

Scientific paper

Synthesis, Antimicrobial Activity and *in silico* Studies on Eugenol Eters

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Received: 28-03-2018

Abstract

The results presented herein represent our continued study based on the modification of phenolic functionality in molecules originated from natural sources by acylation. A small focused library of nineteen eugenyl esters, with four of which are new compounds, is reported. All compounds were subjected to *in vitro* antimicrobial testing. *In silico* studies were carried out calculating physico-chemical, pharmacokinetic and toxicological properties, providing more data as additional guidance for further research.

Keywords: Chemical synthesis; eugenyl esters; *in vitro* antimicrobial activity; *in silico* calculation

1. Introduction

Natural products, especially those derived from plants, represent an excellent source of new bioactive compounds since their structures may be used as natural pharmacophores enabling structural derivatization.¹ Phenylpropenes belong to a vast family of compounds called phenylpropanoids that are biosynthesized in plants from the amino acids phenylalanine and tyrosine *via* shikimate pathway.² Phenylpropenes, such as eugenol, isoeugenol, chavicol and anethole are biosynthesized by flowering plants as defense compounds against animals and microorganisms.³ Because of the antimicrobial properties of phenylpropenes, as well as their pleasant aromas and flavors, since ancient times humans have used plant materials containing such compounds to preserve and flavor their food (cloves, allspice, black pepper, anise, fennel; all of these containing significant amounts of phenylpropenes) and as medicinal agents.³

Eugenol (4-allyl-2-methoxyphenol) is naturally occurring in essential oils of variety of plants belonging to

Lamiaceae, Lauraceae, Myrtaceae and Myristicaceae families. It is one of the major constituents of clove (*Syzygium aromaticum* (L.) Merrill & Perry, Myrtaceae) oil and is largely used both in foods and as flavouring agent.^{4,5} Eugenol and its close analogues have several proven biological activities^{6–16} which make it useful in pharmaceutical production. Additionally, eugenol has been described as an agent with dual effect: antioxidant and pro-oxidant, postulating the existence of beneficial properties in both prevention of cancer formation and in cancer treatment.¹⁷ According to previous *in vitro* and *in vivo* studies eugenol might function as a tumor suppressor in most cancer types inspected.^{17–20}

The activity of eugenol against microorganisms is also well known.^{21,22} The mechanisms of antimicrobial action have been documented in both bacteria and fungi.^{21,23–25} According to a review discussing mechanisms of antimicrobial action of eugenol and essential oils containing eugenol, two main uses of eugenol are particularly indicated: against food-decaying microorganisms and

against bacteria causing oral clinical conditions.²¹ In a review published by Freires *et al.*²⁶ it was concluded that due to its promising antimicrobial activity against streptococci, eugenol should be considered as an anti-cariogenic agent worth of further clinical testing. The mode of action of eugenol against microbials has been thoroughly investigated and reviewed by Marchese *et al.*²¹ In this paper a systematic presentation of the mechanisms against bacteria and fungi known so far is given. Compounds obtained by derivatisation of eugenol have also been reported as antimicrobial agents.^{1,27–30} So far, the most frequently investigated eugenol derivatives in bioassays are glycosides^{27–30} and esters.^{31–33} Like eugenol, eugenyl esters can also represent compounds of promising biological properties. Several papers on isolation and/or synthesis and biological activity of eugenyl esters have reported wide range of activities: antioxidant,^{9,11,13,34} anticancer,³⁵ larvicidal,³⁷ anti-leishmaniasis.³⁸ On the other hand, little data exists on antimicrobial activity, reporting only few compounds involved with respect to the total number synthesized. Antimicrobial assays evaluated antibacterial activity of eugenyl esters of acetic, 3-methylbutanoic, pentanoic and benzoic acids,^{31–33} but no data related to antifungal activity of such derivatives was reported.

The diverse pharmacological activity of eugenol and eugenyl esters, scarce results on antibacterial and no results on antifungal activity of ester derivatives, have determined the aim of our study. By making a modification of a phenolic functionality in eugenol, we have obtained a series of ester compounds (**3a–s**), performed their structural characterization, *in vitro* antimicrobial testing and *in silico* calculation of physico-chemical, pharmacokinetic and toxicological properties. An important contribution of this study is the synthesis of four new compounds (**3l**, **3n**, **3p** and **3r**), followed by the results obtained in antibacterial together with the first results ever obtained in an antifungal assay, and by *in silico* calculations. All together represent an important aspect to guide further research.

2. Materials and Methods

2.1. Chemicals Used

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

2.2. General Synthetic Procedures

Acetyl, benzoyl and palmitoyl chloride (**2a**, **2r**, **2s**) were purchased from Sigma-Aldrich. For the preparation of acyl chlorides **2b–p**, **2r** and of eugenyl esters (Table 1, entries **3a–s**) methods from the literature^{39–42} were utilized and general procedures for the synthesis as reported

in our previously published paper was followed.⁴³ Scheme 1 represents the synthesis of eugenyl esters.

The obtained esters of eugenol **3a–s** were purified by column chromatography, stationary phase Silica Gel 60 (70–230 mesh), mobile phase (hexane/diethyl ether, gradient 9:1 to 6:4). For yields see Table 1. For solid compounds **3q**, **3r** and **3s** melting points were determined in a Stuart Scientific SMP3 apparatus and are uncorrected.

Eugenyl Acetate (3a)

Chromatographic purification gave yellowish oil. C₁₂H₁₄O₃ (*M* = 206.24); yield 96%; MS (EI): *m/z* (%) 206 (M⁺), 164 (100), 149 (27.23), 133 (15.32), 131 (20.72), 121 (11.81), 104 (16.72), 103 (18.52), 91 (23.22), 77 (17.02), 43 (14.18); RI (HP5-MS): 1538; ¹H NMR (500.13 MHz, CDCl₃): δ 6.98 (1H, d, *J* = 8 Hz, **H-6**), 6.77 (2H, m, **H-3** and **H-5**), 6.04 (1H, m, **H-9**), 5.13 (2H, m, **H-10**), 3.85 (3H, s, **H-7**), 3.41 (2H, d, *J* = 6 Hz, **H-8**), 2.34 (3H, s, **H-12**); ¹³C NMR (125.76 MHz, CDCl₃): δ 169.28 (**C-11**), 150.88 (**C-2**), 139.05 (**C-1**), 138.00 (**C-4**), 137.06 (**C-9**), 122.53 (**C-6**), 120.69 (**C-5**), 116.19 (**C-10**), 112.73 (**C-3**), 55.84 (**C-7**), 40.12 (**C-8**), 20.71 (**C-12**). Physical and spectroscopic data were in accordance with those reported.^{37,38}

Eugenyl Propanoate (3b)

Chromatographic purification gave yellowish oil. C₁₃H₁₆O₃ (*M* = 220.26); yield 89%; MS (EI): *m/z* (%) 220 (M⁺), 165 (16.52), 164 (100), 149 (23.02), 133 (12.01), 132 (9.11), 131 (13.91), 104 (10.11), 103 (10.51), 91 (13.21), 57 (14.01); RI (HP5-MS): 1635; ¹H NMR (500.13 MHz, CDCl₃): δ 6.98 (1H, d, *J* = 8 Hz, **H-6**), 6.70–6.90 (2H, m, **H-3** and **H-5**), 5.90–6.07 (1H, m, **H-9**), 5.04–5.23 (2H, m, **H-10**), 3.84 (3H, s, **H-7**), 3.41 (2H, d, *J* = 6 Hz, **H-8**), 2.64 (2H, q, *J* = 8 Hz, **H-12**), 1.31 (3H, t, *J* = 7 Hz, **H-13**); ¹³C NMR (125.76 MHz, CDCl₃): δ 172.72 (**C-11**), 150.96 (**C-2**), 138.87 (**C-1**), 138.13 (**C-4**), 137.13 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.14 (**C-10**), 112.75 (**C-3**), 55.84 (**C-7**), 40.12 (**C-8**), 27.39 (**C-12**), 9.23 (**C-13**). Physical and spectroscopic data were in accordance with those reported.^{37,38}

Eugenyl Butanoate (3c)

Chromatographic purification gave colorless oil. C₁₄H₁₈O₃ (*M* = 234.29); yield 87%; MS (EI): *m/z* (%) 234 (M⁺), 165 (10.91), 164 (100), 149 (19.02), 133 (10.01), 132 (7.91), 131 (11.21), 104 (8.21), 103 (8.41), 91 (10.31), 43 (8.41); RI (HP5-MS): 1729; ¹H NMR (500.13 MHz, CDCl₃): δ 6.97 (1H, d, *J* = 8 Hz, **H-6**), 6.77–6.84 (2H, m, **H-3** and **H-5**), 5.94–6.04 (1H, m, **H-9**), 5.09–5.17 (2H, m, **H-10**), 3.83 (3H, s, **H-7**), 3.41 (2H, d, *J* = 6 Hz, **H-8**), 2.59 (2H, t, *J* = 7 Hz, **H-12**), 1.83 (2H, spt, *J* = 7 Hz, **H-13**), 1.08 (3H, t, *J* = 7 Hz, **H-14**); ¹³C NMR (125.76 MHz, CDCl₃): δ 171.89 (**C-11**), 150.93 (**C-2**), 138.87 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.12 (**C-8**), 35.94 (**C-12**), 18.62 (**C-13**), 13.62 (**C-14**). Physical and spectroscopic data were in accordance with those reported.^{11,38}

Eugenyl 2-Methylpropanoate (3d)

Chromatographic purification gave colorless oil. $C_{14}H_{18}O_3$ ($M = 234.29$); yield 76%; MS (EI): m/z (%) 234 (M^+), 165 (11.11), 164 (100), 149 (16.72), 133 (8.71), 131 (10.01), 103 (8.71), 91 (10.61), 77 (7.71), 71 (7.81), 43 (8.41); RI (HP5-MS): 1679; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.94–7.03 (1H, m, **H-6**), 6.77–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.03 (1H, m, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **H-7**), 3.41 (2H, d, $J = 6.24$ Hz, **H-8**), 2.86 (2H, spt, $J = 6.9$ Hz, **H-12**), 1.35 (6H, d, $J = 6.9$ Hz, $2 \times CH_3$: **H-13** and **H-14**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 175.40 (**C-11**), 150.97 (**C-2**), 138.76 (**C-1**), 138.25 (**C-4**), 137.16 (**C-9**), 122.49 (**C-6**), 120.68 (**C-5**), 116.08 (**C-10**), 112.80 (**C-3**), 55.87 (**C-7**), 40.11 (**C-8**), 34.00 (**C-12**), 19.08 ($2 \times CH_3$: **C-13** and **C-14**). Physical and spectroscopic data were in accordance with those reported.^{33,44}

Eugenyl Pentanoate (3e)

Chromatographic purification gave colorless oil. $C_{15}H_{20}O_3$ ($M = 248.32$); yield 85%; MS (EI): m/z (%) 248 (M^+), 165 (11.01), 164 (100), 149 (15.82), 133 (8.81), 131 (9.61), 104 (7.41), 103 (7.51), 91 (9.31), 57 (14.61), 55 (6.71); RI (HP5-MS): 1829; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.96 (1H, d, $J = 8$ Hz, **H-6**), 6.76–6.83 (2H, m, **H-3** and **H-5**), 5.94–6.03 (1H, m, **H-9**), 5.09–5.16 (2H, m, **H-10**), 3.83 (3H, s, **H-7**), 3.40 (2H, d, $J = 6$ Hz, **H-8**), 2.60 (2H, t, $J = 8$ Hz, **H-12**), 1.78 (2H, quin, $J = 7$ Hz, **H-13**), 1.49 (2H, sxt, $J = 7$ Hz, **H-14**), 1.0 (3H, t, $J = 7$ Hz, **H-15**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 172.07 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.11 (**C-8**), 33.79 (**C-12**), 27.13 (**C-13**), 22.22 (**C-14**), 13.76 (**C-15**). Physical and spectroscopic data were in accordance with those reported.^{11,38}

Eugenyl 3-Methylbutanoate (3f)

Chromatographic purification gave colorless oil. $C_{15}H_{20}O_3$ ($M = 248.32$); yield 85%; MS (EI): m/z (%) 248 (M^+) (3.20), 165 (11.41), 164 (100), 149 (15.72), 133 (7.41), 132 (6.81), 121 (2.90); RI (HP5-MS): 1785; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.96 (1H, d, $J = 6.3$ Hz, **H-6**), 6.78–6.82 (2H, m, **H-3** and **H-5**), 5.98 (1H, s, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **H-7**), 3.41 (2H, d, $J = 6.24$ Hz, **H-8**), 2.48 (2H, d, $J = 7.63$ Hz, **H-12**), 2.29 (1H, m, **H-13**), 1.10 (6H, d, $J = 6.24$ Hz, $2 \times CH_3$: **H-14** and **H-15**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 171.26 (**C-11**), 150.94 (**C-2**), 138.87 (**C-1**), 138.08 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.11 (**C-10**), 112.75 (**C-3**), 55.75 (**C-7**), 43.14 (**C-12**), 40.11 (**C-8**), 25.97 (**C-13**), 22.39 ($2 \times CH_3$: **C-14** and **C-15**). Physical and spectroscopic data were in accordance with those reported.³³

Eugenyl Hexanoate (3g)

Chromatographic purification gave colorless oil. $C_{16}H_{22}O_3$ ($M = 262.34$); yield 82%; MS (EI): m/z (%) 262 (M^+), 165 (10.71), 164 (100), 149 (13.31), 133 (7.11), 132

(6.11), 103 (6.71), 91 (7.91), 55 (6.81), 43 (8.71); RI (HP5-MS): 1932; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.94 (1H, d, $J = 8$ Hz, **H-6**), 6.72–6.83 (2H, m, **H-3** and **H-5**), 5.90–6.03 (1H, m, **H-9**), 5.05–5.17 (2H, m, **H-10**), 3.82 (3H, s, **H-7**), 3.39 (d, $J = 6.6$ Hz, 2H, **H-8**), 2.59 (2H, t, $J = 7.5$ Hz, **H-12**), 1.76 (2H, quin, $J = 7.5$ Hz, **H-13**), 1.47 (2H, sxt, $J = 7.5$ Hz, **H-15**), 0.98 (3H, t, $J = 7.5$ Hz, **H-16**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 172.01 (**C-11**), 150.87 (**C-2**), 138.80 (**C-1**), 138.05 (**C-4**), 137.06 (**C-9**), 122.50 (**C-6**), 120.62 (**C-5**), 116.07 (**C-10**), 112.69 (**C-3**), 55.76 (**C-7**), 40.06 (**C-8**), 33.75 (**C-12**), 27.08 (**C-13**), 22.47 (**C-14**), 22.37 (**C-15**), 13.98 (**C-16**). Physical and spectroscopic data were in accordance with those reported.³⁸

Eugenyl Heptanoate (3h)

Chromatographic purification gave colorless oil. $C_{17}H_{24}O_3$ ($M = 276.37$); yield 82%; MS (EI): m/z (%) 276 (M^+), 165 (11.31), 164 (100), 149 (11.71), 133 (6.01), 132 (5.21), 131 (6.61), 103 (4.90), 91 (6.01), 55 (5.91), 43 (6.51); RI (HP5-MS): 2037; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.96 (1H, d, $J = 8$ Hz, **H-6**), 6.76–6.83 (2H, m, **H-3** and **H-5**), 5.94–6.03 (1H, m, **H-9**), 5.09–5.16 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 7$ Hz, **H-8**), 2.60 (2H, t, $J = 7$ Hz, **H-12**), 1.78 (2H, quin, $J = 7$ Hz, **H-13**), 1.46 (2H, bs, **H-14**), 1.31–1.42 (4H, m, **H-15** and **H-16**), 0.94 (3H, bs, **H-17**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.49 (**C-15**), 28.75 (**C-14**), 25.05 (**C-13**), 22.55 (**C-16**), 14.06 (**C-17**). Physical and spectroscopic data were in accordance with those reported.³⁸

Eugenyl Octanoate (3i)

Chromatographic purification gave yellowish oil. $C_{18}H_{26}O_3$ ($M = 290.40$); yield 86%; MS (EI): m/z (%) 290 (M^+), 165 (16.52), 164 (100), 149 (10.31), 133 (5.41), 132 (4.60), 131 (5.81), 103 (4.60), 91 (5.21), 57 (10.21), 55 (7.71); RI (HP5-MS): 2141; 1H NMR (500.13 MHz, $CDCl_3$): δ 6.96 (1H, d, $J = 8.3$ Hz, **H-6**), 6.76–6.83 (2H, m, **H-3** and **H-5**), 5.94–6.03 (1H, m, **H-9**), 5.09–5.18 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.2$ Hz, **H-8**), 2.59 (2H, t, $J = 7.3$ Hz, **H-12**), 1.79 (2H, quin, $J = 7.3$ Hz, **H-13**), 1.28–1.49 (8H, bm, **H-14–H-17**), 0.88–0.97 (3H, m, **H-18**); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.73 (**C-16**), 29.05 (**C-15**), 28.97 (**C-14**), 25.10 (**C-13**), 22.64 (**C-17**), 14.11 (**C-18**). Physical and spectroscopic data were in accordance with those reported.³⁸

Eugenyl Nonanoate (3j)

Chromatographic purification gave yellowish oil. $C_{19}H_{28}O_3$ ($M = 304.42$); yield 76%; MS (EI): m/z (%) 304 (M^+), 165 (11.31), 164 (100), 149 (9.01), 137 (1.90), 133

(4.40), 132 (4.60), 131 (3.10), 121 (1.50), 107 (1.30), 104 (2.00); RI (HP5-MS): 2246; ^1H NMR (500.13 MHz, CDCl_3): δ 6.96 (1H, d, $J = 8.3$ Hz, **H-6**), 6.70–6.84 (2H, m, **H-3** and **H-5**), 5.92–6.04 (1H, m, **H-9**), 5.03–5.18 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.2$ Hz, **H-8**), 2.59 (2H, t, $J = 7.3$ Hz, **H-12**), 1.78 (2H, quin, $J = 7.5$ Hz, **H-13**), 1.29–1.49 (10H, bm, **H-14–H-18**), 0.83–1.06 (3H, m, **H-19**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.11 (**C-8**), 34.10 (**C-12**), 31.84 (**C-17**), 29.27 (**C-16**), 29.19 (**C-15**), 29.10 (**C-14**), 25.09 (**C-13**), 22.68 (**C-18**), 14.13 (**C-19**). Physical and spectroscopic data were in accordance with those reported.³⁸

Eugenyl Decanoate (3k)

Chromatographic purification gave colorless oil. $\text{C}_{20}\text{H}_{30}\text{O}_3$ ($M = 318.45$); yield 85%; MS (EI): m/z (%) 318 (M^+), 166 (1.00), 165 (11.31), 164 (100), 149 (7.91), 147 (1.00), 137 (1.70), 133 (4.40), 105 (1.50), 103 (4.00); 85 (1.40); RI (HP5-MS): 2348; ^1H NMR (500.13 MHz, CDCl_3): δ 6.96 (1H, d, $J = 7.6$ Hz, **H-6**), 6.78–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.04 (1H, m, **H-9**), 5.10–5.17 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.2$ Hz, **H-8**), 2.59 (2H, t, $J = 7.6$ Hz, **H-12**), 1.79 (2H, quin, $J = 7.6$ Hz, **H-13**), 1.27–1.48 (12H, bm, **H-14–H-19**), 0.92 (3H, t, $J = 6.9$ Hz, **H-20**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.91 (**C-18**), 29.5 (**C-16**), 29.32 ($2 \times \text{CH}_2$: **C-15** and **C-17**), 29.10 (**C-14**), 25.10 (**C-13**), 22.71 (**C-19**), 14.14 (**C-20**). Physical and spectroscopic data were in accordance with those reported.³⁸

Eugenyl Undecanoate (3l)

Chromatographic purification gave colorless oil. $\text{C}_{21}\text{H}_{32}\text{O}_3$ ($M = 332.48$); yield 87%; MS (EI): m/z (%) 332 (M^+), 165 (11.21), 164 (100), 149 (7.31), 133 (3.90), 131 (4.50), 103 (3.70), 91 (4.30), 57 (5.91), 55 (10.01); RI (HP5-MS): 2449; ^1H NMR (500.13 MHz, CDCl_3): δ 6.96 (1H, d, $J = 8.3$ Hz, **H-6**), 6.78–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.03 (1H, m, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.24$ Hz, **H-8**), 2.59 (2H, t, $J = 7.28$ Hz, **H-12**), 1.78 (2H, quin, $J = 7.28$ Hz, **H-13**), 1.28–1.45 (14H, bm, **H-14–H-20**), 0.92 (3H, t, $J = 6.6$ Hz, **H-21**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.94 (**C-19**), 29.61 (**C-16**), 29.54 (**C-17**), 29.35 (**C-15**), 29.32 (**C-18**), 29.10 (**C-14**), 25.10 (**C-13**), 22.72 (**C-20**), 14.14 (**C-21**).

Eugenyl Dodecanoate (3m)

Chromatographic purification gave colorless oil. $\text{C}_{22}\text{H}_{34}\text{O}_3$ ($M = 346.50$); yield 84%; MS (EI): m/z (%) 346 (M^+), 165 (11.31), 164 (100), 149 (6.41), 133 (3.40), 131

(3.90), 103 (3.10), 91 (3.20), 57 (5.01), 55 (8.51); RI (HP5-MS): 2553; ^1H NMR (500.13 MHz, CDCl_3): δ 6.96 (1H, d, $J = 8.3$ Hz, **H-6**), 6.77–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.03 (1H, m, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.9$ Hz, **H-8**), 2.59 (2H, t, $J = 7.28$ Hz, **H-12**), 1.78 (2H, quin, $J = 7.6$ Hz, **H-13**), 1.28–1.51 (16H, bm, **H-14–H-21**), 0.92 (3H, t, $J = 6.6$ Hz, **H-22**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 172.08 (**C-11**), 150.93 (**C-2**), 138.86 (**C-1**), 138.09 (**C-4**), 137.13 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.72 (**C-3**), 55.79 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.94 (**C-20**), 29.65 ($2 \times \text{CH}_2$: **C-16** and **C-18**), 29.54 (**C-17**), 29.37 (**C-15**), 29.32 (**C-19**), 29.10 (**C-14**), 25.10 (**C-13**), 22.72 (**C-21**), 14.14 (**C-22**). Physical and spectroscopic data were in accordance with those reported.^{18,38}

Eugenyl Tridecanoate (3n)

Chromatographic purification gave yellowish oil. $\text{C}_{23}\text{H}_{36}\text{O}_3$ ($M = 360.53$); yield 87%; MS (EI): m/z (%) 360 (M^+), 164 (100), 149 (6.51), 131 (3.10), 103 (3.10), 91 (3.11), 57 (5.10), 55 (8.40); RI (HP5-MS): 2655; ^1H NMR (500.13 MHz, CDCl_3): δ 6.97 (1H, d, $J = 8.3$ Hz, **H-6**), 6.78–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.04 (1H, m, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, $J = 6.94$ Hz, **H-8**), 2.60 (2H, t, $J = 7.28$ Hz, **H-12**), 1.78 (2H, quin, $J = 7.3$ Hz, **H-13**), 1.27–1.54 (16H, bm, **H-14–H-22**), 0.92 (3H, t, $J = 6.6$ Hz, **H-23**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 172.04 (**C-11**), 150.94 (**C-2**), 138.84 (**C-1**), 138.13 (**C-4**), 137.13 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.13 (**C-10**), 112.75 (**C-3**), 55.79 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.95 (**C-21**), 29.38–29.70 ($4 \times \text{CH}_2$: **C-16–C-19**), 29.32 ($2 \times \text{CH}_2$: **C-15** and **C-20**), 29.10 (**C-14**), 25.10 (**C-13**), 22.72 (**C-22**), 14.14 (**C-23**).

Eugenyl Tetradecanoate (3o)

Chromatographic purification gave yellowish oil. $\text{C}_{24}\text{H}_{38}\text{O}_3$ ($M = 374.56$); yield 79%; MS (EI): m/z (%) 374 (M^+), 165 (13.01), 164 (100), 149 (5.81), 133 (3.10), 132 (2.80), 131 (3.50), 69 (2.50), 57 (3.60), 55 (5.51), 43 (2.50); RI (HP5-MS): 2753; ^1H NMR (500.13 MHz, CDCl_3): δ 6.97 (1H, d, $J = 8.32$ Hz, **H-6**), 6.78–6.83 (2H, m, **H-3** and **H-5**), 5.95–6.04 (1H, m, **H-9**), 5.10–5.17 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.41 (2H, d, $J = 6.24$ Hz, **H-8**), 2.60 (2H, t, $J = 7.63$ Hz, **H-12**), 1.78 (2H, m, **H-13**), 1.27–1.50 (18H, bm, **H-14–H-23**), 0.97–0.88 (3H, m, **H-24**); ^{13}C NMR (125.76 MHz, CDCl_3): δ 171.97 (**C-11**), 150.97 (**C-2**), 138.81 (**C-1**), 138.16 (**C-4**), 137.11 (**C-9**), 122.57 (**C-6**), 120.66 (**C-5**), 116.08 (**C-10**), 112.73 (**C-3**), 55.76 (**C-7**), 40.12 (**C-8**), 34.08 (**C-12**), 31.97 (**C-22**), 29.56–29.72 ($5 \times \text{CH}_2$: **C-16–C-20**), 29.71 (**C-18**), 29.67 (**C-20**), 29.62 (**C-17**), 29.56 (**C-19**), 29.42 (**C-22**), 29.34 (**C-15**), 29.11 (**C-14**), 25.12 (**C-13**), 22.74 (**C-23**), 14.14 (**C-24**). Physical and spectroscopic data were in accordance with those reported.¹⁸

Eugenyl Pentadecanoate (3p)

Chromatographic purification gave colorless oil. $\text{C}_{25}\text{H}_{40}\text{O}_3$ ($M = 388.58$); yield 89%; MS (EI): m/z (%) 388

(M⁺), 165 (10.91), 164 (100), 149 (5.61), 131 (3.70), 69 (5.51), 57 (8.41), 55 (14.11), 43 (9.31), 41 (4.10); RI (HP5-MS): 2858; ¹H NMR (500.13 MHz, CDCl₃): δ 6.97 (1H, d, *J* = 8.32 Hz, **H-6**), 6.78–6.83 (2H, m, **H-3** and **H-5**), 5.95–6.04 (1H, m, **H-9**), 5.10–5.17 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.41 (2H, d, *J* = 6.24 Hz, **H-8**), 2.60 (2H, t, *J* = 7.63 Hz, **H-12**), 1.78 (2H, m, **H-13**), 1.27–1.50 (18H, bm, **H-14–H-24**), 0.97–0.88 (3H, m, **H-25**); ¹³C NMR (125.76 MHz, CDCl₃): δ 172.02 (**C-11**), 150.96 (**C-2**), 138.82 (**C-1**), 138.16 (**C-4**), 137.11 (**C-9**), 122.57 (**C-6**), 120.68 (**C-5**), 116.11 (**C-10**), 112.75 (**C-3**), 55.78 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.97 (**C-23**), 29.56–29.75 (6 × CH₂: **C-16–C-21**), 29.42 (**C-22**), 29.34 (**C-15**), 29.11 (**C-14**), 25.12 (**C-13**), 22.74 (**C-24**), 14.16 (**C-25**).

Eugenyl Hexadecanoate (3q)

Chromatographic purification gave white solid. M.p. 39–40 °C. C₂₆H₄₂O₃ (*M* = 402.61); yield 92%; MS (EI): *m/z* (%) 402 (M⁺), 165 (13.41), 164 (100), 149 (5.31), 133 (2.70), 132 (2.50), 131 (3.10), 69 (2.60), 57 (3.70), 55 (5.40), 43 (2.40); RI (HP5-MS): 2976; ¹H NMR (500.13 MHz, CDCl₃): δ 6.96 (1H, d, *J* = 7.63 Hz, **H-6**), 6.77–6.82 (2H, m, **H-3** and **H-5**), 5.95–6.04 (1H, m, **H-9**), 5.10–5.16 (2H, m, **H-10**), 3.83 (3H, s, **C-7**), 3.40 (2H, d, *J* = 6.24 Hz, **H-8**), 2.60 (2H, t, *J* = 7.60 Hz, **H-12**), 1.79 (2H, quin, *J* = 7.50 Hz, **H-13**), 1.27–1.48 (20H, bm, **H-14–H-25**), 0.95–0.85 (3H, m, **H-26**); ¹³C NMR (125.76 MHz, CDCl₃): δ 172.07 (**C-11**), 150.94 (**C-2**), 138.84 (**C-1**), 138.11 (**C-4**), 137.11 (**C-9**), 122.55 (**C-6**), 120.68 (**C-5**), 116.11 (**C-10**), 112.73 (**C-3**), 55.81 (**C-7**), 40.11 (**C-8**), 34.10 (**C-12**), 31.95 (**C-24**), 29.54–29.72 (6 × CH₂: **C-16–C-22**), 29.38 (**C-23**), 29.32 (**C-15**), 29.10 (**C-14**), 25.10 (**C-13**), 22.71 (**C-25**), 14.14 (**C-26**). Physical and spectroscopic data were in accordance with those reported.^{18,38}

Eugenyl Heptadecanoate (3r)

Chromatographic purification gave yellowish solid. M.p. 49–50 °C. C₂₇H₄₄O₃ (*M* = 416.64); yield 76%; MS (EI): *m/z* (%) 416 (M⁺), 165 (12.11), 164 (100), 149 (4.60), 133 (2.50), 132 (2.10), 131 (2.80), 69 (3.00), 57 (4.20), 55 (6.00), 43 (3.00); RI (HP5-MS): 3096; ¹H NMR (500.13 MHz, CDCl₃): δ 6.97 (1H, d, *J* = 8 Hz, **H-6**), 6.74–6.88 (2H, m, **H-3** and **H-5**), 5.95–6.05 (1H, m, **H-9**), 5.04–5.22 (2H, m, **H-10**), 3.84 (3H, s, **C-7**), 3.42 (2H, d, *J* = 7.0 Hz, **H-8**), 2.60 (2H, t, *J* = 7.0 Hz, **H-12**), 1.81 (2H, quin, *J* = 7.0 Hz, **H-13**), 1.28–1.51 (22H, bm, **H-14–H-26**), 0.94 (3H, t, *J* = 7.0 Hz, **H-27**); ¹³C NMR (125.76 MHz, CDCl₃): δ 171.97 (**C-11**), 150.97 (**C-2**), 138.81 (**C-1**), 138.16 (**C-4**), 137.11 (**C-9**), 122.57 (**C-6**), 120.66 (**C-5**), 116.10 (**C-10**), 112.73 (**C-3**), 55.76 (**C-7**), 40.12 (**C-8**), 34.10 (**C-12**), 31.99 (**C-25**), 29.57–29.77 (7 × CH₂: **C-16–C-23**), 29.43 (**C-24**), 29.35 (**C-15**), 29.11 (**C-14**), 25.12 (**C-13**), 22.75 (**C-26**), 14.14 (**C-27**).

Eugenyl Benzoate (3s)

Chromatographic purification gave white solid. M.p. 70–71 °C. C₁₇H₁₆O₃ (*M* = 268.31); yield 96%; MS (EI): *m/z*

(%) 268 (M⁺) (20.82), 269 (3.80), 106 (9.61), 105 (100), 91 (5.41), 78 (3.10), 77 (33.93), 65 (1.90), 51 (4.60); RI (HP5-MS): 2170; ¹H NMR (500.13 MHz, CDCl₃): δ 8.25 (2H, d, *J* = 8.32 Hz, **H-2'** and **H-6'**), 7.66 (1H, t, *J* = 7.28 Hz, **H-4'**), 7.53 (2H, t, *J* = 7.63 Hz, **H-3'** and **H-5'**), 7.10 (1H, d, *J* = 8.32 Hz, **H-6**), 6.87 (1H, s, **H-3**), 6.85 (1H, d, *J* = 8.32 Hz, **H-5**), 5.98–6.06 (1H, m, **H-9**), 5.12–5.18 (2H, m, **H-10**), 3.83 (3H, s, **H-7**), 3.44 (2H, d, *J* = 6.94 Hz, **H-8**); ¹³C NMR (125.76 MHz, CDCl₃): δ 164.91 (**C-11**), 151.13 (**C-2**), 139.08 (**C-4**), 138.22 (**C-9**), 137.14 (**C-1**), 133.42 (**C-4'**), 130.32 (**C-2'** and **C-6'**), 129.55 (**C-1'**), 128.51 (2 × ArC: **C-3'** and **C-5'**), 122.68 (**C-5**), 120.76 (**C-6**), 116.17 (**C-10**), 112.88 (**C-3**), 55.90 (**C-7**), 40.15 (**C-8**). Physical and spectroscopic data were in accordance with those reported.^{11,37}

2. 3. Identification of Synthetized Compounds

2. 3. 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 C₈–C₄₀ alkanes run on HP-5MS column using the above mentioned temperature programme.⁴⁵

2. 3. 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 ¹H and ¹³C, respectively. All NMR spectra were recorded at 298 K in CDCl₃ (isotopic enrichment 99.95%) solution. Chemical shifts are reported on the δ (ppm) scale and are relative to residual CHCl₃ signals (7.24 for ¹H and 77.0 ppm, central line, for ¹³C spectra respectively), and scalar coupling constants are reported in hertz. The experimental error in the measured ¹H–¹H coupling constants was ± 0.5 Hz.

The signals assignment was given by a combination of 1D and 2D COSY and HSQC experiments, using standard Bruker pulse programs.

Acquisition parameters for 1D were as follows: ^1H spectral width of 5000 Hz and 32 K 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 64 K 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 ^1H and 220 ppm for ^{13}C . Delay values were optimized for $^1J_{\text{C,H}}$ 140.0 Hz and $^nJ_{\text{C,H}}$ 3.0 Hz. Zero filling in F1 to 1 K, p/2 shifted sine-bell squared (for gHSQC) or sinebell (for gHMBC) apodization functions were used for processing.

2. 4. Antimicrobial Activity

2. 4. 1. Microbial Strains

The *in vitro* antimicrobial activity of the synthesized compounds was tested 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, fungal organisms: *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. The Gram-negative bacteria *Salmonella abony* NCTC 6017 and *Salmonella typhimurium* ATCC 14028 were obtained from the National Collection of Type Cultures. All microorganisms were maintained at $-20\text{ }^\circ\text{C}$ under appropriate conditions and regenerated twice before use in the manipulations.

2. 4. 2. Screening of Antimicrobial Activity

The minimal inhibitory concentration (MIC) of esters was determined based on a broth microdilution method in 96-well microtitre plates.⁴⁶ 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.*⁴⁷ 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.

2. 5. *In silico* Physico-chemical, Pharmacokinetic and Toxicological Properties of the Synthesized Compounds

In order to obtain a complete picture of the synthesized compounds (3a–s) an *in silico* study was performed. Physico-chemical, pharmacokinetic and toxicological properties of compounds were calculated using the Molinspiration,⁴⁸ admetSAR,⁴⁹ DataWarrior⁵⁰ and Toxtree⁵¹ prediction tools.

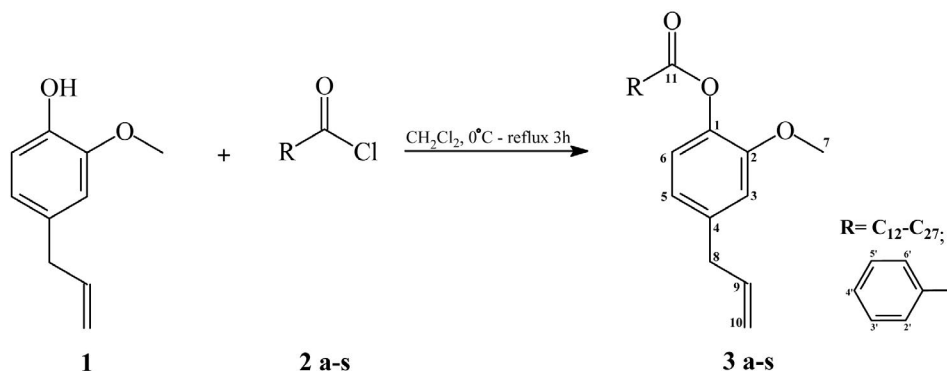
3. Results and Discussion

3. 1. Chemical Synthesis

A small focused library of nineteen eugenyl esters was synthesized. To the best of our knowledge four out of nineteen compounds are new (3l, 3n, 3p and 3r; Scheme 1). All compounds were synthesized according to previously published standard methodology and with high yields ranging from 76% to 96% (Table 1).

3. 2. Antimicrobial Activity

The results obtained in broth microdilution assay are given in Table 2. The assayed samples were less effective than antibiotic/antimycotic used as reference standard and if noted, activity was never greater than the values obtained for the parent compound 1 (MIC/MBC/MFC never exceeded 0.5 mg/mL, Table 2). The results are indicating selective susceptibility of the microorganisms, with *B. subtilis* (3d, 3l, 3n–r), *S. aureus* (3e and 3f), *A. niger* (3f, 3s)



Scheme 1. Synthesis of eugenyl esters

Table 1. Eugenyl esters: chemical entity, yields (%) and entry

R	Yield (%)	Entry
CH ₃	96	3a
CH ₂ CH ₃	89	3b
CH ₂ CH ₂ CH ₃	87	3c
CH(CH ₃) ₂	76	3d
CH ₂ (CH ₂) ₂ CH ₃	85	3e
CH ₂ CH(CH ₃) ₂	85	3f
CH ₂ (CH ₂) ₃ CH ₃	82	3g
CH ₂ (CH ₂) ₄ CH ₃	82	3h
CH ₂ (CH ₂) ₅ CH ₃	86	3i
CH ₂ (CH ₂) ₆ CH ₃	76	3j
CH ₂ (CH ₂) ₇ CH ₃	85	3k
CH ₂ (CH ₂) ₈ CH ₃	87	3l
CH ₂ (CH ₂) ₉ CH ₃	84	3m
CH ₂ (CH ₂) ₁₀ CH ₃	87	3n
CH ₂ (CH ₂) ₁₁ CH ₃	79	3o
CH ₂ (CH ₂) ₁₂ CH ₃	89	3p
CH ₂ (CH ₂) ₁₃ CH ₃	92	3q
CH ₂ (CH ₂) ₁₄ CH ₃	76	3r
Ph	96	3s

and *C. albicans* (3d, 3f, 3n, 3n and 3s) being the most sensitive strains on the synthesized derivatives. On the other hand, all three Gram-negative microorganisms tested (*E. coli*, *S. abony* and *S. typhimurium*) were completely resistant to synthesized compounds tested (initial concentration 2 mg/mL).

Four of our samples (3a, 3e, 3f, 3s) are matching the samples tested in antibacterial assays so far.^{29–31} Eugenyl acetate (3a) was tested in a disc diffusion method on a set of seven Gram-positive and nine Gram-negative bacteria.³⁰ The results have shown notable antimicrobial effect against all sixteen bacteria tested, with a superior performance when compared to pure eugenol.³⁰ Even when different methodologies were employed, two crucial and completely opposed observations can be made; our results have shown that derivatives have never exceeded the activity of the parent compound and that all were inactive against Gram-negative bacteria taken into consideration. Eugenyl benzoate (3s) was tested previously in disc diffusion assay against a smaller panel of laboratory controlled bacterial strains (two Gram-positive, two Gram-negative).

Table 2. The minimal inhibitory (MIC) and minimal bactericidal/fungicidal (MBC/MFC) concentrations (mg/mL) of the eugenol (1) and the synthesized eugenyl esters 3a–s. MIC and MBC/MFC determinations were carried out in triplicate and consistent values were obtained for each microorganism tested.

Cmpd.	Bacterial strains					Fungal strains	
	Gram-positive		Gram-negative			<i>A. niger</i>	<i>C. albicans</i>
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. abony</i>	<i>S. typhimurium</i>		
1	MIC = 0.5	MIC = MBC = 0.5	MIC = 0.5 MBC = 1.0	MIC = 0.5 MBC = 1.0	MIC = MBC = 0.5	MIC = MFC = 0.5	MIC = 0.25 MFC = 1.0
3a	na	na	na	na	na	na	MIC = MFC = 0.5
3b	na	na	na	na	na	na	na
3c	na	na	na	na	na	na	na
3d	MIC = 0.5	na	na	na	na	na	MIC = 0.5
3e	na	na	na	na	na	na	na
3f	na	MIC = 0.5	na	na	na	MIC = 0.5	MIC = 0.5
3g	na	na	na	na	na	na	na
3h	na	na	na	na	na	na	na
3i	na	na	na	na	na	na	na
3j	na	na	na	na	na	na	na
3k	na	na	na	na	na	na	na
3l	MIC = 1.0	na	na	na	na	na	na
3m	na	na	na	na	na	na	na
3n	MIC = 0.5	na	na	na	na	na	MIC = 0.5
3o	MIC = 1.0	na	na	na	na	na	na
3p	MIC = 0.5	na	na	na	na	na	na
3q	MIC = 0.5	na	na	na	na	na	na
3r	MIC = 0.5	na	na	na	na	na	MIC = 1.0
3s	na	na	na	na	na	MIC = 1.0	MIC = 1.0
Doxycycline (µg/mL)	MIC = MBC = 1.56	MIC = 6.25 MBC = 0.78	MIC = MBC = 0.78	MIC = MBC = 6.25	MIC = 6.25 MBC > 50	nt	nt
Nystatin (µg/mL)	nt	nt	nt	nt	nt	MIC = MBC = 6.25	MIC = MBC = 0.78
DMSO 10% aqueous solution		na	na	na	na	na	na na

na, not active; nt, not tested

Stronger antibacterial activity of benzoate was observed compared to its unsubstituted analogue, *i.e.* eugenol.²⁹ Eugenyl pentanoate (**3e**) and 3-methylbutanoate (**3f**) were tested against seventeen different bacterial strains (ATTC, clinical or food isolates) and have shown moderate antibacterial potential, with inhibitory values (MIC) ranging from 0.32 to 6.00 mg/mL.³¹ No noticeable difference in the susceptibility of Gram-negative and Gram-positive bacteria was observed.³¹ For the rest of the synthesized compounds (**3b–d**, **3g–r**) antibacterial results are reported here for the first time. For all compounds synthesized (**3a–s**) results obtained for antifungal assay are the first ever reported (Table 2).

3. 3. *In silico* Study

3. 3. 1. Physico-chemical Properties of the Eugenyl Esters 3a–s

Lipinski's rule of five has been used to evaluate drug likeness and to determine if a pharmacologically or biologically active compound has properties that would make it a likely orally active drug in humans. In our previously published paper we have already summarized factors important for optimal bioavailability of synthesized compounds.⁵² According to the physico-chemical properties calculated by Molinspiration software tool,⁴⁸ compounds **3a–h** and **3s** fulfilled all requirements for good oral bioavailability, including $m_i\text{LogP} < 5$, $n_{\text{ON}} < 10$, $n_{\text{OHNH}} < 5$, $\text{MW} < 500$, $\text{TPSA} < 140 \text{ \AA}^2$ ($< 60 \text{ \AA}^2$), and $n_{\text{rotb}} < 10$ (Supplementary data, Table S1).

3. 3. 2. Pharmacokinetic Properties of the Eugenyl Esters 3a–s

Absorption properties of compounds **3a–s** were predicted by admetSAR⁴⁹ (Supplementary data, Table S2). Based on the results, all of the studied compounds were predicted as compounds able to pass through blood-brain barrier and penetrate into the central nervous system, as compounds capable of being absorbed by intestine, as well as compounds permeable across Caco-2 cells. Compounds **3g–r** were predicted as P-glycoprotein substrates, while compound **3s** was predicted as P-glycoprotein inhibitor. None of the studied compounds was predicted as inhibitor of renal organic cation transporter.

Metabolic properties of the eugenyl esters **3a–s** were predicted by admetSAR⁴⁹ (Supplementary data, Table S3). None of the studied compounds was predicted as CYP2C9 and/or CYP2D6 substrate, while 15 compounds (**3c**, **3e–r**) were predicted as CYP3A4 substrate. Regarding CYP450 inhibition, enzyme CYP1A2 might be inhibited by compounds **3c**, **3e–s**, CYP2C9 by compound **3s**, CYP2C19 by compounds **3b**, **3c**, **3h–s**. However, none of the compounds was predicted as CYP2D6 inhibitor. Most of the investigated compounds were predicted to have low CYP 450 inhibitory promiscuity, except compounds **3a**, **3d** and **3s**.

3. 3. 3. Toxicological Properties of the Eugenyl Esters 3a–s

Using Toxtree prediction tool based on a decision tree approach,⁵¹ we have predicted structural alerts for DNA and protein binding for the studied compounds. Regarding DNA binding alerts, all of the studied compounds were predicted to have the ability to undergo nucleophilic aliphatic substitution ($S_{\text{N}}1$ reactions) and Michael addition. Among the protein binding alerts, compounds **3a–s** were predicted as compounds able to undergo Michael addition and $S_{\text{N}}2$ reactions; **3a–s** were predicted as compounds able to participate in acyl transfer (results given in Supplementary data, Tables S4 and S5).

Based on the toxicological properties predicted by admetSAR⁴⁹ compounds **3a–s** have been characterized as weak hERG (human Ether-à-go-go-Related Gene) inhibitors, non-AMES toxic and non-carcinogenic, but highly toxic for fish, *Tetrahymena pyriformis* and honey bee. Five compounds (**3a–c**, **3e** and **3g**) were predicted as ready biodegradable. According to the risk for acute oral toxicity, all of the studied compounds (**3a–s**) were predicted as Category III, or slightly toxic compounds, with LD_{50} values between 500 and 5000 mg/kg. Depending on the median toxic dose (TD_{50}), all compounds were predicted as “non-required” or non-carcinogenic chemicals (Supplementary data, Table S6).

Toxicological properties of the studied compounds predicted by DataWarrior⁵⁰ indicate that all compounds have high risk for tumorigenic and irritant effects. All compounds were predicted with no risk for mutagenic effects and no risk for reproductive effects (Supplementary data, Table S7).

4. Conclusion

We have synthesized nineteen esters of eugenol, of which four represent new compounds (**3l**, **3n**, **3p** and **3r**). All of the compounds were employed in antimicrobial bioassay, with several compounds tested for the first time in antibacterial (**3b–d**, **3g–r**) and all of the compounds (**3a–s**) ever tested in antifungal activity bioassay. The results have indicated microorganisms' selective susceptibility, with *B. subtilis* (**3d**, **3l**, **3n–r**), *S. aureus* (**3e** and **3f**), *C. albicans* (**3d**, **3f**, **3n**, **3r–s**) and *A. niger* (**3f**, **3s**) as the most susceptible ones, with minimal inhibitory or cidal concentrations (MIC/MBC/MFC) never exceeding the range of activity of eugenol as the parent compound. Based on current results and on our previously published ones,⁴³ having in best case comparable but never greater MIC values than for parent phenolic compound we could not confirm results obtained by previous groups.^{29–31} Our results have shown the importance of free phenolic hydroxyl group in terms of activity against microbials, however more detailed research should be conducted involving also a more

complex viewpoint, as for instance, the disclosure of mechanism of action of eugenyl esters on bacteria and fungi.

The results of our *in silico* study have shown that compounds fulfilling Lipinski's rule of five are **3a–h** and **3s** and were predicted to have good oral bioavailability. All of the studied compounds were predicted as to be 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 substrate and/or inhibitor of various CYP450 enzymes. Compounds **3a–s** were predicted as weak hERG inhibitors, non-AMES toxic and non-carcinogenic, but highly toxic for fish, *T. pyriformis* and honey bee. Further, they were predicted as Category III of risk for acute oral toxicity, as well as compounds with high risk for tumorigenic and irritant effects. Finally, they exhibited at least two structural alerts for both DNA and protein binding, indicating their ability to act as a mutagen or a genotoxic.

Taking in consideration predicted *in silico* properties and estimated drug likeness score, pharmacological and toxicological profile, eugenyl esters might be used as prodrugs of eugenol due to an expected enhanced lipophilicity⁵³ and passive membrane transport.

5. Acknowledgement

The authors acknowledge the Ministry of Science and Technological Development of Serbia for financial support (projects 451-03-821/2012-14, 172044 and 172047) as well as internal project of Faculty of Medicine, University of Niš (project no. 4).

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Povzetek

Rezultati, predstavljeni v tem članku, so nadaljevanje naših študij modifikacij molekul naravnega izvora, s posebnim poudarkom na aciliranju fenolne funkcionalne skupine. Pripravili smo majhno, fokusirano knjižnico devetnajstih estrov evgenola, od katerih so štiri nove spojine. Z vsemi spojinami smo izvedli *in vitro* antimikrobno testiranje. Z *in silico* računskimi študijami smo napovedali fizikalno-kemijske, farmakokinetične in toksikološke lastnosti, kar daje dodatne podatke za boljše usmerjanje raziskav v prihodnosti.