from 20 to 0.004 mg/ml in sterile 96-well plates containing *Mueller Hinton* broth for bacterial cultures and a SD broth for fungal cultures. Then, fungal or bacterial suspensions were added to each well and finally, resazurin solution was added as an indicator to each well. The plates were prepared in triplicate, and placed in an incubator set at 37 °C for 18 – 24 h. The *MIC* was determined visually and defined as the lowest concentration of tested compounds preventing resazurin color change from blue to pink. Streptomycin and ketoconazole were used as positive controls while 5% DMSO was used as a negative control.

Cytotoxicity

Cells and Cell Culture. Human epithelial carcinoma HeLa cells, human lung carcinoma A549 cells and human colon carcinoma LS174 cells and non-malignant human lung fibroblast cell line (MRC5) were obtained from American Type Culture Collection (Manassas, VA, USA). All cancer cell lines were cultured as a monolayer in the RPMI 1640 nutrient medium, with

10% (inactivated at 56 °C) FBS, 3 mM of l-glutamine, and antibiotics, at 37 °C in humidified air atmosphere with 5% CO_2.

In vitro *Cytotoxic Assay. In vitro* assay for cytotoxic activity of tested compounds was performed when the cells reached 70 – 80% of confluence. Stock solution (50 mg/ml) of compounds was dissolved in corresponding medium to the required working concentrations. Neoplastic HeLa cells (5000 cells per well), A549 cells (5000 cells per well), and LS174 cells (5000 cells per well) as well as non-cancerous MRC5 (5000 cells per well) were seeded into 96-well microtiter plates, and 24 h later, after cell adherence, five different, double diluted concentrations of investigated compounds were added to the wells. Final compounds concentrations were 200, 100, 50, 25, and 12.5 μg/ml except for the control wells, where only nutrient medium was added. The cultures were incubated for the next 72 h. The effect on cancer cell survival was determined 72 h after the addition of tested compounds, by the MTT test.[58] Briefly, 20 μl of MTT solution (5 mg/ml PBS) was added to each well and incubated for a further 4 h at 37 °C in 5% CO_2 and humidified air. Subsequently, 100 μl of 10% SDS was added to solubilize the formazan crystals formed from MTT after the conversion by mitochondrial dehydrogenases of viable cells. Absorbencies proportional to the number of viable cells were measured using a microplate reader (*Multiskan EX*, *Thermo Scientific*, Finland) at 570 nm. Each experiment was performed in triplicate and independently repeated at least four times.

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201700077

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Author Contribution Statement

A. Z. Burmudžija, *J. M. Muškinja*, and *Z. R. Ratković* conceived and designed the chemistry experiments and wrote the article. *M. M. Kosanić* and *B. R. Ranković* performed the microbiological experiments. *S. B. Novaković* performed the X-ray experiments and discussion. *S. B. Đorđević* performed the structural measurement experiments. *T. P. Stanojković* and *D. D. Baskić* performed the cytotoxic experiments and discussion.

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