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## Synthesis and tyrosinase inhibitory activities of 4-oxobutanoate derivatives of carvacrol and thymol

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### Abstract

Carvacrol (**1**) and thymol (**2**) were converted to their alkyl 4-oxobutanoate derivatives (**7–20**) in three steps, and evaluated for tyrosinase inhibitory activity. The compounds showed structure-dependent activity, with all alkyl 4-oxobutanoates, except **7** and **20**, showing better inhibitory activity than the precursor 4-oxobutanoic acids (**5** and **6**). In general, thymol derivatives exhibited a higher percent inhibitory activity than carvacrol derivatives at 500  $\mu\text{M}$ . Derivatives containing three and four carbon alkyl groups gave the strongest activity (carvacrol derivatives **9–12**,  $\text{IC}_{50}$  = 128.8–244.1  $\mu\text{M}$ ; thymol derivatives **16–19**,  $\text{IC}_{50}$  = 102.3–191.4  $\mu\text{M}$ ).

### Keywords

Carvacrol; thymol; alkyl 4-oxobutanoates; tyrosinase inhibition

The major components of oregano and thyme essential oils are the isomeric monoterpene phenols, carvacrol (**1**) and thymol (**2**). These phenols exhibit a wide range of biological and pharmacological effects including anticancer, anti-inflammatory, antibacterial, antifungal, anticholinesterase, insecticidal, and antioxidant activities.<sup>1–3</sup> Several derivatives of carvacrol and thymol have been synthesized and show similar or enhanced activity relative to the parent phenols. Ester and carbamate derivatives of both compounds showed increased antifungal<sup>4</sup> and anticholinesterase<sup>5</sup> activities, respectively. Some Schiff base derivatives exhibited similar or better antioxidant activity compared to thymol and ascorbic acid.<sup>6</sup> Heterocyclic derivatives of thymol containing pyridazinone,<sup>7</sup> pyridone,<sup>8</sup> and oxadiazole<sup>9</sup> moieties gave moderate to significant antibacterial activity when compared to standard antibiotics. Similarly, oxadiazole and thiadiazole derivatives of carvacrol displayed significant enhancement of insect growth regulation in a moth species,<sup>10</sup> and moderate improvement in antioxidant activity<sup>11</sup> relative to carvacrol.

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Supplementary data

Experimental procedures, tyrosinase inhibitory assay and data (HRMS, IR, <sup>1</sup>H and <sup>13</sup>C NMR) data for compounds **5–20** are available.

Tyrosinase is the rate-limiting enzyme for the biosynthesis of melanin. Although melanin serves to protect the skin from UV damage, overproduction can lead to skin defects such as melasma, freckles and age spots. As a catechol oxidase, tyrosinase is also implicated in the undesirable browning of fruit. Thus, tyrosinase inhibitors are important in the medical, agricultural and cosmeceutical fields, owing to their ability to mitigate fruit browning and skin defects arising from melanin overproduction.<sup>12–13</sup> The tyrosinase inhibitory activity of carvacrol and thymol have not been well documented. Satooka indicated that thymol affects the redox processes involved in dopachrome, and subsequently melanin formation, but does not directly affect the tyrosinase enzyme activity.<sup>14</sup> However, diesters of carvacrol and thymol incorporating glycolic acid and benzoic or cinnamic acid moieties have shown moderate to potent tyrosinase inhibitory activity when compared with kojic acid,<sup>15–16</sup> a commercially used skin whitening agent. In addition, a diester containing a succinoyl moiety coupled with two kojic acid units gave a better than 2-fold increase in inhibitory activity than kojic acid.<sup>17</sup> Based on the improvements in tyrosinase inhibitory activities upon structural modification of carvacrol, thymol and kojic acid, it was envisioned that alkyl 4-oxobutanoate derivatives of carvacrol and thymol would give enhancement in activity relative to the parent phenols.

4-Oxobutanoate derivatives of carvacrol and thymol were synthesized in three steps (Figure 1). Carvacrol (**1**) and thymol (**2**) were converted to their corresponding ethyl ethers by modification of the procedure reported by Silva et al.<sup>18</sup> Treatment of acetonitrile solutions of the phenols with sodium methoxide and bromoethane afforded ethyl carvacrol (**3**) and ethyl thymol (**4**) in 60% and 93% yields, respectively. The yields and spectroscopic data for compounds **3** and **4** were in good agreement with literature data.<sup>18</sup> With the ethyl ethers in hand, it was anticipated that the 4-oxobutanoic acid moiety could be introduced to the carvacrol/thymol core by Friedel-Crafts acylation with succinic anhydride and aluminum chloride, as reported for butyl thymol.<sup>7</sup> However, attempts to synthesize the 4-oxobutanoic acids using succinic anhydride were unsuccessful in our hands. The use of succinoyl chloride, followed by acidic work up gave the 4-oxobutanoic acids **5** and **6** in 68% and 81% yields, correspondingly. While the bis-carvacrol or bis-thymol  $\gamma$ -diketone could have been obtained as a product of the reaction, there was no evidence of its formation. The carvacrol and thymol 4-oxobutanoic acids were each converted to methyl, ethyl, propyl, allyl, butyl, crotyl and benzyl 4-oxobutanoates by *O*-alkylation, using cesium carbonate and the corresponding alkyl bromides or iodides in acetonitrile.<sup>19</sup> The fourteen new alkyl 4-oxobutanoate derivatives of carvacrol and thymol (**7–20**) were characterized by NMR, IR and HRMS analyses.

Tyrosinase converts L-tyrosine to dopaquinone, which is subsequently converted to dopachrome. The tyrosinase inhibitory assay measures the amount of dopachrome produced in the presence of test compounds.<sup>20</sup> In order to determine structure-activity correlations, compounds **1–20** were evaluated at 500  $\mu$ M for inhibitory activity against mushroom tyrosinase (Table 1). Carvacrol (**1**) and thymol (**2**) gave comparable data, with percent inhibition of 35.7 and 29.4%, respectively. For carvacrol there was a 1.6 fold increase in activity for the ethyl ether derivative (**3**) and a 2.1 fold decrease in activity for the 4-oxobutanoic derivative (**5**). However, there was no significant difference in activity between

thymol and its ethyl ether (**4**) and 4-oxobutanoic acid (**6**) derivatives. The similar activity observed for thymol and its ethyl ether derivative is in contrast to a previous study which suggested that the methyl ether derivative of thymol had a lower inhibitory effect than thymol.<sup>14</sup> The carvacrol and thymol alkyl 4-oxobutanoates showed better inhibitory activities than the parent 4-oxobutanoic acids, except for the methyl ester derivative of carvacrol, **7**, and the benzyl ester derivative of thymol, **20**. In general, the activity was enhanced with an increase in the carbon chain length, with esters containing three and four carbon alkyl groups showing the greatest inhibitory activity (%Inhibition = 72.8–100%). The only exception is the carvacrol allyl 4-oxobutanoate (**10**), which showed significantly lower inhibitory activity (58.2%) than the corresponding propyl **9** (85.6%), butyl **11** (82.3%) and crotyl **12** (72.8%) derivatives. The benzyl ester derivatives showed significantly lower percent inhibition than their three and four carbon counterparts (**13** = 47.0%; **20** = 31.3%).

IC<sub>50</sub> data were obtained for alkyl 4-oxobutanoates (**9–12** and **15–19**) showing greater than 51% inhibition at 500 μM (Table 1). Kojic acid (IC<sub>50</sub> = 21.8 μM), was used as a positive control. The compounds showed moderate inhibitory activity, with IC<sub>50</sub> values ranging from 122.8 to 244.1 μM for the carvacrol series (**9–12**) and 102.3–212.3 μM for the thymol series (**15–19**). Despite the anomalous percent inhibition of the carvacrol allyl 4-oxobutanoate (**10**), it showed the highest inhibitory activity among the carvacrol derivatives (IC<sub>50</sub> = 128.8 μM). For thymol, the crotyl derivative **19** had the highest inhibitory activity (IC<sub>50</sub> = 102.3 μM). While the precise mechanism of action of the 4-oxobutanoates is unknown, the increase in inhibitory activity for the three and four carbon derivatives and decrease for the benzyl group and smaller alkyl groups may indicate interaction with the hydrophobic pocket of the tyrosinase enzyme,<sup>12–13</sup> with optimal binding interactions occurring with three and four carbon alkyl groups.

In conclusion, fourteen alkyl 4-oxobutanoate derivatives of carvacrol and thymol were synthesized and evaluated for their tyrosinase inhibitory effects using mushroom tyrosinase. Although all the alkyl 4-oxobutanoate derivatives showed lower inhibitory activity than kojic acid, the correlations between the structure of the alkyl side chain and tyrosinase inhibitory activity are evident. Esters containing three or four carbon atoms in the alkyl chain were the most effective. Further structural modifications of the carvacrol and thymol derivatives could potentially lead to structures with enhanced tyrosinase inhibitory activities.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

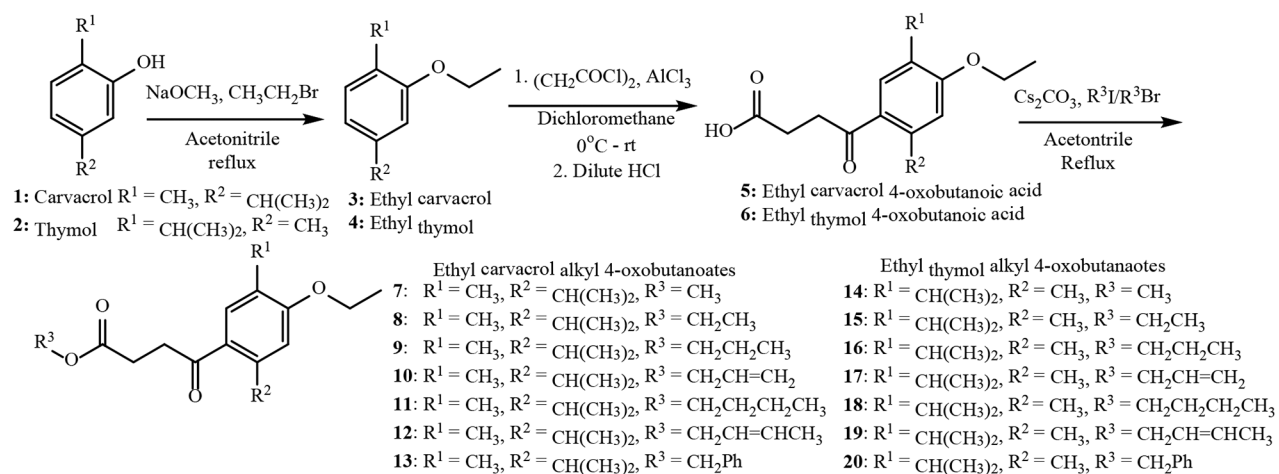
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**Figure 1:**  
 Synthesis of alkyl 4-oxobutanoate derivatives of carvacrol and thymol

**Table 1:**

Tyrosinase inhibitory activities of carvacrol (**1**)/thymol (**2**) and their corresponding derivatives **3**, **5**, **7–13/4**, **6**, **14–20**

Compound	Carvacrol ( <b>1</b> ) %Inhibition	Thymol ( <b>2</b> ) %Inhibition	Carvacrol IC <sub>50</sub> (μM)	Thymol IC <sub>50</sub> (μM)
Parent <b>1/2</b>	35.7±5.4	29.4±3.7	n.d.	n.d.
Ethyl ether <b>3/4</b>	57.5±2.5	28.8±5.6	n.d.	n.d.
4-Oxobutanoic acid <b>5/6</b>	16.9±4.0	30.7±2.7	n.d.	n.d.
Methyl 4-oxobutanoate <b>7/14</b>	31.7±6.2	45.5±2.1	n.d.	n.d.
Ethyl 4-oxobutanoate <b>8/15</b>	50.9±2.1	72.0±5.5	n.d.	212.3±1.7
Propyl 4-oxobutanoate <b>9/16</b>	85.6±6.6	78.8±2.4	217.9±4.3	154.0±3.7
Allyl 4-oxobutanoate <b>10/17</b>	58.2±2.3	100.04±6.7	128.8±1.9	125.6±3.5
Butyl 4-oxobutanoate <b>11/18</b>	82.3±2.1	100±3.9	176.5±2.8	191.4±2.4
Crotyl 4-oxobutanoate <b>12/19</b>	72.8±3.3	99.3±8.0	244.1±3.6	102.3±1.8
Benzyl 4-oxobutanoate <b>13/20</b>	47.0±9.0	31.3±7.1	n.d.	n.d.

Kojic acid (IC<sub>50</sub> = 21.8±1.7 μM)

% Inhibition (500 μM); n.d. = not determined (IC<sub>50</sub> >500 μM)