Scientific paper

Carvacrol Derivatives as Antifungal Agents: Synthesis, Antimicrobial Activity and *in Silico* Studies on Carvacryl Esters

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Abstract

Chemical modifications of natural monoterpenoids to various derivatives have been reported to result in enhancement of biological activities when compared to parent compounds. In this context a well-known biocide and food additive, carvacrol, served as a basic scaffold onto which a phenolic functionality transformation by introducing acyl groups was performed. By using this simple methodology, we obtained a small series of 25 esters. For each of the obtained compounds we have performed structural characterization, *in vitro* antimicrobial testing and *in silico* calculation of physico-chemical, pharmacokinetic and toxicological properties. Despite numerous data on the synthesis and bioactivity of carvacryl ester lower homologues, there are scarce data on esters with acid components higher than C_9, so that among 25 compounds, 10 were reported for the first time (spectral characterization for 12 are herein the first reported). Our research is also the first comprehensive study of carvacryl esters antifungal and of medium/long chain fatty acid esters antibacterial activities. Interesting result is that all the synthesized esters, regardless the nature of the R residue, have shown activity on fungal strain *Aspergilus niger* and on yeast *Candida albicans* comparable to carvacrol. Besides presented experimental data, implementation of *in silico* calculation of physico-chemical, pharmacokinetic and toxicological properties on the prepared compounds, may be valuable information in further research.

Keywords: chemical transformation; carvacrol esters; *in vitro* antimicrobial activity; *in silico* calculation

1. Introduction

Natural products and their scaffolds have a long history of application as valuable starting points for medicinal chemistry and drug discovery.[1] Their structural modification, when compared to parent compounds, has often afforded structures with enhanced pharmacological activities and outstanding therapeutic possibilities.[2,3]

Carvacrol (2-methyl-5-(1-methylethyl)-phenol), a monoterpenoid phenol biosynthetically related to *para*-cymene, frequently occurs in essential oils of many Lamiaceae (*Origanum*, *Thymbra*, *Thymus*, *Satureja*) and Verbenaceae (*Lippia*) plants usually used as spices and for therapy/prevention purposes in folk medicine.[4] A variety of biological properties including antioxidant, antimicrobial, antiviral, insecticidal, antiparasitic, antihypertensive, immunomodulatory and antitumor, resulted from numerous studies overtaken in past 20 years, recently reviewed by Rathod *et al.* and Sharifi-Rad *et al.*[5,6] Moreover, the European Commission has included carvacrol in the list of

chemical flavors and Food and Drug Administration (FDA) approved carvacrol, together with carvacryl ethyl ether, carvacryl acetate and carvacryl propanoate, considering them safe from a toxicological point of view for their use as additives in food products.[7,8]

Although carvacrol is well known as effective in controlling infection diseases, the molecular mechanisms involved in antimicrobial action are not yet completely elucidated. The antibacterial activity of carvacrol has been attributed to its considerable effects on the structural and functional properties of cytoplasmatic membrane, involving outer- and inner membrane disruption and interaction with membrane proteins and intracellular targets.[9,10] The most recent study by Niu *et al.*[11] reported carvacrol could trigger *Candida albicans* apoptosis, causing membrane disruption, inducing ROS production and mitochondrial dysfunction.

Carvacryl derivatives, either natural or synthetic, have also been employed in biological testing with vast range of activities, such as antibmicrobial,[12–15] antiinflamatory,[16] antioxidant,[17–21] anticancer,[17,19,20] larvicidal,[22,23] antihelmintic,[24,25] also acting as enzyme inhibitors (acetylcholinesterase and butyrylcholinesterase,[26] mushroom tyrosinase,[27] *Mycobacterium tuberculosis* chorismate mutase[28]).

There are also a large number of reports on the synthesized esters of carvacrol and on their biological activities.[15,16,22,23,27,29–36] Antimicrobial assays have evaluated activity of a few carvacryl esters of straight chain lower carboxylic acid homologues and diverse heteroaromatic carboxylic acids.[15,29,30,34,37] Interestingly, versus plentiful data on the synthesis and bioactivity of lower homologues, there are sporadic or no data on carvacryl esters with acid components higher than C_9 (except for C_{12} reported in Bassanetti *et al.*[34]).

In the context of diverse biological activities of carvacrol and rich number of promising studies on carvacrol derivatives, a one-step transformation of phenolic functionality by introducing an acyl group was made. We have obtained a series of 25 compounds (**3a–y**), which, after structural characterization, have been involved in *in vitro* antimicrobial testing. This research is the first comprehensive study of the antifungal activity of the synthesized carvacrol derivatives and the first study on antimicrobial activity of carvacryl ester medium/higher homologues. Along with experimental data we provided *in silico* predictions of physico-chemical, pharmacokinetic and toxicological properties for entire group of the synthetized derivatives. Current paper also complements our work on modifying the phenolic function of a few most active natural biocides found in essential oils.[38,39]

2. Experimental

2. 1. Chemicals

All chemicals used were of analytical reagent grade. Unless specified otherwise, all reagents and standards were purchased from Merck (Darmstadt, Germany).

2. 1. 1. General Synthetic Procedures

Acetyl, benzoyl, palmitoyl, stearoyl and oleoyl chloride were purchased from Sigma-Aldrich and were used directly in the synthesis of carvacryl esters. For other acyl chlorides used in the study we have utilized two synthetic approaches depending on whether the transformed acids had less (together with 2-chloroacetyl and trichloroacetyl chloride)[40] or more than 10 carbon atoms,[41] both described by Lazarević *et al.*[38] and Lazarević *et al.*[39]

The material obtained following the above protocols was used directly in the synthesis of esters that was performed as reported in Paolini *et al.*[42] The synthesis of carvacryl (5-isopropyl-2-methylphenyl) esters **3a–y** is represented in Scheme 1. The obtained esters **3a–y** were purified by column chromatography, stationary phase Silica Gel 60 (70–230 mesh), mobile phase (hexane/diethyl ether, gradient 9:1 to 7:3). Data on yields are given in Table 1.

2. 2. Identification of Synthetized Compounds

2. 2. 1. GC-MS Analysis

MS spectra of samples of the synthesized compounds were recorded on a 7890/7000B GC/MS/MS triple quadrupole system (Agilent Technologies, USA, equipped with a Combi PAL auto sampler). The fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Palo Alto, CA, USA) was used. The injector, source and interface operated at 250, 230 and 300 °C, respectively. The temperature program: from 60 for 5 min isothermal to 300 °C at a heating rate of 8 °C/min and on 300 °C for 5 min isothermal. The solutions in hexane were injected in split ratio 10:1. The carrier gas was helium with a flow of 1.0 mL/min. Post run: back flash for 1.89 min, at 280 °C, with helium at 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 50–650, scan time 0.32 s. Semi-quantitative analysis was carried out directly from peak areas in the GC profile. Linear retention indices (RI) were determined based on the retention times of C_8–C_{40} alkanes run on HP-5MS column, using the above mentioned temperature programme.[43]

2. 2. 2. NMR Analysis

NMR spectra were registered on a Bruker AVANCE 500 spectrometer equipped with a 5 mm broadband reverse probe with field *z*-gradient operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. All NMR spectra were recorded at 298 K in $CDCl_3$ (isotopic enrichment 99.95%) solution. Chemical shifts are reported on the δ (ppm) scale and are relative to the residual $CHCl_3$ signals (7.24 for ^{1}H and 77.0 ppm, central line, for ^{13}C spectra, respectively), and scalar coupling constants are reported in Hertz. The experimental error in the measured ^{1}H–^{1}H coupling constants was ±0.5 Hz. The signals assignment was given by a combination of 1D and 2D COSY, HSQC

and HMBC experiments, using standard Bruker pulse programs. Acquisition parameters for 1D were as follows: ^{1}H spectral width of 5000 Hz and 32k data points providing a digital resolution of ca. 0.305 Hz per point, relaxation delay 2 s; ^{13}C spectral width of 29412 Hz and 64k data points providing a digital resolution of ca. 0.898 Hz per point, relaxation delay 2.5 s. The experiments were performed through standard pulse sequences. gCOSY-45 experiments were acquired with 512 t1 increments; 2048 t2 points; spectral/spectrum width 10.0 ppm. The acquisition data for gHSQC and gHMBC experiments were obtained with 512 t1 increments; 2048 t2 points; spectral/spectrum width 10.0 ppm for ^{1}H and 220 ppm for ^{13}C. Delay values were optimized for $^{1}J_{C,H}$ 140.0 Hz and $^{n}J_{C,H}$ 3.0 Hz. Zero filling in F1 to 1k, p/2 shifted sine-bell squared (for gHSQC) or sinebell (for gHMBC) apodization functions were used for processing.

2. 3. Antimicrobial Activity

2. 3. 1. Microbial Strains

Antimicrobial activity of the synthesized compounds was tested *in vitro* against a panel of laboratory control strains belonging to the American Type Culture Collection Maryland, USA: Gram-positive: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538; Gram-negative: *Escherichia coli* ATCC 8739 and *Salmonela typhimurium* ATCC 14028, fungal organisms: *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. The Gram-negative bacteria Salmonella abony NCTC 6017 was obtained from the National Collection of Type Cultures. All microorganisms were maintained at –20 °C under appropriate conditions and regenerated twice before use in the manipulations.

2. 3. 2. Screening of Antimicrobial Activity

The minimal inhibitory concentration (MIC) of esters was determined based on a broth microdilution method performed in 96-well microtitre plates.[44] The inocula of the bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Dimethyl sulphoxide (10% aqueous solution) was used to dissolve and to dilute samples to the highest concentration to be tested (stock concentrations 2 mg/mL). A serial doubling dilution of the samples was prepared in a 96-well microtiter plate, using the method of Sarker *et al.*,[45] with slight modifications. The minimal bactericidal/fungicidal concentration (MBC/MFC) was evaluated as the lowest concentration of tested samples at which inoculated microorganisms were 99.9% killed. Tests were carried out in triplicate. The procedure is described in detail by Lazarević *et al.*[46]

2. 4. *In Silico* Physico-chemical, Pharmacokinetic, and Toxicological Properties of the Synthetized Compounds

Together with experimental data we provided an *in silico* study on physico-chemical, pharmacokinetic and toxicological properties of the synthesized compounds **3a–y**. *In silico* predictions were accomplished using the Molinspiration,[47] admetSAR,[48] DataWarrior,[49] and Toxtree[50] tools.

3. Results and Discussion

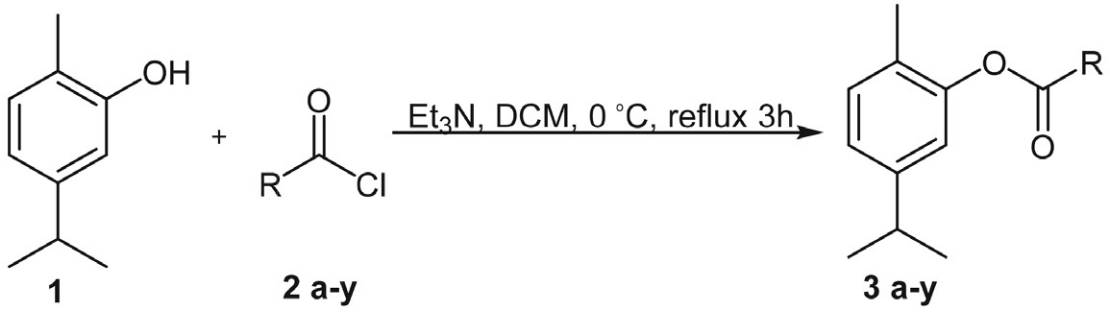
3. 1. Chemical Synthesis

A small focused library of 25 carvacryl esters was synthesized according to previously published standard methodology, given in Scheme 1, with yields ranging from 95 to 52% (Table 1). To the best of our knowledge compounds **3m**, **3o**–**w** are new (Table 1). For solid compounds **3t**, **3v**, **3x** and **3y** melting points were determined in a Stuart Scientific SMP3 apparatus and are uncorrected.

3. 2. Spectral Data on Synthetized Compounds

3. 2. 1. Carvacryl Acetate (3a)[22,30]

Chromatographic purification gave colorless oil. $C_{12}H_{16}O_2$ (M = 192.25); yield 87%; RI (HP5-MS): 1384; ^{1}H



Scheme 1. Synthesis of the assayed esters **3a**–**y**. Reagents and conditions: **2a–y** solution was added dropwise to the solution of **1** and triethylamine (Et_3N), all previously dissolved in dichloromethane (DCM). During the addition, temperature was maintained at 0 °C. After reaching room temperature, the reaction mixture was refluxed for 3 h.[42] For structures and **3a–y** designations, see Table 1.

Table 1. Carvacryl esters **3a**–**y**, mass (g), yield (%) and entry. The reference is related to the previously reported synthesis/antimicrobial research.

Entry	Structure of R in carvacryl esters 3	Mass (g)	Yield (%)	References
3a	CH_3	0.59	87	22, 30
3b	CH_2Cl	0.63	83	22, 27
3c	CCl_3	0.89	91	22, 51
3d	CH_2CH_3	0.59	86	22, 30, 52
3e	$CH{=}CH_2$	0.50	74	53
3f	$CH_2CH_2CH_3$	0.65	89	52, 54
3g	$CH(CH_3)_2$	0.62	85	30
3h	$CH_2(CH_2)_2CH_3$	0.63	82	52, 44
3i	$CH_2CH(CH_3)_2$	0.63	81	30
3j	$CH_2(CH_2)_3CH_3$	0.70	85	23
3k	$CH_2(CH_2)_4CH_3$	0.79	91	52*
3l	$CH_2(CH_2)_5CH_3$	0.78	85	52*
3m	$CH_2(CH_2)_6CH_3$	0.79	82	current study
3n	$CH_2(CH_2)_7CH_3$	0.83	83	
3o	$CH_2(CH_2)_8CH_3$	0.91	87	current study
3p	$CH_2(CH_2)_9CH_3$	0.86	78	34
3q	$CH_2(CH_2)_{10}CH_3$	0.93	81	current study
3r	$CH_2(CH_2)_{11}CH_3$	0.90	76	current study
3s	$CH_2(CH_2)_{12}CH_3$	0.91	74	current study
3t	$CH_2(CH_2)_{13}CH_3$	1.12	92	current study
3u	$CH_2(CH_2)_{14}CH_3$	0.97	73	current study
3v	$CH_2(CH_2)_{15}CH_3$	1.17	85	current study
3w	$CH_2(CH_2)_6CH{=}CHCH_2(CH_2)_6CH_3$	0.71	52	current study
3x	Ph	0.80	95	22, 23, 30, 54
3y	CH_3O-Ph	0.89	95	23

* spectral data are presented in the current paper for the first time

NMR ($CDCl_3$, 500.13 MHz) **δ** 7.14 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 2.90 (spt, *J* = 6.9 Hz, 1H, CH), 2.34 (s, 3H, CH_3) 2.16 (s, 3H, CH_3), 1.25 (d, *J* = 6.9 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 169.3 (C=O), 149.3 (C_{Ar}), 148.1 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.2 (C_{Ar}), 119.8 (C_{Ar}), 33.6 (CH), 23.9 (2 × CH_3), 20.8 (CH_3), 15.8 (CH_3-Ar); MS (EI): *m/z* (%): 192 (M^+) (8.0), 151 (6.4), 150 (55.2), 136 (9.7), 135 (100), 105 (6.8), 105 (5.2), 91 (18.5), 79 (6.6), 77 (9.9), 43 (10.1).

3. 2. 2. Carvacryl 2-Chloroacetate (3b)[22,27]

Chromatographic purification gave colorless oil. $C_{12}H_{15}ClO_2$ (*M* = 226.70); yield 83%; RI (HP5-MS): 1598; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.19 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.08 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar-H), 6.93 (d, *J* = 1.7 Hz, 1H, Ar-H), 4.34 (s, 2H, CH_2), 2.91 (m, 1H, CH), 2.19 (s, 3H, CH_3), 1.26 (d, *J* = 6.9 Hz, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 165.7 (C=O), 148.8 (C_{Ar}), 148.3 (C_{Ar}), 131.1 (C_{Ar}), 126.9 (C_{Ar}), 124.7 (C_{Ar}), 119.3 (C_{Ar}), 40.7 (CH_2), 33.6 (CH), 23.9 (2 × CH_3), 15.7 (CH_3-Ar); MS (EI): *m/z* (%): 226 (M^+) (14.5), 151 (8.2), 150 (80.9), 136 (8.8), 135 (100), 133 (6.5), 105 (10.0), 91 (9.9), 79 (6.8), 77 (18.4).

3. 2. 3. Carvacryl Trichloroacetate (3c)[22,51]

Chromatographic purification gave colorless oil. $C_{12}H_{13}Cl_3O_2$ (*M* = 295.59); yield 91%; RI (HP5-MS): 1731; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.23 (m, 1H, Ar-H), 7.14 (dd, *J* = 8.0 Hz, 1.4 Hz, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 2.95 (spt, *J* = 6.9 Hz, 1H, CH), 2.27 (s, 3H, Ar-CH_3), 1.29 (d, *J* = 6.9 Hz, 6H, $CH(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 160.4 (C=O), 149.0 (C_{Ar}), 148.7 (C_{Ar}), 131.4 (C_{Ar}), 126.8 (C_{Ar}), 125.3 (C_{Ar}), 118.6 (C_{Ar}), 89.9 (C), 33.7 (CH), 23.9 (2 × CH_3), 15.5 (CH_3-Ar); MS (EI): *m/z* (%): 296 (23.0), 294 (24.2), 283 (29.8), 281 (92.1), 279 (M^+) (100.0), 133 (63.1), 117 (35.9), 105 (32.3), 91 (43.6), 77 (21.0).

3. 2. 4. Carvacryl Propanoate (3d)[22,30,52]

Chromatographic purification gave colorless oil. $C_{13}H_{18}O_2$ (*M* = 206.28); yield 86%; RI (HP5-MS): 1479; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.03 (dd, *J* = 7.6, 1.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.93–2.88 (m, 1H, CH), 2.64 (q, 2H, *J* = 7.4 Hz, CH_2), 2.15 (s, 3H, CH_3), 1.33 (t, *J* = 7.4 Hz, 3H, CH_3), 1.26 (d, *J* = 6.9 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125.8 MHz) **δ** 172.7 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 33.6 (CH), 27.7 (CH_2), 23.9 (2 × CH_3), 15.8 (CH_3-Ar), 9.3 (CH_3); MS (EI):

m/z (%): 206 (M^+) (8.3), 151 (7.8), 150 (67.6), 136 (9.9), 135 (100), 133 (6.1), 105 (7.5), 91 (18.6), 79 (6.8), 77 (10.2), 57 (23.7).

3. 2. 5. Carvacryl Acrylate (3e)[53]

Chromatographic purification gave colorless oil. $C_{13}H_{18}O_2$ (*M* = 204.27); yield 74%; RI (HP5-MS): 1466; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.19 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.06 (dd, *J* = 6.2, 1.6 Hz, 1H, Ar-H), 6.95 (s, 1H, Ar-H), 6.65 (dd, *J* = 6.9, 1.3 Hz, 1H, CH_2=CH), 6.4 (dd, *J* = 10.4, 6.9 Hz, 1H, CH=CH_2) 6.05 (dd, *J* = 9.2, 1.4 Hz, 1H, CH_2=CH), 2.88–2.97 (m, 1H, *J* = 6.9 Hz, $CH(CH_3)_2$), 2.18 (s, 3H, CH_3-Ar), 1.28 (d, *J* = 6.9 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 164.3 (C=O), 149.1 (C_{Ar}), 148.1 (C_{Ar}), 132.3 (=C), 130.9 (C_{Ar}), 127.9 (=C<), 127.2 (C_{Ar}), 124.2 (C_{Ar}), 119.8 (C_{Ar}), 33.6 (CH), 23.9 (2 × CH_3), 15.7 (CH_3-Ar); MS (EI): *m/z* (%):204 (M^+) (25.6), 189 (5.6), 150 (44.7), 149 (5.5), 135 (43.6), 105 (7.1), 91 (15.6), 79 (5.8), 77 (9.2), 57 (100).

3. 2. 6. Carvacryl Butanoate (3f)[52,54]

Chromatographic purification gave colorless oil. $C_{14}H_{20}O_2$ (*M* = 220.31); yield 89%; RI (HP5-MS): 1570; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (d, *J* = 8.0 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 2.93–2.88 (m, 1H, CH), 2.59 (t, *J* = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.85 (sxt, *J* = 7.5 Hz, 2H, CH_2), 1.27–1.25 (m, 6H, CH_3), 1.08 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 171.9 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.3 (C_{Ar}), 36.2 (CH_2), 33.6 (CH), 23.9 (2 × CH_3), 18.6 (CH_2), 15.8 (CH_3-Ar), 13.8 (CH_3); MS (EI): *m/z* (%): 220 (M^+) (8.1), 151 (10.6), 150 (91.2), 136 (10.0), 135 (100), 105 (8.6), 91 (19.7), 77 (9.8), 71 (16.8), 43 (19.6).

3. 2. 7. Carvacryl 2-Methylpropanoate (3g)[30]

Chromatographic purification gave colorless oil, $C_{14}H_{20}O_2$ (*M* = 220.31); yield 85%; RI (HP5-MS): 1524; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar--H), 7.04 (dd, *J* = 7.8, 1.9 Hz, 1H, Ar-H), 6.87 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.93–2.84 (m, 2H, CH), 2.16 (s, 3H, CH_3) 1.38 (d, *J* = 6.9 Hz, 6H, 2 × CH_3), 1.26 (d, *J* = 6.9 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 175.3 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.1 (C_{Ar}), 123.9 (C_{Ar}), 119.8 (C_{Ar}), 34.2 (CH), 33.6 (CH), 23.9 (2 × CH_3), 19.1 (2 × CH_3), 15.8 (CH_3-Ar); MS (EI): *m/z* (%): 220 (13.6), 151 (11.1), 150 (100), 136 (8.6), 135 (91.7), 105 (8.5), 91 (18.9), 77 (9.5), 71 (22.8), 43 (32.8).

3. 2. 8. Carvacryl Pentanoate (3h)[52,44]

Chromatographic purification gave colorless oil. $C_{15}H_{22}O_2$ (*M* = 234.34); yield 82%; RI (HP5-MS): 1670; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.04 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.93–2.88 (m, 1H, CH), 2.61 (t, *J* = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.79 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.5 (dq, *J* = 15, 7.4 Hz, 2H, CH_2), 1.26 (d, *J* = 6.9 Hz, 6H, 2 × CH_3), 1.0 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.0 (CH_2), 33.6 (CH), 27.2 (CH_2), 23.9 (2 × CH_3), 22.4 (CH_2), 15.8 (CH_3-Ar), 13.8 (CH_3); MS (EI): *m/z* (%): 234 (6.8), 151 (11.7), 150 (100), 136 (8.5), 135 (86.5), 105 (8.0), 91 (17.2), 85 (11.6), 77 (8.1), 57 (28.9).

3. 2. 9. Carvacryl 3-Methylbutanoate (3i)[30]

Chromatographic purification gave colorless oil. $C_{15}H_{22}O_2$ (*M* = 234.34); yield 81%; RI (HP5-MS): 1619; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.6, 1.7 Hz, 1H, Ar-H), 6.87 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.93–2.88 (m, 1H, CH), 2.49 (d, *J* = 6.9 Hz, 2H, CH_2), 2.34–2.26 (m, 1H, CH), 2.17 (s, 3H, CH_3), 1.26 (d, *J* = 6.9 Hz, 6H, CH_3), 1.11 (d, *J* = 6.6 Hz, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 171.4 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.9 (C_{Ar}), 43.3 (CH_2), 33.6 (CH), 25.8 (CH), 23.9 (2 × CH_3), 22.5 (2 × CH_3), 15.9 (CH_3-Ar); MS (EI): *m/z* (%): 234 (M^+) (8.1), 151 (12.0), 150 (100), 136 (7.9), 135 (80.2), 105 (8.5), 91 (17.9), 85 (13.2), 77 (8.5), 57 (40.3), 41 (7.1).

3. 2. 10. Carvacryl Hexanoate (3j)[23]

Chromatographic purification gave colorless oil, $C_{16}H_{24}O_2$ (*M* = 248.37); yield 85%; RI (HP5-MS): 1770; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.05 (dd, *J* = 7.6, 1.7 Hz, 1H, Ar-H), 6.89 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.94–2.88 (m, 1H, CH), 2.61 (t, *J* = 7.5 Hz, 2H, CH_2), 2.17 (s, 3H, CH_3) 1.85–1.79 (m, 2H, CH_2), 1.48–1.38 (m, 4H, 2 × CH_2), 1.36–1.26 (m, 6H, 2 × CH_3), 0.99–0.91 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.4 (CH_2), 24.8 (CH_2), 23.9 (2 × CH_3), 22,4 (CH_2) 15.8 (CH_3-Ar), 13.9 (CH_3); MS (EI): *m/z* (%) 248 (M^+), 151 (11.9), 150 (100), 135 (73.1), 105 (8.4), 99 (8.8), 91 (17.9), 77 (8.4), 71 (13.1), 55 (8.9), 43 (21.3).

3. 2. 11. Carvacryl Heptanoate (3k)[52]

Chromatographic purification gave colorless oil. $C_{17}H_{26}O_2$ (*M* = 262.39); yield 91%; RI (HP5-MS): 1873; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.9 Hz, 1H, Ar-H), 6.89 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.96–2.88 (m, 1H, CH), 2.61 (t, *J* = 7.5 Hz, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.50–1.44 (m, 2H, CH_2), 1.39–1.37 (m, 4H, 2 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.96–0.91 (m, 3H,

CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.5 (CH_2), 28.9 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.5 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 262 (M^+) 151 (14.3), 150 (100), 135 (67.2), 113 (8.7), 105 (10.4), 91 (22.1), 77 (11.4), 55 (13.6), 43 (31.8), 41 (12.1).

3. 2. 12. Carvacryl Octanoate (3l)[52]

Chromatographic purification gave colorless oil. $C_{18}H_{28}O_2$ (*M* = 276.42); yield 85%; RI (HP5-MS): 1978; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.6 Hz, 1H, Ar-H), 6.89 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.94–2.88 (m, 1H, CH), 2.61 (t, *J* = 7.5 Hz, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.82 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.48–1.31 (m, 8H, 4 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.94 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.7 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.6 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 276 (M^+) 151 (12.3), 150 (100), 135 (52.3), 105 (7.3), 91 (14.4), 77 (6.3), 57 (22.7), 55 (14.0), 43 (11.4), 41 (8.5).

3. 2. 13. Carvacryl Nonanoate (3m)

Chromatographic purification gave colorless oil. $C_{19}H_{30}O_2$ (*M* = 290.45); yield 82%; RI (HP5-MS): 2082; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar-H), 6.9 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.94–2.88 (m, 1H, CH), 2.61(t, *J* = 7.6 Hz, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.82 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.50–1.44 (m, 2H, CH_2), 1.41–1.33 (m, 8H, 4 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.93 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.8 (CH_2), 29.2 (2 × CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22. 7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%): 290 (M^+) 151 (12.2), 150 (100), 136 (4.3), 135 (43.9), 109 (3.9), 91 (6.8), 71 (6.4), 57 (9.0), 55 (7.3), 43 (5.7).

3. 2. 14. Carvacryl Decanoate (3n)

Chromatographic purification gave colorless oil. $C_{20}H_{32}O_2$ (*M* = 304.47); yield 83%; RI (HP5-MS): 2186; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.03 (dd, *J* = 8.0 Hz, 1.4 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 2.93–2.88 (m, 1H, CH), 2.61 (t, *J* = 7.5 Hz, 2H, CH_2), 2.17 (s, 3H, CH_3) 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49–1.29 (m, 14H, 7 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.93 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.5 (CH_2), 29.3 (2 × CH_2), 29.3 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%): 304 (M^+) 151 (12.5), 150 (100), 136 (3.8), 135 (39.0), 109 (4.1), 91 (5.8), 71 (4.5), 57 (5.4), 55 (7.0), 43 (6.1).

3. 2. 15. Carvacryl Undecanoate (3o)

Chromatographic purification gave colorless oil. $C_{21}H_{34}O_2$ (*M* = 318.50); yield 87%; RI (HP5-MS): 2287; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.6, 1.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.9 (quin, *J* = 7. 5 Hz, 1H, CH), 2.6 (t, *J* = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3) 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49–1.28 (m, 16H, 8 × CH_2), 1.26 (d, *J* = 6.9 Hz, 6H, CH_3), 0.92 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 318 (M^+) 151 (13.0), 150 (100), 136 (3.4), 135 (34.9), 109 (4.7), 105 (2.5), 91 (4.6), 57 (6.0), 55 (6.3), 43 (4.9).

3. 2. 16. Carvacryl Dodecanoate (3p)[34]

Chromatographic purification gave colorless oil. $C_{22}H_{36}O_2$ (*M* = 332.53); yield 78%; RI (HP5-MS): 2388; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.6 Hz 1H, Ar-H), 6.88 (d, *J* = 1.4 Hz, 1H, Ar-H), 2.90 (m,1H, CH), 2.60 (t, *J* = 7.6 Hz, 2 H, CH_2), 2.16 (s, 3H, CH_3) 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49–1.43 (m, 2 H, CH_2), 1.4–1.29 (m, 14H, 7 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.92 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.6 (2 × CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 332 (M^+) 151 (12.8), 150 (100), 136 (3.1), 135 (32.1), 109 (5.2), 91 (4.3), 71 (2.6), 57 (7.0), 55 (7.2), 43 (5.2).

3. 2. 17. Carvacryl Tridecanoate (3q)

Chromatographic purification gave colorless oil, $C_{23}H_{38}O_2$ (*M* = 346.55); yield 81%; RI (HP5-MS): 2491; ^1H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.18 (m, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.6 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 2.91 (m, 1H, CH), 2.6 (t, *J* = 7.5 Hz, 2 H, CH_2), 2.16 (s, 3H, CH_3) 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49–1.43 (m, 2H, CH_2), 1.38–1.28 (m, 16H, 8 × CH_2), 1.27 (d, *J* = 6.9 Hz, 6H, CH_3), 0.92 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.7 (2 × CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 25.1 (CH_2), 23.9 (2 x

CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 346 (M^+) 151 (13.3), 150 (100), 136 (2.8), 135 (28.9), 109 (5.2), 91 (3.6), 71 (2.9), 57 (6.4), 55 (6.6), 43 (4.8).

3. 2. 18. Carvacryl Tetradecanoate (3r)

Chromatographic purification gave colorless oil. $C_{24}H_{40}O_2$ (*M* = 360.58); yield 76%; RI (HP5-MS): 2590; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.6 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 2.9 (m, 1H, CH), 2.6 (t, *J* = 7.6 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49-1.43 (m, 2H, CH_2), 1.4-1.28 (m, 18H, 9 × CH_2) 0.91 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.7 (2 × CH_2), 29.6 (2 × CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.9 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 360 (M^+) 151 (12.4), 150 (100), 135 (27.7), 109 (6.0), 91 (4.5), 71 (3.8), 69 (3.7), 57 (9.0), 55 (10.0), 43 (8.3).

3. 2. 19. Carvacryl Pentadecanoate (3s)

Chromatographic purification gave colorless oil. $C_{25}H_{42}O_2$ (*M* = 374.61); yield 74%; RI (HP5-MS): 2694; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* =7.6 Hz, 1H, Ar-H), 7.04 (dd, *J* = 8.0, 1.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 1.7 Hz, 1H, Ar-H), 2.9 (m, 1H, CH), 2.6 (t, *J* = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.49-1.44 (m, 2H, CH_2), 1.4-1.28 (m, 20H, 10 × CH_2), 1.26 (d, *J* = 6.9 Hz, 3H, CH_3), 0.92 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.7 (3 × CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (2 × CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 374 (M^+) 151 (12.5), 150 (100), 135 (25.2), 109 (6.1), 91 (3.9), 71 (3.6), 69 (3.6), 57 (8.6), 55 (9.1), 43 (7.4).

3. 2. 20. Carvacryl Hexadecanoate (3t)

Chromatographic purification gave amorphous white solid. Mp 31–32 °C. $C_{26}H_{44}O_2$ (*M* = 388.63); yield 92%; RI (HP5-MS): 2804; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.7, 1.7 Hz, 1H, Ar-H), 6.88 (d, *J* = 1.6 Hz, 1H, Ar-H), 2.90 (m, 1H, CH), 2.60 (t, *J* = 7.6 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81 (quin, *J* = 7.6 Hz, 2H, CH_2), 1.47-1.28 (bm, 24H, 12 × CH_2), 1.26 (d, *J* = 7.0 Hz, 6H, CH_3), 0.9 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.8 (C_{Ar}), 127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.7 (4 × CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 388 (M^+) 151 (18.7), 150 (100), 136 (2.9), 135 (29.4), 109 (6.8), 71 (2.8), 69 (2.7), 57 (5.5), 55 (5.4), 43 (3.7).

3. 2. 21. Carvacryl Heptadecanoate (3u)

Chromatographic purification gave colorless oil. $C_{27}H_{46}O_2$ (*M* = 402.66); yield 73%; RI (HP5-MS): 2902; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 8.0 Hz, 1H, Ar--H), 7.04 (dd, *J* =7.6, 1.7 Hz, 1H, Ar-H), 6.88 (bs, 1H, Ar--H), 2.9 (m, 1H, CH), 2.60 (t, *J* = 7.6 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.48-1.28 (bm, 26H, 13 × CH_2), 1.26 (d, *J* = 6.9 Hz, 6H, CH_3), 0.92 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.9 (C_{Ar}), 127.2 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 32.0 (CH_2), 29.7 (6 × CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 402 (M^+) 151 (14.0), 150 (100), 135 (21.9), 109 (6.0), 91 (2.6), 71 (2.9), 69 (2.9), 57 (6.4), 55 (6.3), 43 (4.8).

3. 2. 22. Carvacryl Octadecanoate (3v)

Chromatographic purification gave amorphous white solid. Mp 61 °C. $C_{28}H_{48}O_2$ (*M* = 416.69); yield 85%; RI (HP5-MS): 2945; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.04 (dd, *J* = 7.8, 1.9 Hz, 1H, Ar-H), 6.88 (d, *J* =1.8 Hz, 1H, Ar-H), 2.9 (hept, *J* = 6.9 Hz, 1H, CH), 2.6 (t, *J* = 7.6 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.51-1.28 (m, 28H), 1.26 (d, *J* = 6.9 Hz, 6H, 2 × CH_3), 0.92 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.1 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.8 (C_{Ar}), 127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 32.0 (CH_2), 29.7 (7 × CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 416 (M^+), 151 (12.5), 150 (100), 135 (25.6), 109 (7.0), 91 (5.1), 69 (5.5), 57 (13.5), 55 (14.0), 43 (15.9), 41 (5.2).

3. 2. 23. Carvacryl Oleate (3w)

Chromatographic purification gave colorless oil. $C_{28}H_{46}O_2$ (*M* = 414.67); yield 52%; RI (HP5-MS): 2936; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 7.17 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.03 (dd, *J* = 7.8, 1.8 Hz, 1H, Ar-H), 6.87 (d, *J* = 1.8 Hz, 1H, Ar-H), 5.43-5.34 (m, 2H, CH=CH *Z*-configuration), 2.9 (hept, *J* = 7.0 Hz, 1H, CH), 2.6 (t, *J* = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, CH_3), 2.10-2.01 (m, 4H, 2 × CH_2), 1.81 (quin, *J* = 7.5 Hz, 2H, CH_2), 1.61 – 1.53 (m, 2H, CH_2), 1.51-1.41 (m, 2H, CH_2), 1.36 (d, *J* = 7.5 Hz, 6H, 2 × CH_3), 1.33-1.23 (m, 16H, 8 × CH_2), 0.91 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 172.0 (C=O), 149.3 (C_{Ar}), 148.0 (C_{Ar}), 130.8 (C_{Ar}), 130.0 (=C-), 129.7 (=C-),

127.1 (C_{Ar}), 124.0 (C_{Ar}), 119.8 (C_{Ar}), 34.3 (CH_2), 33.6 (CH), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.2 (2 × CH_2), 29.1 (CH_2), 27.7 (CH_2), 27.3 (CH_2), 27.2 (CH_2), 25.1 (CH_2), 23.9 (2 × CH_3), 22.7 (CH_2), 15.8 (CH_3-Ar), 14.1 (CH_3); MS (EI): *m/z* (%) 414.40 (M^+) 151 (12.3), 150 (100), 135 (26.4), 109 (6.1), 83 (4.4), 69 (8.0), 67 (6.3), 57 (4.9), 55 (17.3), 43 (6.1).

3. 2. 24. Carvacryl Benzoate (3x)[22,23,30,54]

Chromatographic purification gave amorphous white solid. Mp 31 °C. $C_{17}H_{18}O_2$ (*M* = 254.33); yield 95%; RI (HP5-MS): 1991; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 8.26 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.68 (m, 1H, Ar-H), 7.56 (m, 2H, Ar-H), 7.23 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.09 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 2.94 (m, 1H, CH), 2.23 (s, 3H, CH_3), 1.29 (d, *J* = 6.9 Hz, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 164.9 (C=O), 149.5 (C_{Ar}), 148.2 (C_{Ar}), 133.5 (C_{Ar}), 130.9 (C_{Ar}), 130.2 (2 × C_{Ar}), 129.6 (C_{Ar}), 129.1 (C_{Ar}) 128.6 (2 × C_{Ar}), 127.4 (C_{Ar}), 124.2 (C_{Ar}), 119.9 (C_{Ar}), 33.6 (CH), 24.0 (2 × CH_3), 15.9 (CH_3-Ar); MS (EI): *m/z* (%): 254 (M^+) (7.3), 106 (7.7), 105 (100), 91 (5.1), 79 (2.1), 78 (3.3), 77 (34.8), 65 (1.2), 51 (6.2), 50 (1.4).

3. 2. 25. Carvacryl 4-Methoxybenzoate (3y)[23]

Chromatographic purification gave amorphous white solid. Mp 31–32 °C. $C_{17}H_{18}O_2$ (*M* = 284.35); yield 95%; RI (HP5-MS): 2302; ^{1}H NMR ($CDCl_3$, 500.13 MHz) **δ** 8.21 (d, *J* = 8.9 Hz, 2H, Ar-H), 7.21 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.07 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.03 (s, 1H, Ar-H), 7.01 (d, 2H, Ar-H), 3.93 (s, 3H, CH_3), 3.51 (q, *J* = 7.0 Hz, 2H contamination EtOAc), 2.93 (m, 1H, CH), 2.21 (s, 3H, CH_3), 1.29 (d, *J* = 6.9 Hz, 6H, 2 × CH_3,), 1.24 (t, *J* = 7.0 Hz, 3H contamination EtOAc); ^{13}C NMR ($CDCl_3$, 125.76 MHz) **δ** 164.6 (C_{Ar}-O),163.8 (C=O), 149.6 (C_{Ar}-OCO), 148.1 (C_{Ar}), 132.2 (2 × C_{Ar}), 130.9 (C_{Ar}), 127.4 (C_{Ar}), 124.0 (C_{Ar}), 122.0 (C_{Ar}), 120.0 (C_{Ar}), 113.9 (2 × C_{Ar}), 55.5 (C-O), 33.6 (CH), 23.9 (2 × CH_3), 15.8 (CH_3-Ar); MS (EI): *m/z* (%): 284 (M^+) (2.6), 136 (8.7), 135 (100), 107 (6.2), 92 (9.0), 91 (3.8), 79 (2.0), 77 (147), 64 (3.4), 63 (2.0).

3. 3. Antimicrobial Activity

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration, which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), using the broth microdilution method. The obtained results are given in Table 2. The assayed samples were less effective than antibiotic/antimycotic used as reference standard, and if observed, activity was never greater than the values acquired for the parent compound **1** (MIC/MBC/MFC never exceeded 0.031 mg/mL). The panel of bacterial strains, represented by Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli*, *S. abony* and *S. typhimurium*) microorganisms, were completely resistant to the synthesized compounds at tested concentration (Table 2), except for compound **3b** that inhibited growth of *B. subtilis* and *S. aureus* at 0.5 and 1 mg/mL, respectively, and inhibited the growth/had cidal effect on *S. typhimurium* at concentrations comparable to **1** (0.50 / 0.25 mg/mL). On the other hand, an interesting experimental fact is that the representatives of the synthesized homologues, regardless of the nature of the R residue, have shown activity on fungal strain *Aspergillus niger* and on yeast *Candida albicans* (for MIC/MFC, see Table 2, entries **3a**–**y**), being antimicrobials comparable to carvacrol.

Compounds **3a**,**d**,**g**,**i**,**x** from our study are matching samples to those tested by Mathela and collaborators,[30] who were making evaluation of antibacterial activity on *Streptococcus mutans* (MTCC 890), *S. aureus* (MTCC 96), *B. subtilis* (MTCC 121), *S. epidermidis* (MTCC 435) and *E. coli* (MTCC 723) and also reported attenuation of the activity in comparison to **1**. Compound **3p** is identical to the sample tested by Bassanetti *et al.*[34] on *E. coli* (isolate and ATCC 25922), *S. typhimurium* (isolate and ATCC 23564), *S. enteritidis* (isolate and ATCC 49220) and *Clostridium perfringens* (isolate and ATCC 13124), with identical observations (regardless of the bacterial strain involved) related to attenuation of the synthetic compound activity in comparison to the parent compound **1**. None of two previous studies did include fungi/yeast strains in antimicrobial bioassays. Moreover, according to the authors' best knowledge and based on the literature search,[55] only one study assaying antifungal (*Botrytis cinerea*) and activity against yeast (*Saccharomyces cerevisiae*), involving only one of the prepared compounds (carvacryl acetate), exists.[37]

Veldhuizen *et al.*[56] comparing **1** with carvacrol-related compounds, indicated structural requirements in exerting antimicrobial activity against pathogenic bacteria such as *E. coli* and *S. aureus*. Further investigations emphasized the correlation between the free-hydroxyl group in the phenolic ring and the antimicrobial potency on ester derivatives obtained by replacing hydroxyl group with acyl moieties. Ultee *et al.*[9] suggested that the crucial role for efficacy of phenolic compounds (*e.g.* carvacrol) is attributed to the presence of OH functional group and to a system of delocalized electrons, allowing compounds to act as proton exchanger, thus reducing the gradient across the cytoplasmic membrane (resulting collapse of the proton-motive force and depletion of the ATP pool lead eventually to cell death, as reported by Ultee *et al.*[9]). The delocalized electron system present in carvacryl derivatives implies that they are proton acceptors, however unable to release a proton through the acyl group to act as a proton exchanger.[37] So far obtained data emphasized that the insertion of acyl groups in the carvacrol aromatic ring results in a weaker antibacterial activity,[57] which was also the result confirmed by Mathela *et al.*[30] and by our current study. However, this single structural modification of phe-

Table 2. The minimal inhibitory (MIC) and minimal bactericidal/fungicidal (MBC/MFC) concentrations of the carvacrol (**1**) and the synthetised **3a–y** esters. The initial concentration of the derivatives applied in broth microdilution assay were 2 mg/mL.

	Bacterial strains					Fungal strains	
	Gram-positive		Gram-negative				
Compound	*B. subtilis*	*S. aureus*	*E. coli*	*S. abony*	*S. typhimurium*	*A. niger*	*C. albicans*
1	MIC = 0.25 MBC = 0.50	MIC = 0.25 MBC = 0.50	MIC = 0.25 MBC = 0.50	MIC = 0.50 MBC = 1.0	MIC = 0.25 MBC = 0.50	MIC = 0.031 MFC = 0.50	MIC = MFC = 0.125
3a	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = MFC = 0.50
3b	MIC = 0.50	MIC = 1.0	na	na	MIC = 0.25 MBC = 0.50	MIC = 0.25 MFC = 0.50	MIC = 0.125 MFC = 0.50
3c	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3d	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3e	na	na	na	na	na	MIC = MFC = 0.50	MIC = 1.0
3f	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3g	na	na	na	na	na	MIC = 0.25 MFC = 1.0	MIC = 1.0
3h	na	na	na	na	na	MIC = 0.25 MFC = 1.0	MIC = 1.0
3i	na	na	na	na	na	MIC = 0.25 MFC = 1.0	MIC = 0.25
3j	na	na	na	na	na	MIC = 0.50 MFC = 1.0	MIC = 0.50
3k	na	na	na	na	na	MIC = MFC = 1.0	MIC = 0.50 MFC = 1.0
3l	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = MFC = 1.0
3m	na	na	na	na	na	MIC = 0.50	MIC = 1.0
3n	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3o	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3p	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 0.50
3q	na	na	na	na	na	MIC = MFC = 0.50	MIC = 0.50 MFC = 1.0
3r	na	na	na	na	na	MIC = MFC = 0.25	MIC = 1.0
3s	na	na	na	na	na	MIC = MFC = 0.25	MIC = 0.25 MFC = 1.0
3t	na	na	na	na	na	MIC = 0.25 MFC = 1.0	MIC = 0.25 MFC = 1.0
3u	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 0.50
3v	na	na	na	na	na	MIC = 0.5	MIC = 0.5

Compound	Bacterial strains: Gram-positive *B. subtilis*	Gram-positive *S. aureus*	Gram-negative *E. coli*	Gram-negative *S. abony*	Gram-negative *S. typhimurium*	Fungal strains: *A. niger*	Fungal strains: *C. albicans*
						MFC = 1.0	MFC = 1.0
3w	na	na	na	na	na	MIC = 0.5 MFC = 1.0	MIC = 0.5 MFC = 1.0
3x	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1.0
3y	na	na	na	na	na	MIC = 0.25 MFC = 0.50	MIC = 1. 0
Positive control (referent standard)							
Doxycycline (μg/mL)	MIC C = MB = 1.56	MIC = 6.25 MBC = 0.78	MIC = MBC = 0.78	MIC = MBC = 12.5	MIC = MBC = 6.25	nt	nt
Nystatin (μg/mL)	nt	nt	nt	nt	nt	MIC = MBC = 6.25	MIC = MBC = 0.78
Negative control (solvent used)							
DMSO 10% aqueous solution		na	na	na	na	na	na na

"na" not active, "nt" not tested, "MIC" minimum inhibitory concentration as the lowest concentration of an antimicrobial agent (synthetic compound) needed to inhibit the visible in vitro growth of a challenge microorganism, "MBC" minimum bactericidal concentration and "MFC" minimum fungicidal concentration; concentrations were evaluated as the lowest concentration at which 99.9% of the inoculated microorganisms were killed.

nolic functionality seems to have different effect on fungi/yeasts (*A. niger* and *C. albicans*) compared to bacteria (Table 2, antibacterial *vs.* antifungal/anticandidal activity). Introducing an acyl group to the carvacrol results in increased lipophilicity of the synthesized compound (Table S1, see octanol–water partition coefficient calculation, represented as m_iLog*P*). Except preserved *A. niger* antifungal potential, for which no remarkable oscillations in values were observed, no significant (balanced) correlations were detected between increased lipophilicity (the chain length) and the antifungal activity (MIC/MFC) among tested carvacryl ester derivatives. Slight (negligible) loss of anti-*A. niger* potential could be observed in those compounds where the parent phenolic (**1**) is substituted with butanoyl, 2-methylpropanoyl, pentanoyl, 3-methylbutanoyl, hexanoyl, octadecanoyl and oleoyl moieties (Table 2. entries **3g**–**k**,**v**,**w**). As for *C. albicans*, the strongest anticandidal activity, except for **1**, was observed for the introduced methanoyl and 2-chloromethanoyl moieties (Table 2, entries **3a**,**b**). There are no striking differences in anticandidal potential of the homologues higher than C_3, and from the Table 2 we can notice that there is anticandidal activity evidenced in all of the synthesized compounds (**3a**–**y**). Interestingly, Damiens *et al.*[36] have observed and stated the importance of (a balanced) hydrophilicity/lipophilicity ratio, though in sesamol derivatives, against a phyto-pathogen fungi *Zymoseptoria tritici* (modulating lipophilicity proved to increase the antifungal biological activity for sesamol derivatives). The phenomena noticed in our research and in Damiens *et al.*[36] does not have to be an isolated incident, it could also be a regularity based on subtle structural changes that would, within certain limits, by enhancing lipophilicity affect bioactivity. It is certain that this aspect deserves further research.

It is interesting to recall and compare the results of the antimicrobial assay we have obtained for acylated thymol (positional isomer of carvacrol) derivatives.[38] Unlike esters of thymol, which effected only growth of *C. albicans*, carvacrol ester derivatives are strongly affecting growth of both, *A. niger* and *C. albicans*, with a more pronounced (cidal) effect on *A. niger*. The stronger effect is most probably related to the orientation/position of the groups in (acylated) positional isomer homologs, and this item could also be worth of further research.

3. 4. *In Silico* Study

3. 4. 1. Physico-chemical Properties

Physico-chemical properties of the studied compounds predicted by the Molinspiration tool[47] are shown in Table S1. It can be seen that **1** and seven synthesized compounds (**3a**,**b**,**d**,**f**,**g**,**e**,**i**) fulfilled all Lipinski's and Veber's rules (m_iLog*P* ≤ 5, TPSA ≤ 140 Å^2, nON ≤ 10, nOHNH ≤ 5, nrotb ≤ 10, M_r ≤ 500) indicating their good oral bioavailability *in vivo*. However, eight compounds (**3c**,**h**,**j**–**m**,**x**,**y**) were predicted with one deviation (m_iLog*P* > 5), and ten compounds (**3n**–**w**) were predicted with two deviations (m_iLog*P* > 5, nrotb > 10) from the Lipinski's and Veber's rules, indicating their poorer bioavalilability.

3. 4. 2. Pharmacokinetic Properties

Absorption properties of the studied compounds predicted by admetSAR[48] are shown in Table S2. All compounds tested were predicted as compounds permeable across Caco-2 cells, capable of being absorbed by intestine, as well as compounds able to pass through blood-brain barrier and penetrate into the central nervous system. None of the tested compounds was predicted as a P-glycoprotein substrate, while a small number (**3r**–**w**,**y**) was predicted as a P-glycoprotein inhibitor.

Metabolic properties of the studied compounds predicted by admetSAR[48] are shown in Table S3. According to the results, the tested compounds differ from each other in their metabolic properties, depending on whether they are potential substrates and/or inhibitors of certain CYP450 isoenzymes. No compound was predicted as CYP450 3A4 substrate or CYP450 2D6/3A4 inhibitor. Only the parent compound **1** was predicted as CYP450 2C9/2D6 substrate. The only compound envisaged as CYP450 2C9 inhibitor is **3x**. Most compounds (19 of 25) are potential inhibitors of CYP450 2C19, and all are potential inhibitors of CYP450 1A2.

3. 4. 3. Toxicological Properties

Toxicological properties of the studied compounds predicted by DataWarrior[49] are shown in Table S4. It can be seen that most of the studied compounds were predicted as non-mutagenic, non-tumorigenic and non-reproductive effective (22, 21 and 22 out of 25 compounds, respectively). Compounds **3b**,**c**,**e** were predicted as highly mutagenic, compounds **3b** and **3c** and highly reproductive effective, while compounds **3b**,**e**,**j** were predicted as highly tumorigenic. All 25 compounds tested were predicted as highly irritant. The results obtained by predicting organ toxicity, organ system toxicity, genotoxicity and ecotoxicity of the studied compounds using admetSAR[48] are shown in Tables S5–S8.

Most of the studied compounds were predicted as potentially non-hepatotoxic, with no risk of eye corrosion or eye irritation, but with the possibility of human ether-à-go-go inhibition. According to the risk of acute oral toxicity, the studied compounds were predicted as category III, or slightly toxic compounds, with LD50 values of 500–5000 mg/kg. Only one compound (**3b**) was predicted as category II, or moderately toxic compound, with LD50 value of 50–500 mg/kg (Table S5).

The results obtained by predicting the compound ability to interact with the hormonal system showed that the studied compounds have low predispositions for estrogen receptor, aromatase and glucocorticoid receptor binding, slightly higher predispositions for thyroid receptor binding and high predispositions for peroxisome proliferator-activated receptor γ binding (Table S6).

Regarding genotoxicity, all of the studied compounds were predicted as non-genotoxic (Table S7), and regarding ecotoxicity, all compounds tested were predicted as non-toxic to avian, but toxic to fish, honey bee and *Tetrahymena pyriformis*. More than a half were predicted as toxic to crustaceans. Finally, the majority was predicted as biodegradable compounds (Table S8).

Structural alerts for DNA and protein binding for the studied compounds, predicted by Toxtree,[50] are presented in Table S9. All of the compounds tested showed at least one structural alert for DNA or protein binding.

4. Conclusion

By chemical synthesis, we have obtained a series of 25 esters, among which 10 compounds are reported for the first time. All of the synthesized compounds were employed in antimicrobial bioassay, exhibiting the greatest activity on fungal strain *A. niger* and on yeast *C. albicans*, where was found that all could be antimicrobials, comparable to carvacrol, and can also be considered as activity holders. While the phenolic hydroxyl group of carvacrol is essential for action against bacteria, it seems that lipophilicity plays an important role in antifungal activity. The pronounced antimicrobial selectivity is certainly a subject deserving more thorough examination either through the mechanism of action or through a greater number of diverse compounds involved in establishing a detailed structure-activity correlation.

Based on our *in silico* study seven compounds (**1** and **3a,b,d,f,g,e,i**) fulfilled all Lipinski's and Veber's rules and were predicted to have good oral bioavailability. All compounds were recognized as compounds able to pass through blood-brain barrier, capable of being absorbed by intestine and permeable across Caco-2 cells. Metabolic properties differ within the studied compounds, depending on whether they act as substrates and/or inhibitors of various CYP450 enzymes. All compounds were predicted as non-genotoxic, and most were predicted as non-mutagenic, non-tumorigenic, non-reproductive effective and non-hepatotoxic. Regarding the risk of acute oral toxicity, they were predicted as slightly toxic compounds. However, some of the compounds showed predispositions to act as potential endocrine disruptors, and all of them showed at least one structural alert for DNA or protein binding.

Taking in consideration the overall results, carvacryl esters are another type of phenolics that, from the aspect of enhanced lipophilicity (improved membrane permeability), could be useful in fungal control.

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5. References

1. T. Rodrigues, D. Reker, P. Schneider, G. Schneider, *Nat. Chem.* **2016**, *8*, 531–541. **DOI:**10.1038/nchem.2479
2. S. Bernardini, A. Tiezzi, V. Laghezza Masci, E. Ovidi, *Nat. Prod. Res.* **2018**, *32*, 1926–1950. **DOI:**10.1080/14786419.2017.1356838
3. A. G. Atanasov, S. B. Zotchev, V. M. Dirsch, *Nat. Rev. Drug. Discov.* **2021**, *20*, 200–216. **DOI:**10.1038/s41573-020-00114-z
4. K. H. C. Baser, *Curr. Pharm. Des.* **2008**, *14*, 3106–3119. **DOI:**10.2174/138161208786404227
5. N. B. Rathod, P. Kulawik, F. Ozogul, J. M. Regenstein, Y. Ozogul, *Trends Food Sci. Tech.* **2021**, *116*, 733–748. **DOI:**10.1016/j.tifs.2021.08.023
6. M. Sharifi-Rad, E. M. Varoni, M. Iriti, M. Martorell, W. N. Setzer, M. Del Mar Contreras, B. Salehi, A. Soltani-Nejad, S. Rajabi, M. Tajbakhsh, J. Sharifi-Rad, *Phytother Res.* **2018**, *32*, 1675–1687. **DOI:**10.1002/ptr.6103
7. Commission European (**2012**) COMMISSION IMPLEMENTING REGULATION (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by regulation (EC) No 2232/96 of the European parliament and of the council, introducing it in Annex I to Regulation (EC) No 1334. Off J Eur Union L 267:161.
8. FDA (**2017**) CFR-Code of federal regulations title 21 FOOD AND DRUGS, volume 3 CITE: 21CFR172.515. CHAPTER I - FOOD AND DRUG ADMINISTRATION DEPARTMENT OF HEALTH AND HUMAN SERVICES SUBCHAPTER B - FOOD FOR HUMAN CONSUMPTION (CONTINUED) PART 172 - FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION.
9. A. Ultee, M. H. J. Bennik, R. Moezelaar, *Appl. Environ. Microbiol.* **2002**; *68*, 1561–1568. **DOI:**10.1128/AEM.68.4.1561-1568.2002
10. A. Ait-Ouazzou, L. Espina, T. K. Gelaw, S. de Lamo-Castellví, R. Pagán, D. García-Gonzalo, *J. Appl. Microbiol.* **2013**, *114*, 173–185. **DOI:**10.1111/jam.12028
11. C. Niu, C. Wang, Y. Yang, R. Chen, J. Zhang, H. Chen, Y. Zhuge, J. Li, J. Cheng, K. Xu, M. Chu, C. Ren, C. Zhang, C. Jia, *Front. Cell. Infect. Microbiol.* **2020**, *10*, 192. **DOI:**10.3389/fcimb.2020.00192
12. L. Marinelli, A. Di Stefano, I. Cacciatore, *Phytochem. Rev.* **2018**, *17*, 903–921. **DOI:**10.1007/s11101-018-9569-x
13. P. F. Pinheiro, L. A. P. Menini, P. C. Bernardes, S. H. Saraiva, J. W. M. Carneiro, A. V. Costa AV, T. R. Arruda, M. R. Lage, P. M. Gonçalves, C. O. Bernardes, E. S. Alvarenga, L. Menini, *J. Agric. Food Chem.* **2018**, *66*, 323–330. **DOI:**10.1021/acs.jafc.7b04418
14. J. U. Patil, K. C. Suryawanshi, P. B. Patil, S. R. Chaudhary, N. S. Pawar, *J. Asian Nat. Prod. Res.* **2010**, *12*, 129–133. **DOI:**10.1080/10286020903455907
15. K. Wang, S. Jiang, Y. Yang, L. Fan, F. Su, M. Ye, *Nat. Prod. Res.* **2019**, *33*, 1924–1930. **DOI:**10.1080/14786419.2018.1480618
16. S. R. B. Damasceno, F. R. A. M. Oliveira, N. S. Carvalho, C. F. C. Brito, I. S. Silva, F. B. M. Sousa, R. O. Silva, D. P. Sousa, A. L. R. Barbosa, R. M. Freitas, J.-V. R. Medeiros, *Life Sci.* **2014**, *94*, 58–66. **DOI:**10.1016/j.lfs.2013.11.001
17. J. Mastelić, I. Jerković, I. Blazević, M. Poljak-Blazi, S. Borović, I. Ivancić-Baće, V. Smrecki, N. Zarković, K. Brcić-Kostic, D. Vikić-Topić, N. Müller, *J. Agric. Food Chem.* **2008**, *56*, 3989–3996. **DOI:**10.1021/jf073272v
18. E. M. Alvarenga, N. A. Sousa, S. de Araújo, J. L. P. Júnior, A. R. Araújo, B. Iles, D. M. Pacífico, G. A. C. Brito, E. P. Souza, D. P. Sousa, J. V. R. Medeiros, *J. Pharm. Pharmacol.* **2017**, 69, 1773–1785. **DOI:**10.1111/jphp.12818
19. J. D. Rajput, S. D. Bagul, R. M. Bendre, *Res. Chem. Intermed.* **2017a**, *43*, 4893–4906. **DOI:**10.1007/s11164-017-2919-2
20 J. D. Rajput, S. D. Bagul, A. A. Hosamani, M. M. Patil, R. S. Bendre, *Res. Chem. Intermed.* **2017b**, *43*, 5377–5393. **DOI:**10.1007/s11164-017-2933-4
21. M. M. de Souza, M. C. Andreolla, T. C. Ribeiro, A. E. Gonçalves, A. R. Medeiros, A. S. de Souza, L. L. G. Ferreira, A. D. Andricopulo, R. A. Yunesc, A. S. de Oliveira, *RSC Med. Chem.* **2020**, *11*, 307–316. **DOI:**10.1039/D0MD00009D
22. V. B. Silva, D. L. Travassos, A. Nepel, A. Barison, E. V. Costa, L. Scotti, M. T. Scotti, F. J. B. Mendonça-Junior, R. La Corte Dos Santos, S. C. de Holanda Cavalcanti, *J. Arthropod. Borne Dis.* **2017**, *11*, 315–330.
23. B. M. de Mesquita, P. G. G. do Nascimento, L. G. S. Souza, I. F. de Farias, R. A. C. da Silva, T. L. G. de Lemos, F. J. Q. Monte, M. T. S. Trevisan, H. C. da Silva, G. M. P. Santiago, *Quím. Nova.* **2018**, *41*, 412–416. **DOI:**10.21577/0100-4042.20170189
24. W. P. P. Andre, W. L. C. Ribeiro, G. S. Cavalcante, J. M. L. dos Santos, I. T. F. Macedo, H. C. B. de Paula, R. M. de Freitas, S. M. de Morais, J. V. de Melo, C. M. L. Bevilaqua, *Vet. Parasitol.* **2016**, *218*, 52–58. **DOI:**10.1016/j.vetpar.2016.01.001
25. W. P. P. André, J. R. de Paiva Junior, G. S. Cavalcante, W. L. C. Ribeiro, J. V. de Araújo Filho, J. M. L. dos Santos, A. P. N. N. Alves, J. P. Monteiro, S. M. de Morais, I. N. G. da Silva, L. M. B. de Oliveira, F. O. M. da Silva Abreu, C. M. L. Bevilaqua, *Rev. Bras. Parasitol. Vet.* **2020**, *29*, e013119. **DOI:**10.1590/s1984-29612019098
26. B. Z. Kurt, I. Gazioglu, A. Dag, R. E. Salmas, G. Kayık, S. Durdagi, F. Sonmez, *Bioorg. Med. Chem.* **2017**, *25*, 1352–1363. **DOI:**10.1016/j.bmc.2016.12.037
27. Z. Ashraf, M. Rafiq, H. Nadeem, M. Hassan, S. Afzal, M. Waseem, K. Afzal, J. Latip, *PLoS One* **2017**, *12*, e0178069. **DOI:**10.1371/journal.pone.0178069
28. R. Alokam, V. U. Jeankumar, J. Padma Sridevi, S. Sai Matikonda, S. Peddi, M. Alvala, P. Yogeeswari, D. Sriram, *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 547–554. **DOI:**10.3109/14756366.2013.823958
29. V. P. Nikumbh, V. S. Tare, P. P. Mahulikar, *JSIR* **2003**, *62*, 1086–1089. **DOI:**10.1093/jnen/62.10.1086
30. C. S. Mathela, K. K. Singh, V. K. Gupta, *Acta Pol. Pharm.* **2010**, *67*, 375–380.
31. L. F. Pires, L. M. Costa, O. A. Silva, A. A. de Almeida, G. S. Cerqueira, D. P. de Sousa, R. M. de Freitas, *Pharmacol. Biochem. Behav.* **2013**, *112*, 42–48. **DOI:**10.1016/j.pbb.2013.09.001
32. M. T. de Santana, V. B. Silva, R. G. de Brito, dos Santos PL, S.

C. de Holanda Cavalcanti, E. O. Barreto, J. N. de Souza Ferro, M. R. V. dos Santos, A. A. de Sousa Araújo, L. J. Quintans-Júnior, *Inflammation* **2014**, *37*, 1575–1587. **DOI:**10.1007/s10753-014-9884-3
33. L. F. Pires, L. M. Costa, A. A. de Almeida, O. A. Silva, G. Santos Cerqueira, D. P. de Sousa, R. M. Pires, P. Satyal, R. M. de Freitas, *Chem.-Biol. Interact.* **2015**, *226*, 49–57. **DOI:**10.1016/j.cbi.2014.12.001
34. I. Bassanetti, M. Carcelli, A. Buschini, S. Montalbano, G. Leonardi, P. Pelagatti, G. Tosi, P. Massi, L. Fiorentini, D. Rogolino, *Food Cont.* **2017**, *73*, 606–612. **DOI:**10.1016/j.foodcont.2016.09.010
35. Y, Song, L. B. Wang, Y. Bei , D.-X. Qin, L.-Y. Ai, Q.-Z. Maa, P.-Y. Lina, *Food Funct.* **2020**, *11*, 1754–1763. **DOI:**10.1039/C9FO02037C
36. A. Damiens, M. T. Alebrahim, E. Léonard, A. Fayeulle, C. Furman, J.-L. Hilbert, A. Siah, M. Billamboz, *Pest. Manag. Sci.* **2021**, *77*, 2403–2414. **DOI:**10.1002/ps.6269
37. A. Ben Arfa, S. Combes, L. Preziosi-Belloy, N. Gontard, P. Chalier, *Lett. Appl. Microbiol.* **2006**, *43*, 149–154. **DOI:**10.1111/j.1472-765X.2006.01938.x
38. J. Lazarević, A. Kolarević, A. Đorđević, G. Stojanović, A. Šmelcerović, P. Ciuffreda, E. Santaniello, *Acta Chim. Slov.* **2017**, *64*, 603–612. **DOI:**10.17344/acsi.2017.3356
39. J. Lazarević, A. Kolarević, G. Stojanović, A. Šmelcerović, P. Ciuffreda, E. Santaniello, *Acta Chim. Slov.* **2018**, *65*, 801–810. **DOI:**10.17344/acsi.2018.4380
40. H. C. Brown, *J. Am. Chem. Soc.* **1938**, *60*, 1325–1328. **DOI:**10.1021/jo701630a
41. W. G. Rose, *J. Am. Chem. Soc.* **1947**, *69*, 1384–1387. **DOI:**10.1021/ja01198a043
42. J. Paolini, A. Muselli, A. F. Bernardini, A. Bighelli, J. Casanova, J. Costa, *Flavour Fragr. J.* **2007**, *22*, 479–487. **DOI:**10.1016/j.chroma.2005.03.131
43. H. Van den Dool, P. D. Kratz, *J. Chromatogr. A* **1963**, *11*, 463–471. **DOI:**10.1016/S0021-9673(01)80947-X
44. NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: Eleventh Informational Supplement, M100-S11. **2003**, National Committee for Clinical Laboratory Standards, Wayne, PA.
45. S. A. Sarker, L. Nahar, Y. Kumarasamy, *Methods* **2007**, *42*, 321–324. **DOI:**10.1016/j.ymeth.2007.01.006
46. J. Lazarević, A. Đorđević, D. Kitić, B. Zlatković, G. Stojanović, *Chem. Biodivers.* **2013**, *10*, 1335–1349. **DOI:**10.1002/cbdv.201200332
47. Molinspiration Cheminformatics, www.molinspiration.com; Accessed May 2020.
48. admetSAR, http://lmmd.ecust.edu.cn/admetsar2; Accessed June 2020.
49. DataWarrior, www.openmolecules.org/datawarrior; Accessed May 2020.
50. Toxtree, v.2.6.13; Accessed May 2020.
51. P. J. Rice, J. R. Coats, *J. Econ. Entomol.* **1994**, *87*, 1172–1179. **DOI:**10.1093/jee/87.5.1172
52. A. B. Sen, S. S. Parmar, *J. Indian Chem. Soc.* **1953**, *30*, 61–63.
53. F. J. Xavier, K. A. Rodrigues, R. G. de Oliveira, C. G. Lima Junior, J. da Câmara Rocha, T. S. L. Keesen, M. R. de Oliveira, F. P. L. Silva, M. L. A. de Almeida Vasconcellos, *Molecules* **2016**, *21*, 1483. **DOI:**10.3390/molecules21111483
54. M. A. M. Mansilla, C. A. R. Vasquez, K. S. Rikardsen, K. Fyrand, B. Wrege, B. O. Hjelstuen, Anti-sea lice compositions and their use, WO2012/001668, PTC/IB2011/052922.
55. SciFinder. Chemical Abstracts Service, n.d. https://scifinder.cas.org; Accessed December 12, 2021.
56. E. J. A. Veldhuizen, J. L. M. Tjeerdsma-Van Bokhoven, C. Zweijtzer, S. A. Burt, H. P. Haagsman, *J. Agric. Food Chem.* **2006**, *54*, 1874–1879. **DOI:**10.1021/jf052564y
57. A. Marchese, C. R. Arciola, E. Coppo, R. Barbieri, D. Barreca, S. Chebaibi, E. Sobarzo-Sánchez, S. F. Nabavi, S. M. Nabavi, M. Daglia, *Biofouling.* **2018**, *34*, 630–656. **DOI:**10.1080/08927014.2018.1480756

Povzetek

Iz literature so poznane kemijske modifikacije naravnih monoterpenoidov v različne derivate, kar lahko okrepi njihove biološke aktivnosti v primerjavi z matičnimi spojinami. Skladno s tem smo karvakrol, znan biocid in dodatek k hrani, uporabili kot ogrodje za uvedbo acilne skupine na prvotno fenolno skupino. S to enostavno metodologijo smo pripravili majhno serijo 25 estrov. Za vsako pripravljeno spojino smo izvedli strukturno karakterizacijo, določili *in vitro* antimikrobno učinkovitost ter *in silico* izračunali nekatere fizikalnokemijske, farmakokinetične ter toksikološke lastnosti. Čeprav obstajajo mnogi podatki o sintezah in bioaktivnostih nižjih karvakrolnih estrskih homologov, so podatki o estrih z daljšimi karboksilnimi kislinami (več kot C_9), zelo redki; izmed 25 spojin jih je kar 10 opisanih prvič (spektroskopske karakterizacije pa so prvič opisane za 12 spojin). Naša raziskava predstavlja prvo podrobno študijo karvakrolnih estrov kot učinkovin proti glivam ter prvo, kjer so karvakrolni estri, sestavljeni iz srednjedolgih ali dolgih verig maščobnih kislin, izkazovali antibakterijske aktivnosti. Zanimivo je, da vse pripravljene spojine, ne glede na naravo ostanka R, izkazujejo aktivnost proti glivi *Aspergilus niger* ter proti kvasovki *Candida albicans*, ki je primerljiva z aktivnostjo karvakrola. Poleg predstavljenih eksperimentalnih podatkov, je tudi uporaba *in silico* računskih metod za določanje fizikalnokemijskih, farmakokinetičnih ter toksikoloških lastnosti pripravljenih spojin, pomembna informacija za nadaljnje raziskave.