Synthesis and antioxidant evaluation for monocarbonyl curcuminoids and their derivatives

ARTÍCULO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA

How to cite this paper:

Reception date:
Received: 15 August 2018
Accepted: 25 November 2018
Published Online: 28 December 2018

DOI:
http://dx.doi.org/10.15649/2346075X.481

Keywords:
Cucumine; Monocarbonylcurcuminoids; Antioxidant; DPPH; Pepronal; 4-thiomethoxy benzaldehyde

ABSTRACT

Introduction: Curcumin is a yellow pigment extracted from the Curcuma longa L, which have a several biological activities and pharmacological properties. Curcuminoids have a wide range as antioxidant not only in a food system, but also for biological systems. Materials and Methods: Acetone, 4-thiomethoxy benzaldehyde, pepronal, thiosemicarbazide, 4-phenylthiosemicarbazide and chloroethylacetate. The two Analogous of monocarbonyl curcuminoids (MCCs) have been synthesized by claisen –Schmidt condensation from the reaction between one mole of acetone with two moles of appropriate aromatic aldehydes (4-thiomethoxy benzaldehyde and pepronal) then synthesized their hetero derivatives. The pyrazols derived from the reaction MCCs with hydrazine or one of their derivative (thiosemicarbazide, 4-phenylhydrazine). Results and Discussion: All synthesized compounds were characterized by various spectroscopic techniques such as FTIR, $^1$HNMR, $^{13}$CNMR, Mass spectroscopies and CHN analysis. The antioxidant activity of synthesized MCCs, 1, 2, 1a, 2a, 3, were determined by the ability to scavenge the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical according to Blois method. The DPPH inhibition activity was measured by spectrophometric method. The polyhydroxy curcuminoid has showed a high activity for scavenging of DPPH radicals, the reason is the hydroxyl phenolic group OH give the compound high activity of scavenging the radical by donating hydrogen atom to the DPPH radicals and inhibition the radical activity by hydrogen atom transfer (HAT). Therefore the scaveng of radical activity will be in the order: 3>2a>1a>2>1 and the half maximal inhibitory concentration (IC$_{50}$) between (17.35-135.2) μmol/L. Conclusions: The proposed structure of the synthesized compounds were confirmed by used a spectroscopic technique such as, FTIR, Mass spectra (EI),$^1$H and $^{13}$C NMR, The antioxidant activity of curcuminoids were studied by using DPPH as a source of radicals. The higher activity of compounds can be attributed to present the phenolic OH group.
INTRODUCTION

Curcumine is a natural extract from rhizome of curcumine longa (Turmeric)\(^1\). The pigment yellow color of extracted contained three isomers of curcuminoids (curcumine, demethoxy curcumine and bis demethoxy curcumine)\(^2\). This natural compound has been used as food pigment and in food industries\(^3\).

**Figure 1 Some examples of Curcumins**

In the last decades the pharmacological activity of curcumine has many applications *in-vivo* and *-viro* as antioxidant\(^4\), anticancer\(^5\), antifungal\(^6\), inflammatory\(^7\), antibacterial\(^8\), and antiviral\(^9\). Because of the curcumine poor solubility in aqueous media\(^10\), which is the most cumin problems of this material leads to poor bioavailability and absorption then cause a rapid metabolism. Therefore, this material attracted a lot of attention in past decades, especially by modifying their structure and preparing new analogues to increase their bioactivity as anticancer and antioxidant\(^11\). In this study, two monocarbonyl curcumine analogous (MCCs) and their derivatives have been designed, and their activity as an antioxidant agents for scavenging of DPPH radicals have been investigated.

MATERIALS AND METHOD

**Materials and Reagents**

All the chemicals and solvents were used with analytical grade (AR) and highest purity which included pepronal and thiomethoxybenzaldehyde (Aldrich), thiosemicarbazide, pyrogallol and chloroacetylchloride (Merck). All the solvents were used equipped by (BDH) company.

**Instrumentation**

FTIR spectra of all compounds were recorded on shimadzu FTIR model Affinity spectrophotometer using KBr pellets in the range (4000-400) cm\(^{-1}\). The Mass spectra were scanned by the EI technique 70eV with an Agilent Technologies 5975 spectrometer. The experimental values of \(^1\)H and \(^13\)CNMR spectra for the studied compounds were scanned on a
Bruker 400MHz spectrometer with a field gradient to operate at 400 MHz for proton observation and 100 MHz for carbon observation using DMSO-d₆ as solvent and TMS as internal standard. Elemental analysis (CHNS) were measured by using elementar Vario MICRO. UV-VISIBLE spectra were measured using a PG-instrument T80+ spectrophotometer.

Synthesis

Preparation of monocarbonyl curcumicoids (MCCs) (1, and 2):

The monocarbonyl curcuminoids 1, and 2 were synthesized by Claisen–Schmidt condensation between acetone (16.65 mmol) with appropriate aromatic aldehydes (33.7 mmol) in (50 mL) ethanol, and (34 mmol) of sodium hydroxide solution as a catalyst. The reaction mixture was stirred for further 10 hours at room temperature. The solid product was collected by filtration, and then was washed by water several times, dried then recrystallized from the appropriate solvent (scheme 1).

(1E,4E)-1,5-bis(benzo[d][1,3]dioxol-5-yl) penta-1,4-dien-3-one (1):

Color: yellow powder, recrystallized from ethyl acetate, yield: 84%, M.P: 186-188°C, ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 6.1(s, 4H, -OCH₂O-), 7.00 (d, 2H, J=5 Hz, CH=C), 7.19 (d, 2H, J=5Hz, aromatic), 7.26 (d, 2H, J=10Hz, aromatic), 7.45(s, 2H, aromatic), 7.71(d, 2H, J= 5Hz, CH=C). ¹³CNMR (100 MHz, DMSO-d₆, δ ppm): 102.1, 107, 109, 124.3, 125.7, 129.7, 142.8, 148.2, 149.8, 189.1. MS (70 eV, m/z): 322[M], 279, 189, 175, 135. FTIR (KBr disk, v, cm⁻¹): 3016, 2919, 1643, 1589, 1489, 1337. Anal. calc. for C₁₉H₁₄O₅: C, 70.80; H, 4.38; found: C, 69.47; H, 4.24.

(E)-5-(benzo[d][1,3]dioxol-5-yl)-3-(2-(benzo[d][1,3]dioxol-5-yl) vinyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (1a):

Color: yellow powder, recrystallized from benzene: petroleum ether, yield: 55.9%, M.P: 208-210 °C, ¹H NMR (400 MHz, DMSO-d₆, δ ppm): 2.97(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring), 5.81(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring), 5.81(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring), 5.81(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring), 5.81(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring), 5.81(dd, H, J=5Hz, CH₂-C pyrazole ring), 3.68(dd, H, J=10Hz, CH₂-C pyrazole ring). FTIR (KBr disk, v, cm⁻¹): 3458, 3336, 3066, 2891, 1761, 1730, 1643, 1576, 1489, 1336. Anal. calc. for C₂₇H₂₈N₄O₆S₂: C, 69.47; H, 5.28; N, 8.92; S, 19.55.

General procedure for the synthesis pyrazoles (1a-c) & (2a-b)

MACs (3.11 mmol) dissolved in (50 mL) of ethanol, and (3.13 mmol) of appropriate hydrazine was added or one of their derivatives (hydrazine monohydrate, thiosemicarbazide, and 4-phenyl hydrazine), then (0.6 g) sodium hydroxide was added to the mixture. The reaction mixture was heated under reflux for 16 hours, then cooled down and poured into iced water, then neutralized by the addition 0.1N HCl. The product was collected by filtration, and then dried and recrystallized from a suitable solvent (scheme 2).
(E)-5-((benzo[d][1,3]dioxol-5-yl)-3-((2-((benzo[d][1,3]dioxol-5-yl) vinyl))-N-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (1b):

Color: yellow powder, recrystallized from benzene: petroleum ether, yield: 49%, M.P.: 198-200°C, 1HNMR (400 MHz, DMSO-d6, δ ppm): 3.00 (dd, H, CH2-C pyrazole ring), 3.68 (dd, H, CH2-C pyrazole ring), 5.43 (dd, H, CH-N pyrazole ring), 5.98 and 6.06 (s, 4H, -OCH2O-), 6.71 (d, 1H, J=10Hz, CH=C), 6.74-7.36 (m, 11H, aromatic), 7.58 (d, 1H, J=8Hz, CH=C), 8.93 (s, 1H, N-H).

13CNMR (100MHz, DMSO-d6, δ ppm): 41.7, 59.9, 106.4, 108.7, 109, 110, 118.6, 119.1, 120.5, 128.7, 128.9, 130.8, 139.7, 146.7, 147.9, 148.4, 151.4, 153.8. FTIR (KBr disk, υ, cm⁻¹): 3383, 3037, 2893, 1685, 1654, 1595, 1489, 1244, 1122, 1039.

Anal. calc. for C26H21N3O4S: C, 66.23; H, 4.49; N, 8.91; S, 6.80, found: C, 65.82; H, 4.31; N, 7.82; S, 6.44.

(E)-5-((4-(methylthio)phenyl)-3-(4-(methylthio)styryl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (2a):

Color: yellow powder, recrystallized from ethanol, yield: 53.3%, M.P.: 138-140°C, 1HNMR (400 MHz, DMSO-d6, δ ppm): 2.44 (s, 3H, -SCH3), 2.9 (s, 3H, -SCH3), 2.98 (dd, H, J=15Hz, CH2-C pyrazole ring), 3.7 (dd, H, J=10Hz, CH2-C pyrazole ring), 5.85 (dd, H, J=5Hz, CH-Npyrazole ring), 7.04-8.08 (m, 10H, aromatic & CH=C), 8.4 (s, 2H, NH2), MS (70 eV, m/z): 499.3[M], 339, 352, 276, 248, 191, 150, 60. FTIR(KBr disk, υ, cm⁻¹): 3116, 2924, 1627, 1604, 1489, 1342, 1265, 1188, 1087. Anal. calc. for C19H14N2S2: C, 67.82; H, 5.92; N, 8.23; S, 18.83, found: C, 67.02; H, 5.92; N, 8.01; S, 17.90.

Procedure for the synthesis of 2-chloro-1-(2,3,4-trihydroxyphenyl)ethan-1-one:

To a cooled solution of stirred of aluminum chloride (24g) dissolved in DCM at 10-15°C, (8g) of pyrogallol in (50mL) DCM was added drop-wise within 20 min. (5mL) of chloroacetyl chloride was added to the reaction mixture which is still at the same temperature. The temperature of reaction mixture raised up to room temperature and further stirring for...
another 20 hours. After that time the reaction was poured onto (100 mL) of dilute hydrochloric acid with stirring for 2 hours at room temperature. The solid material was collected by filtration and washed with water, then the solid suspended in dilute acetic acid solution and heated to 85°C. The mixture was cooled down to room temperature. The product was filtered, washed with water and dried, then recrystallized from benzene (scheme 3).

Color: white powder, recrystallized from benzene, white powder, recrystallized from benzene, yield: 85%, M.P.: 169-170°C, 1H NMR (400 MHz, DMSO-d6, δ ppm): 5.03 (s, 2H, -CH2-), 6.44 (d, J = 5 Hz, aromatic), 7.3 (d, J = 5 Hz, aromatic), 8.77, 10.28, 11.63 (s, 3H, -OH), 13CNMR (100 MHz, DMSO-d6, δ ppm): 47.3, 109, 112, 123, 133, 152.2, 153.4, 195.2. MS (70 eV, m/z): 202.1 [M], 153, 125, 79, 51. FTIR (KBr disk, ν, cm⁻¹): 3498, 3390, 3095, 2989, 1637, 1523.

In the reaction vessel (0.9 g, 2.36 mmol) of compound (1a), and (0.48 g, 2.36 mmol) of compound 2-chloro-1-(2,3,4-trihydroxyphenyl) ethan-1-one dissolved in (20 mL) of dimethyl formamide. The reaction mixture was heated under reflux for 6 hours. The solid product was collected by filtration, dried then recrystallized from ethylacetate (scheme 4).

% inhibition percentage = \frac{A_c - A_s}{A_c} \times 100

Ac: control absorbance, the absorbance of pure DPPH
As: sample absorbance, the absorbance of DPPH mixed with sample

The liner curve was obtained by plotting inhibitor percentage of radical versus concentrations of compounds.

**DPPH Radical Scavenging Assay**

The antioxidant activity of synthesized curcuminoids (1, 1a, 2, 2a, and 3) were determined by measuring the ability of scavenging the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals according to Blois method (12, 13). The DPPH inhibition activity was measured by spectrometric method, by mixing (1 mL, 200 μmol/L) of ethanolic solution of DPPH with (1 mL) of different concentrations (50, 100, and 200) μmol/L of ethanolic solution of synthesized curcuminoids. The absorbance was read at 517 nm as functional of different times by using uv-visible spectrophotometer. In addition, there was a notable change DPPH color graduate from violet to yellow or colorless. The percentage of inhibition was calculated by the following equation (14, 15).
RESULTS AND DISCUSSION

Spectroscopy identification:
The monocarbonyl curcuminoids (MCCs) (1, and 2) prepared according to (Scheme1). The proposed structure has confirmed by using spectroscopic techniques such as FTIR, Mass spectrometry, $^1$H and $^{13}$C NMR spectroscopy as well as the elemental analysis.

Scheme 1 Preparation compound (MCCs).

The (MCCs) prepared according to Claisen–Schmidt condensation reaction between acetone and, aromatic aldehyde, (scheme 2) represents the suggested preposed reaction mechanism.

Scheme 2 Reaction mechanism of (MCCs).
The IR spectra of the prepared compounds exhibit all bands of functional groups. The spectra of compound (1, and 2) have shown stretching vibration bands at $\nu$ (3014, 3016) cm$^{-1}$, and $\nu$ (2912, 2916) cm$^{-1}$ attributed to C-H aliphatic, and C-H aromatic, respectively. The stretching vibration band appeared at $\nu$(1643, 1643.35) cm$^{-1}$ attributed to ketone group as well as band of C=C group at $\nu$(1598, 1603) cm$^{-1}$. The $^1$HNMR spectra analysis for (MCCs) at room temperature in DMSO-d$_6$ confirmed the proposed structure. The spectrum of compound (1) has shown singlet signal for CH$_2$-O at $\delta$ (6.10 ppm), doublet signals of vinylic proton at $\delta$ (7.0, and 7.71) ppm and signals at $\delta$(7.19, 7.26, and 7.45) ppm which attributed to CH-C aromatic. The $^{13}$CNMR spectra showed signals at $\delta$ (102) ppm, and $\delta$ (188) ppm assigned to CH$_2$-O and ketone groups, respectively. The pyrazole derivatives of (MCCs) was synthesized from the reaction curcuminoids with hydrazine and their derivatives according to (scheme 3).

![Scheme 3 Preparation pyrazoles of (MCCs).](image)

The IR spectra of pyrazole compounds show a new band at $\nu$)1620-1627( cm$^{-1}$ attributed to the C=N in pyrazol ring$^{[10]}$, the bands at $\nu$ (3066-3014) cm$^{-1}$ related to C-H aromatic and at $\nu$ (1604-1598) cm$^{-1}$ assigned of stretching vibration of C=C group. In addition, the band of carbonyl group disappeared in spectra of curcuminoi ds derivatives (pyrazoles). The $^1$HNMR spectra analysis for pyrazoles show new signals due to the interaction between neighbor protons in pyrazole ring, two doublet of doublet signals at $\delta$ (2.49-2.97) ppm, and $\delta$ (3.68-4.0) ppm attributed to C-CH$_2$-C of pyrazol ring as well as doublet of doublet signal at $\delta$ (5.05-5.85) ppm assigned of CH=N of pyrazol ring$^{[12]}$. In addition exhibited signals attributed to the aliphatic, aromatic, and vinylic protons. The $^{13}$CNMR spectra of pyrazoles display signals of the carbon skeleton for pyrazoles. The spectra characterized signals attributed to aliphatic carbon, vinylic carbon, aromatic carbon, and C=N of pyrazol ring. Mass spectra (EI) results show the proposed formula. The elemental analysis was used to determine the molecular formula of the prepared compounds.
compounds. In all cases, the differences in values of elemental percentage between measured and calculated formula are within acceptable values. The compound (3) was prepared from the reaction of compound (1a) with 2-chloro-1-(2,3,4-trihydroxyphenyl) ethan-1-one, according to (scheme 4).

Scheme 4 preparation thiazolo thiazole of (MCCs).

The compound 2-chloro-1-(2,3,4-trihydroxyphenyl) ethan-1-one was prepared according to Frielde–Crafts reaction. The proposed structure was confirmed by using spectroscopic techniques. The IR spectrum shows strong band at $\nu$(3390) cm$^{-1}$ and band at $\nu$(1637) cm$^{-1}$ attributed to hydroxyl group, and carbonyl group, respectively. The $^1$HNMR spectrum shows signal at $\delta$(5.03) ppm, doublet signals at $\delta$ (6.44, and 7.3) ppm attributed to aromatic proton as well as three singlet signals at $\delta$ (8.77,10.28, and 11.63) assigned to phenolic proton. The $^{13}$CNMR spectrum shows eight signals one of them at $\delta$ (196) ppm attributed to carbonyl carbon. The Mass spectrum results improved the proposed formula. The pyrazolo thiazol derivatives characterized by spectroscopic techniques, the IR spectrum shows bands at $\nu$(3527) cm$^{-1}$, $\nu$(3032) cm$^{-1}$, $\nu$(2908) cm$^{-1}$, $\nu$(1621) cm$^{-1}$, $\nu$(1604) cm$^{-1}$ and $\nu$(1087, 1037) cm$^{-1}$ attributed to C-OH, C-H aromatic, C-H aliphatic, C=N, C=C, and O-CH$_2$-O groups. The $^1$HNMR spectrum shows signals at $\delta$ (10.46, 8.98, and 8.06) ppm attributed to phenolic proton, signals at $\delta$ (6.23-7.38) ppm attributed to olefinic and aromatic protons, as well as signal at $\delta$ (6.28) ppm assigned to O-CH$_2$-O proton and three doublet of doublet signals at $\delta$ (3.12, 3.84, and 5.58) ppm attributed to protons of pyrazol ring.

Antioxidant activity assay

The in-vitro free radical inhibition activity of (MCCs) and some derivatives were analyzed by the DPPH method, the percentage of inhibition activity and the result of IC$_{50}$ are gathered in (Table1), and (figure 7). The inhibition percentage activity of synthesized compounds (1, 1a, 2, 2a, and 3) concentration (50-200) μmol/L are measured by the decreasing of DPPH absorbance at 517 nm with time (figures 1-6). The compounds showed lower in scavenging activity in comparison with ascorbic acid, expect the compound (3) which showed higher inhibition activity of 66.8% at 50 μmol/L, 78.57% at 100 μmol/L, and 95% at 200 μmol/L. The other compounds (1, 1a,
2, and 2a) showed lower inhibition at 200 μmol/L. Therefore the compound 3 showed a high inhibition activity due to the phenolic OH groups(18) that able to scavenge the radicals. The scavenge of radicals activity of compounds (1, 1a, 2, 2a, and 3) will be in the order of: 3 > 2a > 1a > 2 > 1. As well as half maximal inhibitory concentration (IC₅₀) between (17.35-135.2) μmol/L.

Figure 2: DPPH free radical scavenging activity of compounds (1) at 50-200 μmol/L concentrations showing percentage inhibition.

Figure 3: DPPH free radical scavenging activity of compounds (1a) at 50-200 μmol/L concentrations showing percentage inhibition.
Figure 4: DPPH free radical scavenging activity of compounds (2) at 50-200 µmol/L concentrations showing percentage inhibition.

![Graph showing DPPH free radical scavenging activity](image)

Figure 5: DPPH free radical scavenging activity of compounds (2a) at 50-200 µmol/L concentrations showing percentage inhibition.

![Graph showing DPPH free radical scavenging activity](image)
Figure 6: DPPH free radical scavenging activity of compounds (3) at 50-200 µmol/L concentrations showing percentage inhibition.

Figure 7: DPPH free radical scavenging activity of ascorbic acid at 200 µmol/L concentrations showing percentage inhibition.
Figure 8: The percentage inhibition of DPPH free radical scavenging activity of compounds (1, 1a, 2, 2a, 3, and Vit.C) at 50-200 µmol/L concentrations.

Tabla 1: In-vitro antioxidant activities of compounds (1, 1a, 2, 2a, and 3).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage of Inhibition</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 µmol/L</td>
<td>100 µmol/L</td>
<td>200 µmol/L</td>
<td>IC 50 µmol/L</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>66.89</td>
<td>78.57</td>
<td>95.09</td>
<td>18.74</td>
</tr>
<tr>
<td>1a</td>
<td>60.38</td>
<td>64.74</td>
<td>66.99</td>
<td>60.12</td>
</tr>
<tr>
<td>2</td>
<td>57.51</td>
<td>63.44</td>
<td>65.91</td>
<td>20.97</td>
</tr>
<tr>
<td>2a</td>
<td>63.241</td>
<td>65.91</td>
<td>74.01</td>
<td>31.59</td>
</tr>
<tr>
<td>1</td>
<td>25.39</td>
<td>30.73</td>
<td>36.561</td>
<td>135.2</td>
</tr>
<tr>
<td>3</td>
<td>66.01</td>
<td>83.69</td>
<td>98.42</td>
<td>17.35</td>
</tr>
</tbody>
</table>

CONCLUSIONS

In this study, the monocarbonyl curcuminoids (MCCs) was synthesized according to Claisen–Schmidt condensation reaction between acetone and aromatic aldehydes, followed by synthesis of their derivatives. The IR, $^1$HNMR, $^{13}$CNMR, and Mass spectra as well as elemental analysis of the studied-compounds are considered as the essential identification. The antioxidant activity of curcuminoids were studied by using DPPH as a source of radicals. The higher activity of compounds can be attributed to present the phenolic OH group. IC$_{50}$ value between (17.35-135.2) µmol/L.
REFERENCES


