Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):468-474

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and biological evaluation of 2′-hydroxy-*retro*-chalcone derivatives as antituberculosis agent

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ABSTRACT

Two new series of 2′-hydroxy-retro-chalcone derivatives have been synthesized and fully characterized by IR, 1H NMR, 13C NMR, and mass spectral data. All of these derivatives were evaluated for their antituberculosis activity against Mycobacterium tuberculosis H37Rv. Eight of the tested compounds inhibited the growth of the mycobacterial strain. Among them, compound (E)-1-[4-(decyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one was found to be the most active with MIC value of 27 µM. The results of this study suggest that chalcones are a class of compounds worthy of further investigation as an alternative inexpensive and synthetic therapeutic antituberculosis agent.

Keywords: 2′-hydroxy-*retro*-chalcone derivatives, antituberculosis activity, biological evaluation, *Mycobacterium tuberculosis* H37Rv

INTRODUCTION

Chalcones are precursors of various plant metabolites and in the synthesis of many biologically important compounds. The synthesis of chalcones and their derivatives have been actively investigated in the past decade and have attracted considerable attention due to their numerous biological properties. They have been shown to exhibit an impressive array of biological activities, such as antimalarial [1-3], anticancer [4-8], antituberculosis [9], antimicrobial [10-15], antileishmanial [16], anti-inflammatory [17,18], antifilarial [19], antifungal [20,21], larvicidal [22], anticonvulsant [23], and antioxidant [24-26].

The increasing interest in antituberculosis screening of chalcones arises from the urgent need to develop new drugs against the advancing multi-drug resistance strains of the etiologic agent. In a related study, Lin et al. [9] reported that derivatives of chalcone with one ring substituted with a heteroatom and with or without hydrophobic substitutions exhibited greater than 90% inhibitory activity against *Mycobacterium tuberculosis H37Rv*. On the contrary, the presence of additional hydrophilic substituents, such as methoxyl, hydroxyl and amino groups, resulted in a dramatic decrease of tuberculosis activity. In another recent related study, Ngainia et al. [13] evaluated the effects of the hydroxyl and the alkyl groups of the synthesized chalcone derivatives against wild-type *Escherichia coli* ATCC 8739. All the synthesized compounds exhibited significant antimicrobial activities and the optimum inhibition was dependent on the position of the hydroxyl group as well as the length of the alkyl chains.

In this study, we synthesized two series of novel 2′-hydroxy-*retro*-chalcone derivatives (*E*)-1-(4-alkyloxyphenyl)-2-(hydroxyphenyl)-prop-2-en-1-one containing alkyl chains of various lengths (C_7-C_{12} , C_{14}, and C_{19}) and possessing

different subtituents. These compounds were screened against *Mycobacterium tuberculosis* H37Rv to evaluate the effects of the various added substituents as well as the length of the alkyl chain.

EXPERIMENTAL SECTION

Materials and equipments

TLC was performed on pre-coated Merck 60 GF_{254} silica gel plates (absorbent thickness, 0.25 mm). Column chromatography was performed on silica gel (Merck Kieselgel 60, 70–230 mesh ASTM). Melting points were recorded on a Stuart Scientific SMP1 apparatus. NMR spectra were recorded in acetone-d_6, with TMS as internal standard at 25^{o}C, using a Bruker Avance 500 and 300 MHz spectrometer. Chemical shifts were reported in ppm (δ). Splitting patterns were assigned as: *s* for *singlet*, *br s* for *broad singlet*, *d* for *doublet*, *t* for *triplet*, *dd* for *doublet of doublets*, and *m* for *multiplet*. HRESIMS spectra were performed using a Micro TOF-Q mass spectrometer. IR (KBr) spectra were recorded using a Perkin-Elmer system 2000 FT-IR spectrometer. All of the chemicals, including 5-bromosalicylaldehyde and 5-methoxysalicylaldehyde, used in this study were purchased from Sigma-Aldrich, USA.

General procedure for the synthesis of various p- alkyloxyphenyl-ethanone (3a-g)

12.5 mmol of sodium carbonate was added to a stirred solution of 5 mmol of 4-hydroxyacetophenone in 100 mL dimethylformamide. The solution was stirred at room temperature for 15 minutes. 5 mmol of bromoheptane (2 mL) was then added drop wise to this mixture while stirring and the reaction were heated at reflux for 5 hours. The solution was stirred and heated at reflux for 5 hours. The reaction mixture was then allowed to cool to room temperature for 40 minutes. The solution was then poured into 500 mL of an ice water mixture to get brownish precipitates. These precipitates were filtered and washed with cold water. The compounds (**3a-g**) were purified and recrystallized from the mixture of ethyl acetate and ethanol [27].

General method for the synthesis of first series of chalcone subtituted derivatives from 5-bromosalicylaldehyde (4a-g)

Title compounds (**4a-g**) were obtained by the condensation of substituted acetophenone compounds (**3a-g**) with 5-bromosalicyladehydes (**4**). A solution of 1-[4-(alkoxy)phenyl]-ethanone (0.5 mmol) and 5-bromosalicylaldehyde (0.5 mmol) in absolute ethanol was heated at 80^{o}C. Potassium hydroxide (5 mL) was added to this solution while stirring and the mixture was refluxed for 24-36 h. Then 10 % HCl was added to neutralize the reaction solution. The resulting mixture was extracted with ethyl acetate and washed with brine solution then dried over $MgSO_4$. After removal of the solvent under vacuum, crude product was purified by column chromatography using silica gel (eluent: *n*-hexane/acetone: 8:2 v/v) to afford compounds (**4a-g**).

SPECTRAL DATA

(E)-1-[4-(heptoxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4a)

Yellow solid. Yield: 35.4%. IR KBr (cm^{-1}): 3411 (OH), 2924 (C-H), 1638 (C=O), 1602 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Aceton-d_6): 8.15 (2H, *d*, *J* = 8.0 Hz), 8.07 (1H, *d*, *J* = 15.5 Hz), 7.99 (1H, *d*, *J* = 2.5 Hz), 7.96 (1H, *d*, *J* = 15.5 Hz), 7.38 (1H, *dd*, *J* = 2.5, 9.0 Hz), 7.05 (2H, *d*, *J* = 8.0 Hz), 6.97 (1H, *d*, *J* = 9.0 Hz), 4.10 (2H, *t*, *J* = 6.5 Hz), 1.78-1.82 (2H, *m*), 1.45-1.49 (2H, *m*), 1.35-1.39 (2H, *m*), 1.27-1.32 (4H, *m*), 0.89 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.3, 164.0, 157.0, 137.7, 134.6, 131.9, 131.7, 131.6, 125.5, 123.6, 119.1, 115.2, 112.4, 68.7, 32.5, 30.3, 29.4, 26.7, 23.3, 14.3; MS (ESI): *m/z* (100%) 415.0935 $(M-H)^+$. $C_{22}H_{25}B_rO_3$ $(M-H)^+$ requires 415.0914.

(E)-1-[4-(octyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4b)

Yellow solid. Yield: 15.0%. IR KBr (cm^{-1}): 3087 (OH), 2921 (C-H), 1645 (C=O), 1601 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Aceton-d_6): 8.15 (2H, *d*, *J* = 9.0 Hz), 8.07 (1H, *d*, *J* = 16.0 Hz), 8.00 (1H, *d*, *J* = 2.5 Hz), 7.96 (1H, *d*, *J* = 16.0 Hz), 7.39 (1H, *dd*, *J* = 2.5, 9.0 Hz), 7.06 (2H, *dd*, *J* = 2.0, 9.0 Hz), 6.97 (1H, *d*, *J* = 9.0 Hz), 4.11 (2H, *t*, *J* = 6.5 Hz), 1.77-1.81 (2H, *m*'), 1.47-1.52 (2H, *m*), 1.33-1.38 (2H, *m*), 1.28-1.33 (6H, *m*), 0.88 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 500 MHz, Aceton-d_6): 188.32, 164.03, 156.94, 137.67, 134.60, 131.92, 131.64, 131.56, 125.51, 123.61, 119.07), 115.20, 112.37, 68.98, 32.55, 30.23, 30.08, 26.71, 23.30, 14.33; MS (ESI): m/z (100%) = 453.1009 $(M+Na^+)$. $C_{23}H_{27}B_rO_3$ $(M+Na^+)$ requires 453.1036.

(E)-1-[4-(nonyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4c)

Yellow solid. Yield: 47.7%. IR KBr (cm^{-1}): 3402 (OH), 2921 (C-H), 1645 (C=O), 1603 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.16 (2H, *d*, *J* = 8.0 Hz), 8.10 (1H, *d*, *J* = 16.0 Hz), 8.01 (1H, *d*, *J* = 2.5 Hz), 7.97 (1H, *d*, *J* = 16.0 Hz), 7.40 (1H, *dd*, *J* = 2.5, 9.0 Hz), 7.07 (2H, *d*, *J* = 8.0 Hz), 6.98 (1H, *d*, *J* = 9.0 Hz), 4.12 (2H, *t*, *J* = 6.5 Hz), 1.79-1.84 (2H, *m*), 1.47-1.52 (2H, *m*), 1.36-1.40 (2H, *m*), 1.27-1.33 (8H, *m*), 0.89 (3H, *t*, *J* = 7.0 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.3, 164.0, 157.0, 137.7, 134.6, 131.9, 131.7, 131.6, 125.5, 123.6, 119.1, 115.2,

112.4, 69.0, 32.6, 30.3, 30.1, 29.9, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 443.1203 $(M-H)^+$. $C_{24}H_{29}B_rO_3$ $(M-H)^+$ requires 443.1227.

(E)-1-[4-(decyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4d)
Yellow solid. Yield: 22.8%. IR KBr (cm^{-1}): 3433 (OH), 2920 (C-H), 1646 (C=O), 1601 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.17 (2H, *d*, *J* = 8.0 Hz), 8.10 (1H, *d*, *J* = 16.0 Hz), 8.02 (1H, *d*, *J* = 2.5 Hz), 7.98 (1H, *d*, *J* = 16.0 Hz), 7.40 (1H, *dd*, *J* = 2.5, 9.0 Hz), 7.08 (2H, *d*, *J* = 8.0 Hz), 6.98 (1H, *d*, *J* = 9.0 Hz), 4.13 (2H, *t*, *J* = 6.5 Hz), 1.79-1.85 (2H, *m*), 1.47-1.53 (2H, *m*), 1.36-1.41 (2H, *m*), 1.26-1.33 (10H, *m*), 0.89 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.3, 164.0, 156.9, 137.6, 134.6, 131.9, 131.6, 131.6, 125.5, 123.6, 119.1, 115.2, 112.4, 69.0, 32.6, 30.2, 30.1, 29.9, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 457.1395 $(M-H)^+$. $C_{25}H_{31}B_rO_3$ $(M-H)^+$ requires 457.1384.

(E)-1-[4-(dodecyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4e)
Yellow solid. Yield: 27.2%. IR KBr (cm^{-1}): 3420 (OH), 2921 (C-H), 1644 (C=O), 1605 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.02 (2H, *d*, *J* = 9.0 Hz), 7.95 (1H, *d*, *J* = 16.0 Hz), 7.87 (1H, *d*, *J* = 2.5 Hz), 7.83 (1H, *d*, *J* = 16.0 Hz), 7.26 (1H, *dd*, *J* = 2.5, 8.5 Hz), 6.93 (2H, *d*, *J* = 9.0 Hz), 6.84 (1H, *d*, *J* = 8.5 Hz), 3.99 (2H, *t*, *J* = 6.5 Hz), 1.64-1.69 (2H, *m*), 1.33-1.38 (2H, *m*), 1.22-1.27 (4H, *m*), 1.14-1.17 (22H, *m*), 0.75 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.2, 164.0, 156.9, 137.6, 134.6, 131.9, 131.7, 131.5, 125.5, 123.5, 119.0, 115.2, 112.4, 69.0, 32.6, 30.3, 30.4, 30.4, 30.3, 30.1, 29.4, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 485.1694 $(M-H)^+$. $C_{27}H_{35}B_rO_3$ $(M+H)^+$ requires 485.1697.

(E)-1-[4-(tetradecyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4f)
Yellow solid. Yield: 29.6%. IR KBr (cm^{-1}): 3420 (OH), 2920 (C-H), 1642 (C=O), 1605 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.17 (2H, *d*, *J* = 9.0 Hz), 8.09 (1H, *d*, *J* = 16.0 Hz), 8.02 (1H, *d*, *J* = 2.5 Hz), 7.98 (1H, *d*, *J* = 16.0 Hz), 7.41 (1H, *dd*, *J* = 2.5, 8.5 Hz), 7.08 (2H, *d*, *J* = 9.0 Hz), 6.99 (1H, *d*, *J* = 8.5 Hz), 4.14 (2H, *t*, *J* = 6.5 Hz), 1.79-1.85 (2H, *m*), 1.48-1.54 (2H, *m*), 1.35-1.40 (2H, *m*), 1.27-1.31 (18H, *m*), 0.89 (3H, *t*, *J* = 7.0 Hz,); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.3, 164.0, 156.9, 137.6, 134.6, 131.9, 131.6, 131.6, 125.5, 123.6, 119.1, 115.2, 112.4, 69.0, 32.6, 30.4, 30.4, 30.3, 30.2, 30.1, 29.4, 26.7, 23.3, 14.3; MS (ESI): *m/z* (100%) 537.1914 $(M+Na)^+$. $C_{29}H_{39}B_rO_3$ $(M+Na)^+$ requires 537.1914.

(E)-1-[4-(nonadecyloxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (4g)
Yellow solid. Yield: 6.8%. IR KBr (cm^{-1}): 3437 (OH), 2918 (C-H), 1675 (C=O), 1606 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.15 (2H, *d*, *J* =8.0 Hz), 8.07 (1H, *d*, *J* = 16.0 Hz), 8.00 (1H, *d*, *J* = 2.5 Hz), 7.97 (1H, *d*, *J* = 16.0 Hz), 7.39 (1H, *dd*, *J* = 2.5, 8.5 Hz), 7.06 (2H, *d*, *J* =8.0 Hz), 7.00 (1H, *dd*, *J* = 2.5, 8.5 Hz), 4.09 (2H, *t*, *J* = 6.5 Hz), 1.77-1.82 (2H, *m*), 1.46-1.50 (2H, *m*), 1.35-1.39 (4H, *m*), 1.26-1.31 (36H, *m*), 0.87 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.2, 163.9, 156.9, 137.6, 134.6, 131.9, 131.7, 131.5, 125.5, 123.5, 119.0, 115.2, 112.4, 69.0, 32.7, 30.4, 30.4, 30.3, 30.2, 29.6, 29.4, 26.7, 23.4, 14.4; MS (ESI): *m/z* (100%) 583.2796 $(M-H)^+$. $C_{34}H_{49}B_rO_3$ $(M-H)^+$ requires 583.2792.

General method for the synthesis of second series of chalcone subtituted derivatives from 5-methoxysalicylaldehyde (5a-g)
The experimental procedure conducted for the synthesis of (*E*)-3-(5-bromo-2-hydroxyphenyl)-1-(4-(alkoxy)phenyl)prop-2-en-1-one **(4a-g)** was followed by reacting appropriately 1-[4-(alkoxy)phenyl]-ethanone (**3a-g**) (0.5 mmol) in minimum amount of absolute ethanol with 5-methoxysalicylaldehyde (**5**) (0.5 mmol) in the presence of potassium hydroxide. Purification of the crude product by column chromatography using *n*-hexane/acetone (8:2 v/v) afforded compounds **(5a-g)**.

SPECTRAL DATA

(E)-1-(4-(heptyloxy)phenyl)-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5a)
Yellow solid. Yield: 13.7%. IR KBr (cm^{-1}): 3393 (OH), 2928 (C-H), 1638 (C=O), 1602 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.15 (1H, *d*, *J* = 15.5 Hz), 8.13 (2H, *d*, *J* = 8.5 Hz), 7.90 (1H, *d*, *J* = 15.5 Hz), 7.37 (1H, *d*, *J* = 2.5 Hz), 7.07 (2H, *d*, *J* = 8.5 Hz), 6.94 (1H, *d*, *J* = 9.0 Hz), 6.89 (1H, *dd*, *J* = 2.5, 9.0 Hz), 4.13 (2H, *t*, *J* = 6.5 Hz), 3.81 (3H, *s*), 1.79-1.85 (2H, *m*), 1.47-1.52 (2H, *m*), 1.36-1.41 (2H, *m*), 1.29-1.35 (4H, *m*), 0.91 (3H, *t*, *J* = 7.0 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.6, 163.9, 154.2, 152.1, 139.5, 132.2, 131.5, 123.4, 122.5, 119.1, 118.0, 115.2, 113.0, 69.0, 56.1, 32.5, 29.9, 29.8, 26.7, 23.3, 14.3; MS (ESI): *m/z* (100%) 367.1884 $(M-H)^+$. $C_{23}H_{28}O_4$ $(M-H)^+$ requires 367.1915.

(E)-1-[4-(octyloxy)phenyl]-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5b)
Yellow solid. Yield: 23.3%. IR KBr (cm^{-1}): 3402 (OH), 2926 (C-H), 1655 (C=O), 1602 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.16 (1H, *d*, *J* = 15.5 Hz), 8.13 (2H, *d*, *J* = 8.5 Hz), 7.90 (1H, *d*, *J* = 15.5 Hz), 7.38 (1H, *d*, *J*

= 2.5 Hz), 7.08 (2H, *d*, *J* = 8.5 Hz), 6.93 (1H, *d*, *J* = 9.0 Hz), 6.90 (1H, *dd*, *J* = 2.5, 9.0 Hz), 4.13 (2H, *t*, *J* = 6.5 Hz), 3.82 (3H, *s*), 1.80-1.86 (2H, *m*), 1.48-1.53 (2H, *m*), 1.38-1.43 (4H, *m*), 1.30-1.35 (4H, *m*), 0.90 (3H, *t*, *J* = 7.0 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.5, 163.9, 154.2, 152.0, 139.4, 132., 131.5, 123.4, 122.5, 119.1, 118.0, 115.2, 113.0, 69.0, 56.1, 32.6, 30.0 (3C), 26.7, 23.3, 14.3; MS (ESI): *m/z* (100%) 405.2021 $(M+Na)^+$. $C_{27}H_{35}NO_5$ $(M+Na)^+$ requires 405.2036.

(E)-1-[4-(nonyloxy)phenyl]-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5c)

Yellow solid. Yield: 14.7%. IR KBr (cm^{-1}): 3407 (OH), 2925 (C-H), 1656 (C=O), 1601 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.15 (1H, *d*, *J* = 15.5 Hz), 8.13 (2H, *d*, *J* = 9.0 Hz), 7.89 (1H, *d*, *J* = 15.5 Hz), 7.37 (1H, *d*, *J* = 2.5 Hz), 7.07(2H, *d*, *J* = 9.0 Hz), 6.94 (1H, *d*, *J* = 9.0 Hz), 6.89 (1H, *dd*, *J* = 2.5, 9.0 Hz), 4.11 (2H, *t*, *J* = 6.5 Hz), 3.81 (3H, *s*), 1.78-1.83 (2H, *m*), 1.47-1.52 (2H, *m*), 1.37-1.42 (4H, *m*), 1.30-1.35 (4H, *m*), 0.89 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.5, 163.9, 154.2, 152.0, 139.4, 132.2, 131.5, 123.4, 122.5, 119.1, 118.0, 115.2, 113.1, 68.9, 56.1, 32.6, 30.3, 30.1, 30.0, 29.9, 26.7, 23.3, 14.3; MS (ESI): *m/z* (100%) 419.2177 $(M+Na)^+$. $C_{25}H_{32}O_4$ $(M+Na)^+$ requires 419.2193.

(E)-1-(4-(decyloxy)phenyl)-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5d)

Yellow solid. Yield: 12.6%. IR KBr (cm^{-1}): 3440 (OH), 2924 (C-H), 1645 (C=O), 1601 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.16 (1H, *d*, *J* = 16.0 Hz), 8.14 (2H, *d*, *J* = 9.0 Hz), 7.90 (1H, *d*, *J* = 16.0 Hz), 7.39 (1H, *d*, *J* = 3.0 Hz), 7.07 (2H, *d*, *J* = 9.0 Hz), 6.94 (1H, *d*, *J* = 9.0 Hz), 6.90 (1H, *dd*, *J* = 3.0, 9.0 Hz), 4.10 (2H, *t*, *J* = 6.5 Hz), 3.82 (3H, *s*), 1.82 (2H, *m*), 1.47-1.52 (2H, *m*), 1.37-1.41 (4H, *m*), 1.27-1.34 (8H, *m*), 0.89 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.5, 163.9, 154.2, 152.0, 139.4, 132.1, 131.5, 123.4, 122.4, 119.1, 117.9, 115.1, 113.0, 68.9, 56.0, 32.6, 30.3, 29.3, 30.1, 29.9, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 409.2383 $(M-H)^+$. $C_{26}H_{34}O_4$ $(M-H)^+$ requires 409.2384.

(E)-1-(4-(dodecyloxy)phenyl)-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5e)

Yellow solid. Yield: 28.0%. IR KBr (cm^{-1}): 3420 (OH), 2922 (C-H), 1647 (C=O), 1600 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.16 (1H, *d*, *J* = 16.0 Hz), 8.13 (2H, *d*, *J* = 9.0 Hz), 7.90 (1H, *d*, *J* = 16.0 Hz), 7.38 (1H, *d*, *J* = 3.0 Hz), 7.08 (2H, *d*, *J* = 9.0 Hz), 6.94 (1H, *d*, *J* = 9.0 Hz), 6.90 (1H, *dd*, *J* = 3.0, 9.0 Hz), 4.11 (2H, *t*, *J* = 6.5 Hz), 3.82 (3H, *s*), 1.77-1.83 (2H, *m*), 1.43-1.47 (2H, *m*), 1.38-1.43 (4H, *m*), 1.30-1.35 (12H, *m*), 0.89 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.5, 163.9, 154.2, 152.4, 139.4, 132.1, 131.5, 123.4, 122.4, 119.1, 117.9, 115.1, 112.9, 68.9, 56.0, 32.6, 30.4, 30.4, 30.3, 30.1, 29.9, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 437.2663 $(M-H)^+$. $C_{28}H_{28}O_4$ $(M-H)^+$ requires 437.2697.

(E)-1-[4-(tetradecyloxy)phenyl]-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5f)

Yellow solid. Yield: 12.1%. IR KBr (cm^{-1}): 3426 (OH), 2920 (C-H), 1650 (C=O), 1604 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.01 (1H, *d*, *J* = 16.0 Hz), 7.99 (2H, *d*, *J* = 9.0 Hz), 7.76 (1H, *d*, *J* = 16.0 Hz), 7.24 (1H, *d*, *J* = 3.0 Hz), 6.93 (2H, *d*, *J* = 9.0 Hz), 6.80 (1H, *d*, *J* = 9.0 Hz), 6.75 (1H, *dd*, *J* = 3.0, 9.0 Hz), 3.96 (2H, *t*, *J* = 6.5 Hz), 3.81 (3H, *s*), 1.64-1.69 (2H, *m*), 1.33-1.37 (2H, *m*), 1.23-1.27 (4H, *m*), 1.14-1.18 (16H, *m*), 0.75 (3H, *t*, *J* = 6.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.6, 163.9, 154.2, 152.0, 139.5, 132.2, 131.5, 123.4, 122.5, 119.1, 118.0, 115.1, 113.0, 68.9, 56.1, 32.6, 30.4 (3C), 30.1, 29.9, 26.7, 23.3, 14.4; MS (ESI): *m/z* (100%) 465.3016 $(M-H)^+$. $C_{23}H_{28}O_4$ $(M-H)^+$ requires 465.3016.

(E)-1-[4-(nonadecyloxy)phenyl]-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (5g)

Yellow solid. Yield: 3.9% IR KBr (cm^{-1}): 3401 (OH), 2921 (C-H), 1643 (C=O), 1605 (C=C); ^{1}H NMR (δ, ppm, 500 MHz, Acetone-d_6): 8.01 (1H, *d*, *J* = 16.0 Hz), 7.99 (2H, *d*, *J* = 9.0 Hz), 7.75 (1H, *d*, *J* = 16.0 Hz), 7.24 (1H, *d*, *J* = 3.0 Hz), 6.93 (2H, *d*, *J* = 9.0 Hz), 6.79 (1H, *d*, *J* = 9.0 Hz), 6.75 (1H, *dd*, *J* = 3.0, 9.0 Hz), 3.98 (2H, *t*, *J* = 6.5 Hz), 3.82 (3H, *s*), 1.63-1.67 (2H, *m*), 1.35-1.40 (2H, *m*), 1.24-1.28 (4H, *m*), 1.13-1.18 (26H, *m*), 0.85 (3H, *t*, *J* = 7.5 Hz); ^{13}C NMR (δ, ppm, 125 MHz, Acetone-d_6): 188.1, 163.4, 153.7, 151.6, 139.0, 131.0, 130.9, 122.9, 122.0, 118.6, 117.5, 114.6, 112.5, 68.1, 55.6, 31.4, 30.1, 29.9, 29.6, 26.7, 23.3, 13.6; MS (ESI): *m/z* (100%) 535.3913 $(M-H)^+$. $C_{35}H_{52}O_4$ $(M-H)^+$ requires 535.3913.

Antituberculosis activity assay

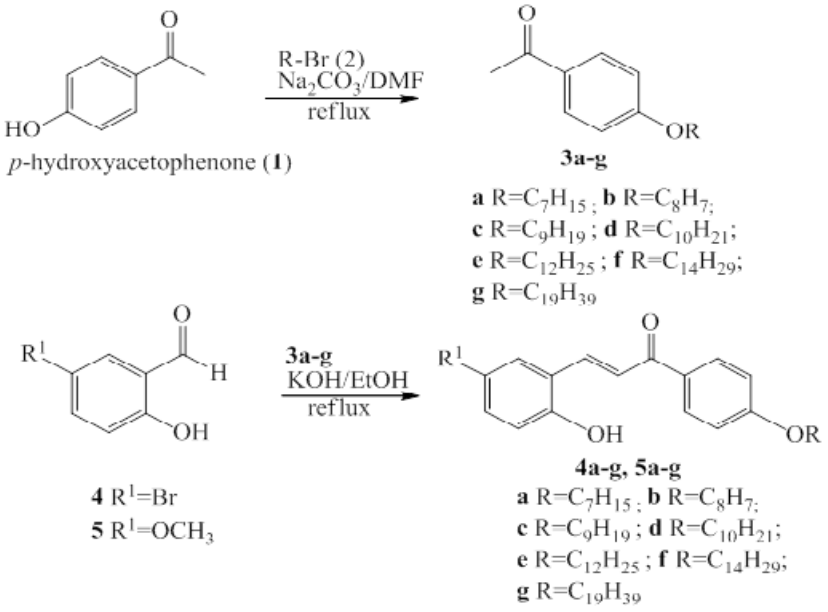
The *M. tuberculosis* inoculum was prepared from a log phase culture in Middlebrook 7H9 broth (Difco, USA) supplemented with albumin, dextrose, and catalase (ADC) and its turbidity was adjusted to McFarland standard no. 1 (approximately 3×10^7 CFU/ mL). The bacterial suspension was further diluted 1:20 in Middlebrook 7H9 broth supplemented with OADC (oleic acid, albumin, dextrose and catalase). The antituberculosis activity was performed by a colorimetric tetrazolium microplate assay (TEMA) as reported by Arshad et al. [28].

RESULTS AND DISCUSSION

Chemistry

The general synthetic route employed to prepare the chalcone derivatives was based on Claisen–Schmidt condensation. Two series of fourteen chalcone derivatives (**4a–g** and **5a-g**) were prepared by the condensation of substituted benzaldehydes and appropriately substituted acetophenone in equimolar ratio to form the expected compounds, using potassium hydroxide as a catalyst (Scheme 1). All the synthesized compounds were confirmed by chromatography and characterized spectroscopically. The first step of the synthesis involved the preparation of compounds **3a–g** as shown in Scheme 1. In the first series, compounds (**5a–g**) were synthesized by treating 5-bromosalicylaldehyde (**5**) with a series of *p*-alkoxyacetophenone **(3a-g**) in the presence of potassium hydroxide in ethanol. Purification by column chromatography using silica gel (eluent: *n*-hexane/acetone: 8:2 v/v), produced a series of the (*E*)-1-[4-(alkoxy)phenyl]-3-(2-hydroxy-5-bromophenyl)prop-2-en-1-one (**4a–g**) compounds. Interestingly, infra red spectroscopic analysis of chalcone derivatives **4a–g** revealed the presence of new bands of vinyl (C=C) and carbonyl (C=O) functional groups with absorptions at 1600-1606 and 1638-1646 cm^{-1} respectively, as well as other characteristic absorption bands. These absorption bands were characteristics for α,β-unsaturated ketone. The ^{1}H NMR of **4a-g** indicated the presence of two doublets of vinylic protons (CH=CH) at δ 7.83–8.09 (2H, *d*, *J* = 15.5-16.0 Hz, H-2 & H-3) and one set of long alkoxyl chains at δ 0.87–0.89 ppm and 3.99–4.12 ppm. Furthermore, the ^{13}C NMR spectroscopic analysis also confirmed structural identity, with resonances observed at δ 123.59-137.70 and 14.33-68.98 ppm. In addition, high resolution mass spectrometry (EI) showed accurate molecular ion peaks for all derivatives. Mass spectroscopy displayed the molecular weight of the synthesized compounds and fully supported structural assignment.

A second series of chalcone derivatives **5a-g** was prepared by a related route (Scheme 1). A series of *p*-alkoxyacetophenone **(3a-g**) were treated with 5-methoxysalicylaldehyde (**5**) in the presence of potassium hydroxide in ethanol and afforded a series of the (*E*)-1-[4-(alkoxy)phenyl]-3-(2-hydroxy-5-methoxyphenyl)prop-2-en-1-one (**5a–g**) compounds. The structures of this series of derivatives were established on the basis of spectroscopic data including IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometric data. The spectroscopic analysis of this series revealed the same diagnostic absorption bands and resonances. All the chalcone derivatives displayed the molecular ion peak equivalent to the molecular weight of the proposed compounds. Hence m/z value confirmed the molecular weight of the respective synthesized compounds.



Scheme 1. Synthesis route of the chalcone derivatives 4a–g; 5a–g

Antituberculosis activity

The two series of fourteen chalcone derivatives (**4a–g** and **5a-g**) were screened against *Mycobacterium tuberculosis* H37Rv strain ATCC 25618. The results of antituberculosis activity of these compounds are shown in Table 1. In the present study, seven of the synthesized chalcones were found to be active against the mycobacterial strain with MIC values in the range of 93 – 27 μM.

In the first series (**4a-g**), compound **4d** exhibited the highest antituberculosis activity with the lowest MIC value of 27 µM, followed by **4a** and **4b** with the MIC values of 30 and 29 µM**,** respectively. In addition **4c** also exhibited promising activity with MIC value of 56 µM. The results for this series indicate that the antituberculosis activity was not dependent on the length of the alkyl chain. These results also indicate a general trend of activity, that is, the compounds with a bromine substituent demonstrated stronger antituberculosis activity than those with a methoxyl substituent at the same position. It was apparent that the presence of bromine in the structure enhanced the activity of these compounds (**4a-g**).

Table 1. MIC values of the tested compounds

Compounds	MIC in µM
4a	30
4b	29
4c	56
4d	27
4e	102
4f	97
4g	85
5a	135
5b	65
5c	126
5d	121
5e	114
5f	53
5g	93
Isoniazid	1.3

In the second series, three (**5b, f, g)** out of seven compounds exhibited antituberculosis activity in the range of 93 – 53 µM. The most active compounds were **5b** (8 carbon chain), and **5f** (14 carbon chain), affirming that the alkyl chain length did not play a significant role in the antituberculosis activity. In general, the compounds in this series (**5a-g**) exhibited lower activity compared with the first series (**4a-g**). This implied that the presence of hydrophilic substituents, such as methoxyl, resulted in a decrease of antituberculosis activity. In addition, the hydrophobicity of the long alkyl chain substituent for the chalcone derivatives could be a contributing factor for their antituberculosis activity.

CONCLUSION

In conclusion, we have reported the synthesis, structure elucidation and biological evaluation of fourteen chalcone derivatives containing various alkyl chains length and different subtituents. Study of their antituberculosis activity revealed that the chalcone derivatives with a bromine substituent exhibited stronger antituberculosis activity than those with a methoxyl substituent. However, it was found that the presence of bromine and the hydrophobicity of the long alkyl chain substituent for the chalcone derivatives could be contributing factors for their antituberculosis activity. The results of this study could be useful for future efforts to synthesize and evaluate chalcone derivatives in order to enhance their antituberculosis activity.

Acknowledgments
The authors thank the Universiti Sains Malaysia (USM) for funding this research project (RUT: 1001/PKIMIA/855006 and RUI: 1001/PBIOLOGI/815084). We are also thankful to Assoc. Prof. Dr. David Larsen and Mr. Ian Stewart from the University of Otago, New Zealand, for the HRESIMS spectra. Noviany thanks the Directorate General of Higher Education, the Ministry of Research Technology and Higher Education of Indonesia and the MTCP scholarship funded by Ministry of Higher Education of Malaysia as well as USM fellowship for financial support.

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