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2-HYDROXY-4-METHOXYBENZALDEHYDE: LARVICIDAL STRUCTURE-ACTIVITY STUDIES

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ABSTRACT. 2-Hydroxy-4-methoxybenzaldehyde (**1**), a compound isolated from *Mondia whytei* (Hook) Skeels (Asclepiaceae) roots exhibited larvicidal activity (LD_{50} 22 μg/mL). A total of 18 other derivatives and closely related congeners revealed varying levels of larvicidal activity. Several closely related congeners, like 2-benzyloxy-4-methoxybenzaldehyde (**2**), 2-hydroxybenzaldehyde (**12**), 2-benzyloxybenzaldehyde (**3**) and benzylphenyl ether (**4**), showed marked improvement in activity (LD_{50} 10, 9, 4.8, 1.2 μg/mL, respectively) against *Anopheles gambiae* larvae. 2-Benzoyloxy-4-methoxybenzaldehyde (**5**) exhibited similar activity level (LD_{50} 28 μg/mL) as **1**.

KEY WORDS: 2-Hydroxy-4-methoxybenzaldehyde, Larvicides, Structure-activity relationships, 2-Hdroxybenzaldehyde, 2-Benzyloxybenzaldehyde, Benzylphenyl ether *Anopheles gambiae*,

INTRODUCTION

Each year 300-500 million people are infected and more than 1 million, mostly children under the age of 5, die of malaria [1]. Malaria parasites have become increasingly resistant to the most commonly used and affordable drugs like chloroquine [2], amodiaquine [3], mefloquine [4]. *In vitro* resistance to artemisinin has also been reported [5]. The search for cost effective vaccines is on, but several hurdles are yet to be surmounted making it a long-term disease control strategy [6].

Malaria was eradicated in the USA, USSR, southern Europe and most of the Caribean Islands mainly through vector control between the 1940s and 1960s [7-8]. Emphasis needs to be placed on vector control once again. Several organochlorine-based insecticides have been used in the control of arthropod disease vectors. The use of DDT both as an adulticide and larvicide since its discovery led to spectacular suppression of arthropod diseases [9]. Other compounds that have had positive impact on vector control include organophosphates, carbamates and pyrethrins [10]. Unfortunately, these compounds have been found to affect non-target organisms, accumulate in the food chain and persist in the environment [11]. Furthermore, mosquitoes have developed resistance to most of the synthetic insecticides [12-13].

Natural products have also been exploited for vector control as exemplified by the insecticidal properties of pyrethrins [14]. Other potential insecticides include azadirachtin, from neem tree [15]; rotenone, from several *Derris* species [16]; and ryanodine, from *Ryania speciosa* [17]. The excellent repellent and insecticidal properties of the synthetic pyrethroids as a result of qualitative structure-activity relationship studies [18-19] have inspired a cascade of research on other natural compounds as templates for more potent synthetic insecticides [20-21].

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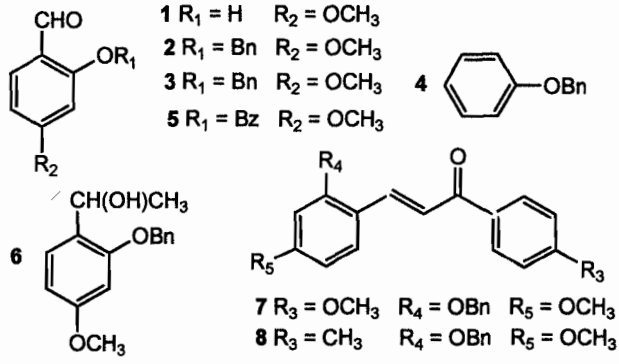
2-Hydroxy-4-methoxybenzaldehyde (**1**) has been previously isolated from the roots of *Mondia whytei* (Hook) Skeels (Asclepiaceae) [22]. It was shown to be a tyrosinase inhibitor [23] and responsible for the characteristic taste of the plant roots [24]. With an LD_{50} value of 22 μg/mL, the compound prompted us to undertake structure-activity studies to probe the functional groups responsible for activity. We hereby report the mosquito larvicidal activity of 2-hydroxy-4-methoxybenzaldehyde (**1**), 18 derivatives and closely related congeners.



RESULTS AND DISCUSSION

The benzyl ethers, **2-4** were synthesized in good yields by the solvent-based benzylation method in ethanol [25]. The respective phenols were reacted with benzyl chloride in presence of potassium carbonate. The singlets in ^{1}H NMR at δ 5.27 and 5.07 indicated the presence of the benzylic methylene protons confirming successful benzylation to give **2-4**.

Benzoylation of **1** under the Schotten-Baumann conditions [26] realized the phenyl ester **5** as confirmed by the presence of the peak at δ 154.0 in ^{13}C NMR. The Grignard reaction of **2** with methylmagnesium bromide yielded **6** in good amounts as confirmed by the absence of the peak between δ 9-10 due to the aldehydic proton in **2** and the presence of a doublet at δ 1.26 (3H, J = 5.6 Hz) in ^{1}H NMR attributed to the terminal methyl group in **6**. The peak at δ 4.93 was assigned to the hydroxyl group. The presence of the alcohol (**6**) was confirmed by the peak at δ 62.2 in ^{13}C NMR.

The chalcones, **7** and **8**, were obtained in good yields by the Claisen-Schmidt aldol condensation of **2** with 4-methoxyacetophenone and 4-methylacetophenone, respectively, in the presence of sodium hydroxide [27]. *Trans*-olefinic coupling constants (14.4 and 15.8 Hz, respectively) in ^{1}H NMR of **7** and **8** confirmed the geometry of the double bond.

The larvicidal activities of 2-hydroxy-4-methoxybenzaldehyde (**1**), derivatives (**2** and **5**) and congeners (**3-4**, **6-19**) against *Anopheles gambiae* are summarized in Table 1. Interestingly, the phenyl ester (**5**) had similar larvicidal activity levels (LD_{50} 28 μg/mL) to 2-hydroxy-4-methoxybenzaldehyde (**1**) (LD_{50} 22 μg/mL). The benzyl ether (**2**) had improved activity. The activity of the benzyl ether (**2**) (LD_{50} 10 μg/mL) was significantly lowered when the aldehyde group was converted into the alcohol (**6**) (LD_{50} 75 μg/mL, 7.5 times) via Grignard reaction to a level much lower than for **1** (3 times). The absence of the 4-methoxyl coupled with protection of the hydroxyl group as in **3** resulted in remarkable increase of activity (LD_{50} 4.8 μg/mL, 4 times). These observations indicated the significance of the aldehyde and hydroxyl groups for the

larvicidal activity of 2-hydroxy-4-methoxybenzaldehyde (**1**). This was further confirmed by the improved activity observed for 2-hydroxybenzaldehyde (**12**) (LD_{50} 9 μg/mL). However, both groups are necessary for the larvicidal activity as confirmed by the significant reduction in activity observed for benzaldehyde (**9**) and phenol (**10**) at 55 and 54 μg/mL, respectively, while methoxybenzene (**11**) was inactive (LD_{50} >100 μg/mL). Surprisingly, benzyl phenyl ether (**4**) (LD_{50} 1.2 μg/mL) was found to be 45 times more active than benzaldeyde (**9**) and phenol (**10**), 20 times more than 2-hydroxy-4-methoxybenzaldehyde (**1**) and 4 times more than 2-benzyloxybenzaldehyde (**3**). This suggests that even the aldehyde group has some antagonistic properties on larvicidal activity.

Table 1. LD_{50} values of synthetic compounds against *Anopheles gambiae s.s.*

Compound number	Name	LD_{50} (μg/mL)
1	2-Hydroxy-4-methoxybenzaldehyde	22
2	2-Benzyloxy-4-methoxybenzaldehyde	10
3	2-Benzyloxybenzaldehyde	4.8
4	Benzyl phenyl ether	1.2
5	2-Benzoyloxy-4-methoxybenzaldehyde	28
6	2-Benzyloxy-4-methoxy-1-(1'-hydroxyethyl)benzene	75
7	2-Benzyloxy-4,4'-dimethoxychalcone	59
8	2-Benzyloxy-4-methoxy-4'-methylchalcone	>100
9	Benzaldehyde	55
10	Phenol	54
11	Methoxybenzene	>100
12	2-Hydroxybenzaldehyde	9
13	2-Methoxyphenol	>100
14	3-Methoxyphenol	77
15	4-Methoxyphenol	62
16	4-Hydroxybenzaldehyde	>100
17	4-Methoxybenzaldehyde	32
18	4-Hydroxy-3-methoxybenzaldehyde	>100
19	4-Methoxysalicylic acid	58

The initial results suggested that the presence of a hydroxyl *ortho* to a carbonyl group boosted the activity as observed in **12**. However, shifting it to the *para* position lowered the activity as demonstrated in **16**. Similarly, a methoxyl group *para* to the carbonyl on the other hand showed antagonistic effect as exhibited by **17**. The same observation was made when the methoxyl group was at *ortho*, *meta* or *para* position to the hydroxyl groups as in **13-15**. The antagonistic effect of the methoxyl group was further revealed on the trisubstituted compounds like **5**, **7**, **8** and **18**. Likewise, the carboxylic acid group was also evidently antagonistic as manifested in **19**. The increased activity on benzylation suggests that the benzyl group enhances activity more than the hydroxyl even in the absence of *m*-carbonyl and *o*-methoxyl groups.

The ester moiety on the other hand has a slight diminishing effect on activity as observed in **5** whose activity was marginally lower than **2**. The presence of the carbonyl is important in eliciting larvicidal activity as elaborated by its absence and the subsequent low activity of **6** which is about 3 times lower than **2**. This was further demonstrated by a substantial decrease in activity of the chalcones (**7** and **8**). However, between the chalcones, **7** had significantly higher activity than **8**. The difference may be attributed to the *p*-methoxyl and *p*-methyl groups on ring 2. The *p*-methoxyl group may have a potentiating effect on the activity contrary to what is observed with the simpler derivatives.

EXPERIMENTAL

General procedures

Solvents and reagents of over 99% purity were used as supplied from Sigma-Aldrich and Merck-BDH. Analytical thin layer chromatography was performed on Merck pre-coated silica gel 60 F_{254} (5 x 10 cm and 0.20 mm film thickness) and visualization done under UV light and/or sprayed with 25% sulphuric acid in methanol and oven dried. Column chromatography was done on silica gel (0.040-0.063 mm, 230-400 mesh (Merck) and eluted with different solvent systems.

Melting points were determined on Sanyo Gallenkamp electronic melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were obtained from a Shimadzu double beam spectrophotometer (UV-180). Infrared (IR) spectra were run on Shimadzu Fourier transform spectrometer as KBr disks. Nuclear magnetic resonance (NMR) spectra were run on Varian Mercury Gemini 200 MHz spectrometer. ^{1}H and proton noise decoupled ^{13}C NMR spectra were recorded in $CDCl_3$ and $(CD_3)_2SO$, at 200 and 50 MHz, respectively, and are expressed in δ values (ppm) relative to tetramethylsilane. Coupling constants (*J*) in ^{1}H NMR spectra were measured in Hz. COSY spectra were used to determine neighbouring protons. DEPT was used to determine the multiplicity of ^{13}C NMR peaks. Electron impact mass spectra (EIMS) were obtained from Fission VG Platform II spectrometer at 70 eV.

Larvicidal assays were done according to the WHO [28] protocol at different concentrations (1–100 μg/mL) in triplicates. Mortality was recorded after 24, 48 and 72 hours of exposure of the larvae to various concentrations of the compounds in water under a controlled temperature holding room (38 ^{O}C) and humidity. Mortality was determined using the formula:

$$\% \text{ Mortality} = [Y/X] \times 100$$

Y = mean death count, X = initial larvae population. Abbot's formula [29] was used to correct the percentage mortality. Expected probits and the lethal doses were calculated according to Busvine [30].

Synthetic procedures

2-Benzyloxy-4-methoxybenzaldehyde (2). A mixture of 2-hydroxy-4-methoxybenzaldehyde (**1**) (0.46 g, 3 mmol), benzyl chloride (0.38 g, 3 mmol), sodium bicarbonate (0.42 g, 5 mmol) and ethanol (25 mL) was stirred under reflux for 5 h. The mixture developed a deep green colour that later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* and purified by column chromatography to give 0.39 g (53%) of **2** (R_f 0.33, 8:2 hexane:EtOAc, SiO_2). Alternatively, after refluxing for 5 h, the mixture was cooled for 18 h in a refrigerator forming long needle-like crystals. The crystals were separated by filtration under suction, washed with cold ethanol to give white needle-like crystals of **2** (R_f 0.33) in 83%. Found: m.p. 54-56 ^{O}C; UV λ_{max} (DMSO): 324 nm; IR ν_{max} (KBr): 3020, 2867, 2830, 2780, 1670, 1577, 1502 cm^{-1}; ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 10.22 (*s*, 1H, -CHO), 7.66 (*dd*, 1H, *J* = 8.8, 1.2 Hz, H-5), 7.30-7.51 (*m*, 5H, C_6H_5), 6.79 (*d*, 1H, *J* = 1.2 Hz, H-3), 6.65 (*d*, 1H, *J* = 8.8 Hz, H-6), 5.27 (*s*, 2H, H-7), 3.83 (*s*, 3H, OCH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$): δ 187.9 (*s*, CHO), 166.6 (*s*, C-4), 163.2 (*s*, C-2), 137.1 (*s*, C-1'), 130.6 (*d*, C-6), 129.2 (*d*, C-2',6'), 128.7 (*d*, C-4'), 128.2 (*d*, C-3', 5'), 119.3 (*s*, C-1), 107.8 (*d*, C-5), 100.4 (*d*, C-3), 70.7 (*t*, C-7), 56.5 (*q*, -OCH_3); EIMS: *m/z* 242 [M^+] (1), 213 (20), 151 (24), 91 (100), 77 (8), 65 (82), 51 (26), 39 (30).

2-Benzyloxybenzaldehyde (3). Benzyl chloride (2.06 g, 16 mmol), 2-hydroxybenzaldehyde (**12**) (2 g, 16 mmol), sodium bicarbonate (1.5 g, 18 mmol) and ethanol (25 mL) were mixed and stirred under reflux 5 h. The mixture developed a deep green colour that later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* to give 1.74 g (50%) of **3** as white needle-like crystals (R_f 0.53, 8:2, hexane:EtOAc, SiO_2). Found: m.p. 42-43 ^{O}C; UV λ_{max} (DMSO): 324 nm; IR ν_{max} (KBr): 3059, 2877, 2750, 1681, 1596, 1500, 1238, 1161 cm^{-1}; ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 10.41 (*s*, 1H, -CHO), 7.67 (*dd*, 1H, *J* = 8.4, 8.6 Hz, H-5), 7.61 (*d*, 1H, *J* = 8.4 Hz, H-6), 7.37 (*d*, 1H, *J* = 7.4 Hz, H-3), 7.27-7.42 (*m*, 5H, H-2', H-3', H-4', H-5', H-6'), 7.07 (*dd*, 1H, *J* = 8.6, 7.4 Hz, H-4), 5.27 (*s*, 2H, OCH_2); ^{13}C NMR (200 MHz, $(CD_3)_2SO$): δ 189.9 (*d*, -CHO), 161.3 (*s*, C-2), 137.13 (*d*, C-1'), 137.05 (*s*, C-6), 129.2 (*d*, C-2', C-6'), 128.7 (*d*, C-4), 128.5 (*d*, C-4'), 128.2 (*d*, C-3', 5'), 125.2 (*s*, C-1), 121.6 (*s*, C-5), 114.7 (*d*, C-3), 70.5 (*t*, C-7); EIMS: *m/z* 212 [M$^+$] (9), 183 (23), 121 (58), 92 (48), 91 (100), 77 (10), 65 (87), 51 (21) 39 (51).

Benzylphenyl ether (4). Phenol (**10**) (0.46 g, 5 mmol), benzyl chloride (0.63 g, 5 mmol), sodium bicarbonate (0.42 g, 5 mmol) and ethanol (25 mL) were mixed and stirred under reflux for 5 h. The mixture developed a deep green colour that later became brown. The mixture was cooled to room temperature, potassium chloride removed by filtration and washed with ethanol. The filtrate was evaporated *in vacuo* to give 0.68 g (76%) of **4** as white needle-like crystals (R_f 0.78, 8:2, hexane: EtOAc, SiO_2). Found: m.p. 34-35 ^{O}C; UV λ_{max} (DMSO): 277 nm; IR ν_{max} (KBr): 3035, 2862, 1245 cm^{-1}; ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 7.27-7.42 (*m*, 7H, H-2', H-3', H-4', H-5', H-6', H-3, H-5), 6.99 (*d*, 2H, *J* = 8.4 Hz, H-2, H-6), 6.92 (*m*, 1H, H-4), 5.07 (*s*, 2H, H-7); ^{13}C NMR (200 MHz, $(CD_3)_2SO$): δ 159.0 (*s*, C-1), 137.8 (*s*, C-1'), 130.2 (*d*, C-3, 5), 129.1 (*d*, C-2', 6'), 128.5 (*d*, C-4'), 128.4 (*d*, C-3', 5'), 121.4 (*d*, C-4), 115.4 (*d*, C-2, 6), 69.7 (*t*, C-7); EIMS: *m/z* 184 [M$^+$] (29), 92 (32), 91 (100), 77 (14), 65 (89), 51 (37), 39 (73).

2-Benzoyloxy-4-methoxybenzaldehyde (5). Benzoyl chloride (1.5 g, 12 mmol) was added dropwise to a stirred solution of 2-hydroxy-4-methoxybenzaldehyde (**1**) (1 g, 6.6 mmol) in 20 mL of anhydrous pyridine over a period of 10 min. The solution was warmed to 50 ^{O}C, stirred for 40 min., poured into a mixture of 20 g ice and 50 mL of 1 M HCl and stirred until a fine crystalline suspension was formed. The crude product was collected by filtration, washed with ice-acid water and re-crystallized from 80% MeOH to give 0.95 g (57%) of **5** as white crystalline product (R_f 0.32, 8:2 hexane: EtOAc, SiO_2). Found: m.p. 76-77 ^{O}C; UV λ_{max} (DMSO): 304 nm; IR ν_{max} (KBr): 2767, 1693, 1566, 1500, 1265, 1217, 1132 cm^{-1}; ^{1}H NMR (200 MHz $(CD_3)_2SO$): δ 10.04 (*s*, 1H, -CHO), 8.21 (*d*, 2H, *J* = 7.2 Hz, H-2', H-6'), 7.88 (*d*, 1H, *J* = 8.6Hz, H-6), 7.66 (*dd*, 1H, *J* = 7.2, 7.5 Hz, H-4'), 7.52 (*dd*, 2H, *J* = 7.0, 7.5 Hz, H-3', H-5'), 6.92 (*dd*, 1H, *J* = 8.6, 2.1 Hz, H-5), 6.80 (*d*, 1H, *J* = 2.1 Hz, H-3), 3.87 (*s*, 3H, OCH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$): δ 187.2 (*s*, -CHO), 165.2 (*s*, C-4), 164.8 (*s*, C-2), 154.0 (*s*, C-7), 134.0 (*s*, C-4'), 132.0 (*d*, C-6), 130.3 (*d*, C-2', 6'), 130.1 (*d*, C-1'), 128.7 (*d*, C-3', 5'), 121.8 (C-1), 112.6 (*d*, C-5), 108.6 (*d*, C-3), 55.9 (*q*, OCH_3); EIMS: *m/z* 256 [M$^+$] (1), 151 (16), 122 (86), 105 (97), 77 (100), 51 (62).

2-Benzyloxy-4-methoxy-1-(1'-hdroxyethyl)benzene (6). 2-Benzyloxy-4-methoxybenzaldehyde (**2**) (0.05 g, 0.2 mmol) was dissolved in anhydrous ether (10 mL) and put in a dropping funnel. Methyl magnesium bromide (1.4 M solution in ether) (1.43 mL, 2 mmol) was put in a three-neck flask in which a stream of dry nitrogen was flowing and the solution of 2-benzyloxy-4-methoxybenzaldehyde (**2**) added slowly with vigorously stirring. The mixture was stirred for 4 h, 5 mL mixture of distilled water/HCl (50:50) added and the organic layer separated, dried with anhydrous $CaSO_4$ and evaporated *in vacuo*. Analysis of the concentrate by TLC revealed two

spots (R_f 0.30 and 0.33, 8:2 hexane:EtOAc, SiO_2). These were separated by column chromatography using hexane/ethyl acetate. The product (**6**) (R_f 0.30) was recovered in 57% (0.03 g) yield. Found: m.p. 46-48 ^{o}C; UV λ_{max} (DMSO): 305 nm; IR ν_{max} (KBr): 3284, 2934, 2831, 1504, 1255, 1195, 1082, 1014 cm^{-1}; ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 7.40-7.43 (*m*, 5H, H-2', H-3', H-4', H-5', H-6'), 7.34 (*d*, 1H, *J* = 8.4 Hz, H-6), 6.58 (*s*, 1H, H-3), 6.52 (*d*, 1H, *J* = 8.4 Hz, H-5), 5.11 (*s*, 2H, -OCH_2-), 5.01 (*q*, 1H, J = 5.6 Hz, H-8), 4.92 (br *s*, 1H, OH), 3.71 (*s*, 3H, OCH_3), 1.26 (*d*, 3H, *J* = 5.6 Hz, -CH_3); ^{13}C NMR (200 MHz, $(CD_3)_2SO$): δ 158.6 (*s*, C-2), 154.8 (*s*, C-4), 137.1 (*s*, C-1'), 128.2 (*d*, C-2', 6'), 127.8 (*d*, C-1), 127.5 (*d*, C-6), 127.0 (*d*, C-3', 5'), 125.9 (*d*, C-4'), 104.5 (*d*, C-5), 99.0 (*d*, C-3), 69.0 (*t*, C-7), 62.2 (*d*, C-8), 55.0 (*q*, OCH_3), 24.9 (*q*, C-9); EIMS: *m/z* 258 [M^+] (4), 240 (8), 150 (14), 91 (100), 77 (8), 65 (16).

2-Benzyloxy-4,4'-dimethoxychalcone (7). Sodium hydroxide (0.5 g, 12.5 mmol), 5 mL of distilled water and 5 mL of ethanol were placed in a 50 mL round bottom flask equipped with a magnetic stirrer. The flask was immersed in an ice bath and 0.60 g (3.6 mmol) of *p*-methoxyacetophenone dissolved in ethanol (10 mL) added while stirring followed by drop-wise addition of 2-benzyloxy-4-methoxybenzaldehyde (**2**) (1 g, 4 mmol). The temperature of the mixture was maintained at 25 ^{o}C and stirring continued for 4 h. The stirrer was removed and the reaction mixture refrigerated for 18 h. The product was filtered under suction, washed with 40 mL of cold distilled water followed by ice-cold ethanol and re-crystallized from boiling ethanol to give pale 1.2 g (89%) of yellow crystals of **7** (R_f 0.29, 8:2 hexane:EtOAc, SiO_2). Found: m.p. 112-112.5 ^{o}C; UV λ_{max} (DMSO): 376 nm; IR ν_{max} (KBr): 3350, 3060, 3049, 3000, 1649, 1598, 1249, 1050 cm^{-1}; ^{1}H NMR (200 MHz, $(CD_3)_2SO$): δ 7.81-7.96 (*m*, 2H, H-4", H-5), 7.90 (*d*, 2H, *J* = 10.2 Hz, H-3', H-5'), 7.84 (*d*, 1H, *J* = 14.4 Hz, H-7), 7.50 (*d*, 2H, *J* = 10.2 Hz, H-2', H-6'), 7.49 (*d*, 1H, *J* = 14.4 Hz, H-8), 7.44-7.53 (*m*, 2H, H-3", H-5"), 7.00 (*d*, 1H, *J* = 8.6 Hz, H-2" H-6"), 6.79 (*s*, 1H, H-3), 6.65 (*d*, 1H, *J* = 9.0 Hz, H-6), 5.23 (*s*, 2H, OCH_2), 3.85 (*s*, 3H, 4-OCH_3), 3.83 (*s*, 3H, 4'-OCH_3); ^{13}C NMR (200 MHz, $CD_3)_2SO$): δ 187.3 (C, C-9), 162.8 (*s*, C-4'), 162.6 (*s*, C-4), 159.1 (*s*, C-2), 138.5 (*d*, C-7), 136.5 (*s*, C-1"), 131.5 (*d*, C-1), 130.7 (*s*, C-6), 130.4 (*d*, C-2', 6'), 128.6 (*d*, C-2'', 6"), 128.13 (*d*, C-4''), 128.07 (*d*, C-3'', C-5''), 119.4 (*d*, C-8), 116.2 (*s*, C-1'), 113.8 (*d*, C-3', C-5'), 106.4 (*d*, C-5), 99.5 (*d*, C-3), 70.0 (*t*, C-7"), 55.5 (*t*, 4-OCH_3), 55.4 (*q*, 4'-OCH_3); EIMS: *m/z* 374 [M^+] (3), 267 (40), 135 (81), 91 (100), 77 (22), 65 (17).

2-Benzyloxy-4-methoxy-4'-methylchalcone (8). A mixture of sodium hydroxide (0.3 g, 7.5 mmol), distilled water (5 mL) and ethanol (5 mL) were placed in a 50 mL round bottom flask equipped with a magnetic stirrer. The flask was immersed in an ice bath and 0.16 g (1.1 mmol) of *p*-methylacetophenone dissolved in ethanol (10 mL) added while stirring followed by drop-wise addition of 2-benzyloxy-4-methoxybenzaldehyde (**2**) (0.3 g, 1.2 mmol). The temperature of the mixture was maintained at 25 ^{o}C and stirring continued for 4 h. The stirrer was removed and the reaction mixture refrigerated for 18 h. Pale yellow needle-like crystals (R_f 0.42, 8:2 hexane:EtOAc, SiO_2) of **8** were re-crystallized from boiling ethanol in 95% (0.36 g) yield. Found: m.p. 133-134 ^{o}C; UV λ_{max} (DMSO): 374 nm; IR ν_{max} (KBr): 3350, 3050, 3028, 3000, 2950, 2850, 1651, 1598, 1257, 1049 cm^{-1}; ^{1}H NMR (200 MHz $(CD_3)_2SO$): δ 7.71-7.93 (*m*, 5H, H-5, H-7, H-2', H-6', H-4"), 7.47-7.53 (*m*, 2H, H-3", H-5"), 7.49 (*d*, 1H, *J* = 10.8 Hz, H-3', H-5'), 7.48 (*d*, 1H, *J* = 16.4, H-8), 7.29 (*d*, 1H, *J* = 8.0 Hz, H-2", H-6"), 6.78 (*s*, 1H, H-3), 6.65 (*d*, 1H, *J* = 8.4 Hz, H-6), 5.22 (*s*, 2H, -OCH_2-), 3.83 (*s*, 3H, 4-OCH_3), 2.38 (*s*, 3H, 4' -CH_3); ^{13}C NMR (200 MHz, $(CD_3)_2$ SO): δ 188.1 (*s*, C-9), 162.5 (*s*, C-4), 158.9 (*s*, C-2), 142.8 (*s*, C-4'), 138.9 (*d*, C-7), 136.2 (*s*, C-1''), 135.1 (*s*, C-1'), 131.5 (*s*, C-1), 129.0 (*d*, C-2', 6'), 128.4 (*d*, C-3', C-5'), 128.0 (*d*, C-2", C-6"), 127.9 (*d*, C-3", 5", 4"), 119.2 (*d*, C-8), 115.9 (*s*, C-1'), 106.3 (*d*, C-5), 99.4 (*d*, C-3), 69.9 (*t*, C-7"), 55.5 (*q*, 4-OCH_3), 21.2 (*q*, 4'-CH_3); EIMS: *m/z* [M^+1] 359 (1), 358 (4), 251 (37), 119 (85), 91 (100), 65 (43), 51 (7).

CONCLUSIONS

Compound **4** showed the highest activity (LD_{50} 1.2 μg/mL), being 45 times higher than **10**, 8 times **12** and 20 times **1**. Next was found to be **3** (LD_{50} 4.8 μg/mL) and **2** (LD_{50} 2.2 μg/mL) suggesting that the benzyl group plays a vital role in the enhancement of activity. The carbonyl and hydroxyl groups are the most important functional groups for the larvicidal activity of this group of compounds especially when at a 1,2-position relative to each other. The methoxyl group has a major antagonistic effect on the larvicidal activity of 2-hydroxy-4-methoxy-benzaldehyde (**1**). The aldehyde group has slight antagonistic effect on the larvicidal activity of 2-hydroxy-4-methoxybenzaldehyde (**1**). The benzyl group is a powerful larvicidal protagonist. These compounds present a potentially useful class of larvicidal agents that need to be further studied.

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