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SYNTHESIS AND FIRST-TIME ASSESSMENT OF *o*-EUGENOL DERIVATIVES AGAINST *Mycobacterium tuberculosis*

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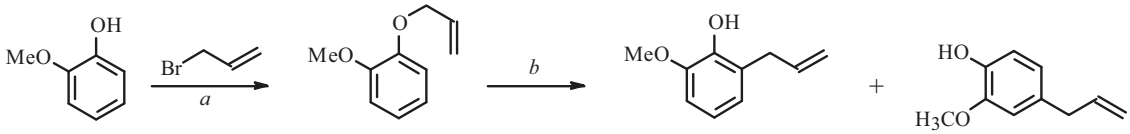
In this work, we report the first-time assessment of o*-eugenol, 6-allyl-2-methoxyphenol, and their selected derivatives, against* Mycobacterium tuberculosis *$H_{37}RV$, using the MABA susceptibility test. The bromo, nitro,* O*-alkylated, and reduced derivatives were obtained by standard methods and were characterized by spectroscopic and mass spectral data. Structure–activity relationships were investigated, with the most active derivatives being 4,5-dibromo-2-methoxy-6-propylphenol (139 μM) and 2-methoxy-3-nitro-6-propylphenol (237 μM). This study provides important information on the rational design of new lead anti-TB drugs based on* o*-eugenol derivatives.*

Keywords: eugenol, *o*-eugenol, synthesis, tuberculosis, *Mycobacterium tuberculosis*, nitration, bromination, large scale.

Eugenol (**1**, 4-allyl-2-methoxyphenol) (Scheme 1), is a natural aromatic product, obtained as an oil from various plant species such as cloves (*Syzygium aromaticum*), and cinnamon (*Cinnamomum tamala*). Its biosynthetic path involves the amino acid tyrosine [1], and this natural product has several applications in different fields, e.g., as an oxidant, a pheromone, a stabilizing agent, an anesthetic, an antiseptic, an antibacterial, a flavoring agent, an essential oil, and in perfumery [2].

An isomer of eugenol is *o*-eugenol, (**2**, 2-allyl-6-methoxyphenol) (Scheme 1), a natural aromatic product either, found in species such as *Miscanthus x giganteus* [3], in cade oil of *Juniperus oxycedrus* L. [4, 5], and *Mosla chinensis* [6]. However, this isomer is readily available synthetically as the major product from a reaction involving guaiacol (**3**, 2-methoxyphenol) and allyl bromide in a two-step process, which also produces eugenol (**1**) as a minor product. A study done with this regioisomer, *o*-eugenol, has already highlighted the antinociceptive and anti-inflammatory activity [7], but little is known about other biological uses of this compound as well as its derivatives.

Despite the broad spectrum of applications of eugenol (**1**), few studies have been made on the use of **1** or **2** and their derivatives in the search for new antitubercular agents. Tuberculosis (TB) continues today as a significant global public health problem, being responsible for 1.7 million deaths worldwide in 2016 [8] (WHO, 2017). The resurgence of this disease is due to different factors, with a major one being the emergence of bacteria becoming increasingly resistant to the standard array of drugs used to treat this disease [9].



3 **3a** **2** **1**

a. K_2CO_3; acetone, reflux, 8 h, 85%; *b*. 200°C, 90 min

Scheme 1

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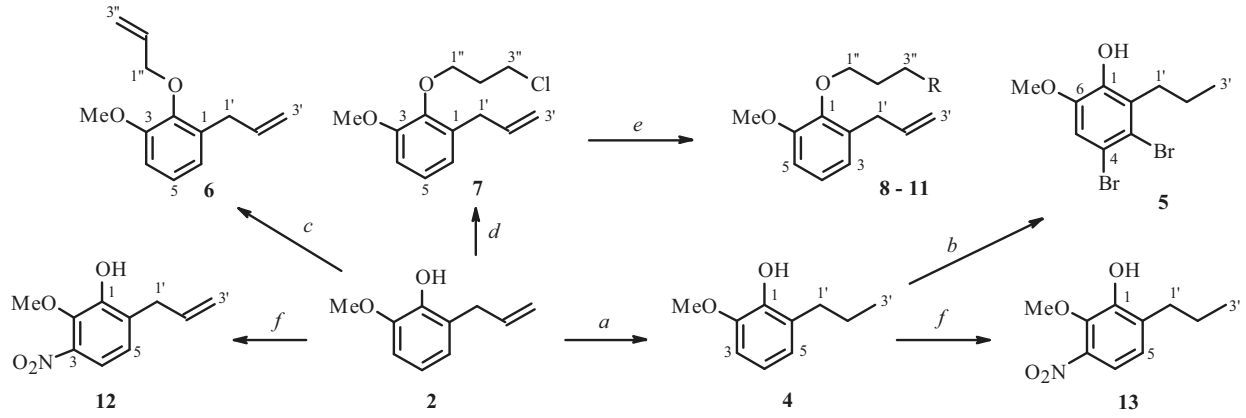


8: R = -N(CH_2CH_2)$_2$NH; **9:** R = -NHCH_3; **10:** R = -NH(CH_2)$_2CH_3$; **11:** R = -NH(CH_2)$_3CH_3$

a. H_2, Pd/C, EtOH, r.t., 21 h, 72%; *b.* Br_2, CH_2Cl_2, –70°C, 1 h, 79%; *c.* allyl bromide, K_2CO_3, acetone, reflux, 14 h, 89%; *d.* 1-bromo-3-chloropropane, K_2CO_3, acetone, reflux, 25 h, 86%; *e.* piperazine, methylamine, propylamine or butylamine, K_2CO_3, CH_3CN or MeOH, reflux or r.t., 18 – 67 h, 15 – 39%; *f.* HNO_3, CH_2Cl_2, r.t., **12**: 90 min, 37%; **13**: 1 h, 51%

Scheme 1

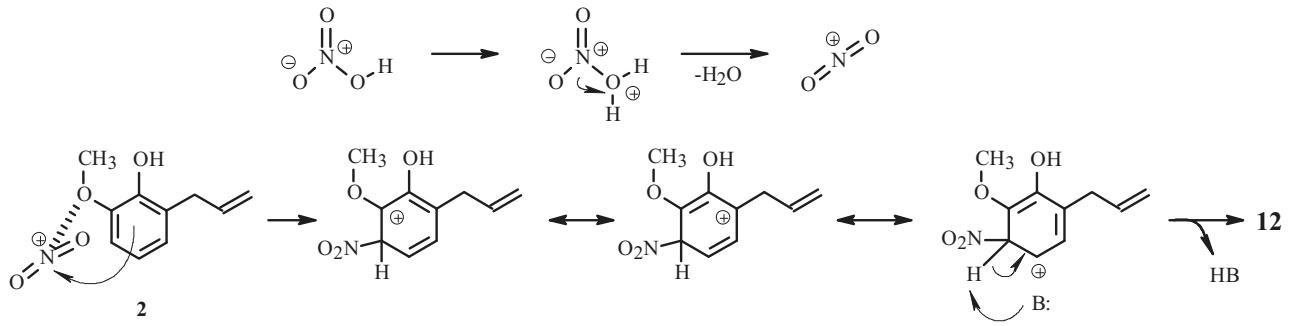
Thus new drugs and strategies are required, with aims to combat the resistant strains, reduce treatment time with fewer side effects, and reduce costs. Therefore, we have initiated a study of the anti-TB potential of *o*-eugenol (**2**) and its derivatives. We now report our findings.

Compounds **1** and **2** were obtained synthetically in 10 and 84% yields, respectively, following a published procedure [10], via a two-step process involving an initial allylation of guaiacol (**3**) with allyl chloride or bromide, followed by a Claisen rearrangement. The *o*-eugenol (**2**) was prepared on a large scale (59 g) (Scheme 1).

The *o*-eugenol derivatives **4–13** were synthesized, as shown in Scheme 2, and their anti-TB activity was tested *in vitro* against *M. tuberculosis* ATTC 27294 using the Microplate Alamar Blue Assay (MABA) [11].

For the nitration of *o*-eugenol and its reduced derivative **4**, the reactions were carried out using nitric acid in dichloromethane at room temperature, since the classical sulfuric/nitric acid nitrating agent resulted in decomposition. The products *o*-eugenol and **4** were regioselectively nitrated in the *ortho* position to the methoxy group, furnishing **12** and **13** in 37 and 51% yields, respectively (Scheme 2). The preferential formations of the *o*-substituted products using HNO_3/CH_2Cl_2 can be explained by the formation of a "pre-complex" involving the active NO_2^+ cation with the methoxy group as indicated by Strazzolini et al. [12] and is illustrated in Scheme 3.

All the new compounds were generally characterized from spectral data, including ^{1}H NMR, ^{13}C NMR, and IR spectra, and by mass spectrometry. Specifically, in the IR spectra, compounds **4**, **5**, **12**, and **13** showed O–H stretching vibrations at 3394–3536 cm^{-1}, while only compound **11** showed the characteristic N–H stretching vibration above 3600 cm^{-1}. In general, the ^{1}H NMR spectra showed aromatic protons at 6.70–7.02 ppm in the expected multiplicities, the C–H vinylic proton of compounds **7–12** as a multiplet at 5.91–6.13 ppm, while the CH_2 vinylic protons were found at 5.02–5.40 ppm, and the chemical shifts of the hydroxyl group of compounds **4**, **5**, **12**, and **13** appeared as singlets at 5.05–6.31 ppm. The methoxy group protons of compounds **4–13** were found as singlets in the range 3.83–3.99 ppm.



Scheme 3. Mechanistic proposal for formation of **12** or **13**.

TABLE 1. Evaluation of the Antimycobacterial Activity of Compounds **1–13** and Ethambutol (reference drug)

Compound	MIC[a], μM	LogP[b]	LD_{50}[c]	Compound	MIC[a], μM	LogP[b]	LD_{50}[c]
1	Res	2.57	1930	**8**	Res	3.32	500
2	610	2.57	880	**9**	Res	2.56	372
3	Res	1.33	520	**10**	Res	3.39	184
3a	Res	2.47	2200	**11**	361	3.8	372
4	Res	2.84	2000	**12**	478	2.65	1930
5	139	4.23	686	**13**	237	2.9	400
6	Res	3.53	1670	Ethambutol	15	0.06	998
7	Res	3.65	1670				

[a] The maximum concentration tested equal to 100 μg/mL, considering any value above that as resistant; [b] calculated using ChemBioDraw Program version 12.02.1076; [c] the predicted toxicity (mg/kg) was done using the virtual lab program, PRO-TOX II.

From Table 1, it can be observed that the hydroxyl group seems important for the anti-TB activity, as shown by the active phenolic compounds **5** (139 μM), **12** (478 μM), and **13** (237 μM) and the inactivity of compounds **7–10**. However, the inactivity of eugenol (**1**) and the reduced activity of *o*-eugenol (**2**), both phenolic compounds, suggests that other factors are important, such as the presence of strong electron withdrawing groups, which are found in compounds **5** (two bromo groups), **12**, and **13** (both having nitro groups). The influence of functional groups such as bromine and nitro on different compounds can occur in different ways. They confer varying degrees of lipophilicity and solubility to the molecules [13]. On the other hand, both can have a positive effect on a given biological target if their electron withdrawing effect beneficially influences the interaction of the substituted compounds with the receptor in question [13]. For various phenolic derivatives, such as those described herein, it may be that the effect of such groups, through the electron withdrawing effect, is to increase the hydrogen binding donor capacity of free hydroxyl, allowing an increase to activity due to higher interaction with the receptor in question [13].

An *ortho* allyl group influences the activity as shown by comparing results for *o*-eugenol (610 μM) and eugenol (resistant, with a value MIC > 100 μg/mL). Comparison of the activities of **12** and **13** indicates that replacing the allyl group by propyl does increase the activity. The lipophilicity of the compounds is another factor that should be generally considered. The derivative that displayed the best activity is compound **5** (139 μM), which has the highest log P value. However, no correlation between activities and log P can be realistically drawn from the data presented in Table 1. Compounds with values of LD_{50} greater than 200 are considered nontoxic.

EXPERIMENTAL

General. Melting points were determined using an MQAPF-302 (MicroQuimica Ltd., Santa Catarina, Brazil) apparatus and are uncorrected. NMR spectra were analyzed using 400 and 500 MHz Bruker AC spectrometers with tetramethylsilane as internal standard. Infrared spectra were obtained using a Thermo Nicolet 6700 spectrometer. High-resolution mass spectra were acquired on a Bruker compact-Tof. The progress of the reactions was monitored by thin-layer chromatography (TLC) on 2.0 cm × 6.0 cm aluminum sheets (silica gel 60, HF-254, Merck) with a thickness of 0.25 mm, using ultraviolet light irradiation. For column chromatography, Merck silica gel (70–230 or 230–400 mesh) was used. Solvents and reagents were used without further purification.

1-(Allyloxy)-2-methoxybenzene (3a). A reaction mixture of guaiacol (**3**) (63 g, 0.5 mol), allyl bromide (66 g, 0.55 mol), and K_2CO_3 (69 g, 0.5 mol) in CH_3COCH_3 (100 mL) was refluxed for 8 h. After rotary evaporation, H_2O (200 mL) was added, and the mixture was extracted using Et_2O (2 × 100 mL). The organic phase was collected, washed with 10% w/v NaOH solution (2 × 100 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to afford **1** (70.8 g, 85%) as a yellow oil. Spetral data were compared with the corresponding published data [14].

Syntheses of *o*-Eugenol (2) and **Eugenol (1)**. Compound **3a** (70 g, 0.43 mol) was heated at 200°C with stirring for 1 h and 30 min, $PhCH_3$ (100 mL) was added, and the mixture was extracted using 10% w/v NaOH solution. The basic extract was acidified using dilute H_2SO_4 (200 mL 1:1) and extracted using EtOAc (3 × 100 mL). The organic extracts were evaporated, dried over anhydrous Na_2SO_4, and distilled under reduced pressure (75–77°C/0.5 mmHg). The oil containing a mixture of the

regioisomers eugenol and *o*-eugenol was submitted to chromatographic purification on silica gel (SiO_2, 70–230 mesh, *n*-hexane–EtOAc, 75.5:2.5) to separate *o*-eugenol (**2**), which was obtained as a yellow oil (58.8 g, 84%). Eugenol (**1**) was obtained as a yellow oil (7.0 g, 10%). Spetral data were compared with the corresponding published data [14].

2-Methoxy-6-propylphenol (4). A solution of *o*-eugenol (**2**, 450 mg, 2.7 mmol) in EtOH (6 mL) was hydrogenated in the presence of palladium on active carbon (10%, 1 mol%) under stirring at room temperature for 21 h. Then, the reaction medium was filtered through Celite, and the filtrate was evaporated under reduced pressure to provide **4**, a colorless oil (330 mg, 72%). IR spectrum (ν, cm^{-1}): 3496 (OH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.70–6.69 (3H, m, H-3, 4, 5), 5.05 (1H, br.s, OH), 3.87 (3H, s, OCH_3), 2.61 (2H, t, J = 7.6, H-1′), 1.64 (2H, sex, J = 7.6, H-2′), 0.96 (3H, t, J = 7.3, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 146.29 (C-2), 143.55 (C-1), 128.51 (C-6), 122.39 (C-5), 119.04 (C-4), 108.17 (C-3), 55.95 (OCH_3), 31.90 (C-1′), 22.91 (C-2′), 14.05 (C-3′). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 166 (M^+, 84), 138 (21), 137 (100), 122 (17), 77 (13). HR-ESI-MS *m/z* 165.09 $[M - H]^-$ (calcd for $C_{10}H_{13}O_2$, 165.09).

3,4-Dibromo-6-methoxy-2-propylphenol (5). A solution of compound **4** (150 mg, 0.9 mmol) in CH_2Cl_2 (5 mL) was cooled to –70°C in a dry ice/acetone bath; Br_2 (176 mg, 1.1 mmol) was added to this solution, and the reaction mixture was stirred for 1 h. An aqueous solution of $Na_2S_2O_3$ (5 mL of 10% w/v solution) was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by a chromatographic column on silica gel (70–230 mesh, 10% v/v acetone–*n*-hexane) to furnish **5** as a yellow oil (231 mg, 79%). IR spectrum (ν, cm^{-1}): 3536 (OH). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.01 (1H, s, H-5), 5.72 (1H, s, OH), 3.87 (3H, s, OCH_3), 2.84 (2H, t, J = 7.85, H-1′), 1.57 (2H, sex, J = 7.37, H-2′), 0.99 (3H, t, J = 7.37, H-3′). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 145.84 (C-6), 143.24 (C-1), 130.27 (C-2), 118.68 (C-3), 114.27 (C-4), 112.88 (C-5), 56.30 (OCH_3), 33.25 (C-1′), 21.84 (C-2′), 14.05 (C-3′). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 324 (M^+, 100), 293 (52), 197 (50), 126 (43). HR-ESI-MS *m/z* 322.9112 $[M - H]^-$ (calcd for $C_{10}H_{11}Br_2O_2$, 322.9105).

1-Allyl-2-(allyloxy)-3-methoxybenzene (6). A mixture of *o*-eugenol (**2**, 500 mg, 3 mmol), K_2CO_3 (1.240 mg, 9 mmol), and allyl bromide (400 mg, 3.3 mmol) in 20 mL of CH_3COCH_3 (20 mL) was refluxed for 14 h. After rotary evaporation, H_2O (15 mL) was added and the whole extracted with Et_2O (2 × 15 mL). The organic phase was washed with aqueous NaOH solution (10% w/v, 2 × 15 mL). The organic solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to furnish a yellow oil (550 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.99 (1H, t, J = 7.9, H-5), 6.77–6.80 (2H, m, H-4, 6), 6.08–6.12 (1H, m, H-2″), 5.94–6.00 (1H, m, H-2′), 5.37 (1H, dd, J = 17.2, 1.6, H-3″$_{trans}$), 5.22 (1H, dd, J = 10.4, 1.3, H-3″$_{cis}$), 5.03–5.07 (2H, m, H-3′), 4.48 (2H, dt, J = 5.7, 1.25, H-1″), 3.85 (3H, s, OCH_3), 3.42 (2H, d, J = 6.6, H-1′). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 152.83 (C-2), 145.84 (C-3), 137.30 (C-2′), 134.48 (C-2″), 134.16 (C-1), 123.86 (C-6), 121.95 (C-5), 117.13 (C-3″), 115.52 (C-3′), 110.42 (C-4), 73.75 (C-1″), 55.73 (OCH_3), 34.23 (C-1′). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 204 (M^+, 72), 163 (100), 135 (40), 103 (72), 91 (43). HR-ESI-MS *m/z* 227.1062 $[M + Na]^+$ (calcd for $C_{13}H_{16}NaO_2$, 227.1048).

1-Allyl-2-(3-chloropropoxy)-3-methoxybenzene (7). A reaction mixture of *o*-eugenol (**2**, 300 mg, 1.8 mmol), K_2CO_3 (760 mg, 5.5 mmol), 1-bromo-3-chloropropane (860 mg, 5.5 mmol), and CH_3COCH_3 (15 mL) was refluxed for 25 h. After evaporation of the solvent under reduced pressure, H_2O (15 mL) was added and extraction was effected with Et_2O (2 × 15 mL). The organic phase was washed with 10% w/v NaOH solution (2 × 15 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give a colorless oil (380 mg, 86%), without further purification. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.99 (1H, t, J = 7.9, H-5), 6.76–6.80 (2H, m, H-4, 6), 5.90–6.00 (1H, m, H-2′), 5.02–5.07 (2H, m, H-3′), 4.07 (2H, t, J = 5.8, H-1″), 3.80–3.83 (5H, m, OCH_3, H-3″), 3.41 (2H, d, J = 6.5, H-1′), 2.18–2.30 (1H, m, H-2″). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 152.74 (C-2), 145.81 (C-3), 137.23 (C-2′), 133.98 (C-1), 123.98 (C-6), 122.04 (C-5), 115.59 (C-3′), 110.41 (C-4), 69.21 (C-1″), 55.69 (OCH_3), 41.92 (C-3″), 34.03 (C-1′), 33.46 (C-2″). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 240 (M^+, 100), 164 (80), 149 (42), 131 (44), 103 (42). HR-ESI-MS *m/z* 263.0819 $[M + Na]^+$ (calcd for $C_{13}H_{17}ClNaO_2$, 263.0815).

1-(3-(2-Allyl-6-methoxyphenoxy)propyl)piperazine (8). A reaction mixture of compound **7** (300 mg, 1.2 mmol), K_2CO_3 (520 mg, 3.8 mmol), and 210 mg of piperazine (2.4 mmol) in CH_3CN (10 mL) was refluxed, with magnetic stirring for 19 h. The solvent was removed under reduced pressure, H_2O (20 mL) was added, and the mixture was extracted with EtOAc (3 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by a chromatographic column on silica gel (230–400 mesh; 10% v/v MeOH–$CHCl_3$) to furnish the product as a yellow oil (127 mg, 35%). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.98 (1H, t, J = 7.9, H-4), 6.76–6.78 (2H, m, H-3, 5), 5.93–5.98 (1H, m, H-2′), 5.02–5.07 (2H, m, H-3′), 3.97 (2H, t, J = 6.4, H-1″), 3.83 (3H, s, OCH_3), 3.41 (2H, d, J = 6.4, H-1′), 2.94 (4H, t, J = 4.7, H-5″), 2.57 (2H, t, J = 7.4, H-3″), 1.95 (3H, qt, J = 6.4, H-2″). ^{13} C NMR (125 MHz, $CDCl_3$, δ, ppm):

152.81 (C-1), 146.07 (C-6), 137.36 (C-2′), 134.03 (C-2), 123.77 (C-3), 121.99 (C-4), 115.51 (C-3′), 110.43 (C-5), 71.20 (C-1″), 55.73 (C-3″), 55.71 (C-4″), 54.04 (OCH_3), 45.80 (C-5″), 34.07 (C-1′), 27.41 (C-2″). Mass spectrum (EI, 70 eV), *m/z* ($I_{rel.}$, %): 290 (37), 127 (12), 99 (M^+, 100), 84 (12), 70 (14). HR-ESI-MS *m/z* 291.2105 $[M + H]^+$ (calcd for $C_{17}H_{27}N_2O_2$, 291.2073).

3-(2-Allyl-6-methoxyphenoxy)-*N*-methylpropan-1-amine (9). To a solution of compound **7** (300 mg, 1.2 mmol) in MeOH (5 mL) was added a 40% aqueous solution of CH_3NH_2 (7.5 mL). The reaction mixture was stirred at room temperature for 48 h, H_2O (10 mL) was added, and the mixture was extracted with EtOAc (3 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, affording a residue which was purified by a chromatography column on silica gel (230–400 mesh; 10% v/v MeOH–$CHCl_3$) to furnish **9** as a yellow oil (56 mg, 19%). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.02 (1H, t, J = 7.9, H-4), 6.78–6.80 (2H, m, H-3, 5), 5.90–5.98 (1H, m, H-2′), 5.02–5.06 (2H, m, H-3′), 4.02 (2H, t, J = 5.6, H-1″), 3.90 (3H, s, OCH_3), 3.40 (2H, d, J = 6.1, H-1′), 3.12 (2H, t, J = 6.2, H-3″), 2.65 (3H, s, H-4″), 2.13 (2H, qt, J = 5.97, H-2″). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm, J/Hz): 147.61 (C-1), 140.72 (C-6), 132.35 (C-2′), 129.20 (C-2), 119.54 (C-3), 117.52 (C-4), 111.03 (C-3′), 105.52 (C-5), 66.74 (C-1″), 51.09 (OCH_3), 44.05 (C-3″), 29.84 (C-4″), 29.15 (C-1′), 23.16 (C-2″). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 164 (44), 103 (20), 91 (24), 72 (100), 70 (30). HR-ESI-MS *m/z* 236.1776 $[M + H]^+$ (calcd for $C_{14}H_{22}NO_2$, 236.1651).

3-(2-Allyl-6-methoxyphenoxy)-*N*-propylpropan-1-amine (10). A reaction mixture of compound **7** (200 mg, 0.8 mmol), propylamine (229 mg, 1.7 mmol), K_2CO_3 (740 mg, 12.5 mmol), and CH_3CN (3.5 mL) was refluxed with stirring for 67 h. After evaporation of the solvent under reduced pressure, H_2O (10 mL) was added, and the mixture was extracted with EtOAc (3 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by a chromatographic column on silica gel (230–400 mesh; 10% v/v MeOH–$CHCl_3$) to furnish **10** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.01 (1H, t, J = 7.9, H-4), 6.77–6.80 (2H, m, H-3, 5), 5.92–5.98 (1H, m, H-2′), 5.06 (1H, t, J = 1.44, H-3′), 5.02–5.05 (1H, m, H-3′), 4.23 (1H, br.s, NH), 4.01 (2H, t, J = 5.8, H-1″), 3.88 (3H, s, OCH_3), 3.40 (2H, d, J = 6.5, H-1′), 3.06 (2H, t, J = 6.7, H-3″), 2.78 (2H, t, J = 7.5, H-4″), 2.09 (2H, qt, J = 6.1, H-2″), 1.68 (2H, sex, J = 7.4, H-5″), 0.95 (3H, t, J = 7.4, H-6″). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 152.42 (C-1), 145.57 (C-6), 137.13 (C-2′), 134.00 (C-2), 124.22 (C-3), 122.23 (C-4), 115.73 (C-3′), 110.28 (C-5), 71.74 (C-1″), 55.80 (OCH_3), 50.70 (C-4″), 47.04 (C-3″), 33.91 (C-1′), 28.42 (C-2″), 21.82 (C-5″), 11.54 (C-6″). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 263 (29), 234 (23), 100 (48), 72 (M^+, 100), 70 (22). HR-ESI-MS *m/z* 264.1986 $[M + H]^+$ (calcd for $C_{16}H_{26}NO_2$, 264.1964).

***N*-(3-(2-Allyl-6-methoxyphenoxy)propyl)butan-1-amine (11)**. A solution of compound **7** (200 mg, 0.8 mmol) and butylamine (3 mL, 30 mmol) was stirred at 70°C for 18 h. After addition of H_2O (15 mL), the reaction mixture was extracted with EtOAc (3 × 15 mL). The organic phases were collected, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by a chromatographic column on silica gel (230–400 mesh; 10% v/v MeOH–$CHCl_3$) to furnish **11** as a yellow oil (90 mg, 39%). ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.00 (1H, t, J = 7.9, H-4), 6.77–6.79 (2H, m, H-3, 5), 5.93–5.99 (1H, m, H-2′), 5.02–5.06 (2H, m, H-3′), 4.00 (2H, t, J = 5.9, H-1″), 3.87 (3H, s, OCH_3), 3.41 (2H, d, J = 6.5, H-1′), 2.95 (2H, t, J = 6.7, H-3″), 2.72 (2H, t, J = 7.4, H-4″), 2.02 (2H, qt, J = 6.3, H-2″), 1.56 (2H, qt, J = 7.4, H-5″), 1.36 (2H, sex, J = 7.6, H-6″), 0.92 (3H, t, J = 7.35, H-7″). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 152.60 (C-1), 145.82 (C-6), 137.23 (C-2′), 134.01 (C-2), 124.00 (C-3), 122.12 (C-4), 115.63 (C-3′), 110.34 (C-5), 71.62 (C-1″), 55.76 (OCH_3), 49.25 (C-4″), 47.15 (C-3″), 33.97 (C-1′), 31.52 (C-5″), 29.51 (C-2″), 20.41 (C-6″), 13.93 (C-7″). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 277 (63), 234 (51), 114 (68), 86 (M^+, 100), 70 (31). HR-ESI-MS *m/z* 278.2148 $[M + H]^+$ (calcd for $C_{16}H_{26}NO_2$, 278.2120).

6-Allyl-2-methoxy-3-nitrophenol (12). Fuming HNO_3 (230 mg, 3.6 mmol) was added carefully to a solution of *o*-eugenol (**2**, 200 mg, 1.2 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 1 h and 30 min. After addition of an aqueous solution of K_2CO_3 (20 mL, 10% w/v), the reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL). The reunited organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue obtained was subjected to column chromatographic purification on silica gel (230–400 mesh, EtOAc–*n*-Hex 2%) to furnish **12** as a yellow solid (94 mg, 37%), mp 67–68°C (EtOAc). IR spectrum (ν, cm^{-1}): 3394 (OH). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.79 (1H, d, J = 2.5, H-5), 7.66 (1H, d, J = 2.5, H-4), 6.31 (1H, s, OH), 5.90–5.98 (1H, m, H-2′), 5.08–5.16 (2H, m, H-3′), 3.99 (3H, s, OCH_3), 3.45 (2H, d, J = 6.6, H-1′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 149.36 (C-1), 145.86 (C-2), 140.56 (C-3), 134.82 (C-6), 126.16 (C-2′), 119.07 (C-5), 116.99 (C-3′), 104.41 (C-4), 56.58 (OCH_3), 33.50 (C-1′). Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 209 (M^+, 100), 147 (15), 131 (15), 103 (29), 91 (22). HR-ESI-MS *m/z* 208.0579 $[M - H]^-$ (calcd for $C_{10}H_{10}NO_4$, 208.0610).

2-Methoxy-3-nitro-6-propylphenol (13). Fuming HNO_3 (230 mg, 3.6 mmol) was added carefully to a solution of compound **4** (200 mg, 1.2 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 1 h. After addition of an aqueous solution of K_2CO_3 (30 mL, 10% w/v), the reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL).

The organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was subjected to purification by column chromatographic on silica gel (230–400 mesh, EtOAc–*n*-Hex 2%) to furnish the product as a yellow solid (130 mg, 51%), mp 59–61°C (EtOAc). IR spectrum (ν, cm^{-1}): 3408 (OH). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.78 (1H, d, J = 2.6, H-5), 7.64 (1H, d, J = 2.6, H-4), 6.27 (1H, s, OH), 3.98 (3H, s, OCH_3), 2.67 (2H, t, J = 7.65, H-1′), 1.67 (2H, sex, J = 7.6, H-2′), 0.97 (3H, t, J = 7.3, H-3′). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 149.55 (C-1), 145.76 (C-2), 140.40 (C-3), 128.56 (C-6), 119.15 (C-5), 104.02 (C-4), 56.52 (OCH_3), 31.49 (C-1′), 22.42 (C-2′), 13.83 (C-3′). Mass spectrum (EI, 70 eV), *m/z* ($I_{rel.}$, %): 211 (M^+, 83), 183 (17), 182 (100), 136 (19), 93 (12). HR-ESI-MS *m/z* 210.0724 $[M - H]^-$ (calcd for $C_{10}H_{12}NO_4$, 210.0766).

The antimycobacterial activities of the compounds guaiacol (**3**), intermediate **3a**, *o*-eugenol (**2**), eugenol (**1**), and derivatives **4–13** have been assessed against *M. tuberculosis* ATTC 27294 using the Micro plate Alamar Blue Assay (MABA). This methodology is nontoxic, uses a thermally-stable reagent, and shows good correlation with proportional BACTEC radiometric methods [15, 16]. The method is described as follows: 200 µL of sterile deionized water was added to all outer-perimeter wells of 96 sterile well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ) to minimize evaporation of the medium in the test wells during incubation. The 96 plates received 100 µL of Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA), and successive dilution of the compounds **1–13** was made directly on the plate. The final drug concentrations tested were 3.12 to 100 µg/mL. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. Then 25 µL of a freshly prepared 1:1 mixture of Alamar Blue (Accumed International, Westlake, Ohio) reagent and 10% Tween 80 was then added to the plate and the whole incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented a color change from blue to pink. MIC values represent means of three separate experiments, and the variation coefficient of the method is 9.8 %. The toxicity was predicted using the virtual lab program PRO-TOX II.

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REFERENCES

1. P. Manitto, D. Monti, and P. Gramatica, *J. Chem. Soc., Perkin Trans. 1*, 1, 727 (1974).
2. T. S. Kaufman, *J. Braz. Chem. Soc.*, **26** (6), 1055 (2015).
3. C. E. Greenhalf, D. J. Nowakowski, N. Yates, I. Shield, and A. V. Bridgwater, *Biomass Bioenergy,* **56**, 247 (2013).
4. G. T. McQuate, Y. S. Keum, C. D. Sylva, Q. X. Li, and E. B. Jang, *J. Econ. Entomol.*, **97** (3), 862 (2004).
5. Y. -S. Keum, G. T. McQuate, and Q. X. Li, *Biochem. Syst. Ecol.*, **35** (4), 188 (2007).
6. H. Cao, L. Zuguang, and C. Xiaozhen, *Chin. J. Chem.*, **29** (10), 2187 (2011).
7. D.V. Fonseca, P. R. R. Salgado, H. C. A. Neto, A. M. F. O. Golzio, M. R. D. C. Filho, C. G. F. Melo, F. C. Leite, M. R. Piuvezam, L. C. M. Pordeus, J. M. B. Filho, and R. N. Almeida, *Int. Immunopharmacol.*, **38**, 402 (2016).
8. Global Tuberculosis Report 2017, Switzerland: World Health Organization (WHO), 2017. ISBN: 978-92-4-156551-6.
9. C. Lienhardt, M. Raviglione, M. Spigelamn, R. Hafner, E. Jaramillo, M. Hoelscher, A. Zumla, and J. Gheuens, *J. Infect. Dis.*, **205**, S241 (2012).
10. C. F. H. Allen and J. W. Gates, *Org. Synth.*, **25**, 49 (1945).
11. S. G. Franzblau, R. S. Witzig, J. C. McLaughlin, P. Torres, G. Madico, A. Hernandez, M. T. Degnan, M. B. Cook, V. K. Quenzer, R. M. Ferguson, and R. H. Gilman, *J. Clin. Microbiol.*, **36** (2), 362 (1998).
12. P. Strazzolini, A. G. Giumanini, A. Runcio, and M. Scuccato, *J. Org. Chem.*, **63** (4), 952 (1998).
13. C. Wermuth, D. Aldous, P. Raboisson, and D. Rognan, *The Practice of Medicinal Chemistry*, Academic Press, Amsterdam, 2015, 902 pp.
14. Y.-L. Lin, J.-Y. Chengy, and Y.-H. Chu, *Tetrahedron*, **63**, 10949 (2007).
15. J. D. Vanitha and C. N. Paramasivan, *Mycobacteriology*, **49** (3), 179 (2004).
16. R. S. Reis, I. Neves, S. L. S. Lourenco, L. S. Fonseca, and M. C. S. Lourenco, *J. Clin. Microbiol.*, **42** (5), 2247 (2004).