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Research Article

ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF SYNTHESIZED MANNICH BASE DERIVATIVES OF (2*E*,6*E*)-2-[(4-HYDROXY-3-METHOXYPHENYL)METHYLIDENE]-6-(PHENYL METHYLIDENE)CYCLOHEXAN-1-ONE

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ABSTRACT

Objective: To further understand this compound, we synthesized and evaluated the antioxidant and anti-inflammatory activity of a series of its Mannich base derivatives.

Methods: We synthesized the compounds via the previously reported Mannich reaction method. Their structures were elucidated by Fourier-transform infrared,^{1}H-NMR,^{13}C-NMR, and high-resolution mass spectra. The derivatives' anti-inflammatory and antioxidant activities were tested using the inhibition of protein denaturation method and the 2,2-diphenyl-2-picrylhydrazyl free radical scavenging assay.

Results: The IC_{50} values for the anti-inflammatory activity of the 2,6-dimethylmorpholine, pyrrolidine, 1-methylpiperazine, and dimethylamine Mannich base derivatives 2a–d were 10.67, 10.72, 37.75, and 1.93 μM, respectively; for (2*E*,6*E*)-2-({4-hydroxy-3-methoxyphenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one (1), diclofenac sodium, and curcumin, the IC_{50} values were 56.29, 1.52, and 8.43 μM, respectively. The IC_{50} values for the antioxidant activity of compounds 2a–2d were 229.62, 57.29, 280.43, and 219.22 μM, respectively; for compound 1, quercetin, and curcumin, the IC_{50} values were 144.22, 27.28, and 26.45 μM, respectively.

Conclusion: Substituting Mannich bases into (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl) methylidene]-6-(phenylmethylidene)cyclohexan-1-one enhanced its anti-inflammatory activity, but lowered its antioxidant activity. Compound 2d, (2*E*,6*E*)-2-({3-[(dimethylamino)methyl]-4-hydroxy-5-methoxyphenyl}methylidene)-6-(phenyl methylidene)cyclohexan-1-one, exhibited potent anti-inflammatory activity comparable to diclofenac sodium and four times higher than curcumin. However, further investigation of this compound's mechanism of action and toxicity is warranted.

Keywords: Asymmetrical mono-carbonyl analogs of curcumin, Mannich bases, Antiinflammatory, Antioxidant.

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INTRODUCTION

Curcumin, a phenolic compound found in *Curcuma* spp., exhibits a variety of biological activities, including anti-inflammatory and antioxidant activities [1]. However, because of its low solubility, low stability (both chemically and metabolically), and low bioavailability, its clinical applications have been limited [2,3]. Many curcumin analogs have been synthesized and investigated in attempts to improve its chemical properties and biological activity [4].

Several series of symmetrical monocarbonyl analogs of curcumin (MACs), containing a cyclohexanone or cyclopentanone linker between the two phenyl rings, reportedly have superior anti-inflammatory and antioxidant activity, higher chemical stability, and improved pharmacokinetic profiles compared to curcumin [3,4]. On the other hand, several asymmetrical MAC (AMACs) reportedly exhibit potent anti-inflammatory, antioxidant, and antitumor activities [5-8]. Finally, our group reported that, while AMACs containing a morpholine Mannich base exhibit low antioxidant activity, two compounds exhibit potent anti-inflammatory activity [9]. In particular, substituting a Mannich base (aminomethylation) into a compound can provide an important pharmacophore moiety to potentiate the compound's biological activity [10].

To understand the effect of Mannich base substitution on the biological activity of compounds, and to discover a new antioxidant and anti-inflammatory compound, we synthesized a series of Mannich base derivatives of one of the AMACs, (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]-6-(phenylmethylidene)cyclohexan-1-one, and evaluated their antioxidant and anti-inflammatory activity.

MATERIALS AND METHODS

Chemistry

Materials and general procedures

All chemicals and solvents were purchased from commercial sources (Sigma-Aldrich, St. Louis, USA; Merck, Darmstadt, Germany; and Mallinckrodt, St. Louis, USA) and used without further purification. The synthesized compounds' purity was determined by thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck, Germany). Melting points were determined via the open-ended capillary method, using the Analogue Melting Point Apparatus SMP11 (Stuart Scientific, UK), and were uncorrected. Infrared (IR) spectra were recorded on an Fourier-transform IR (FT-IR) spectrophotometer (8400S; Shimadzu, Japan); nuclear magnetic resonance spectra were recorded on an NMR spectrometer (A500a, Agilent, USA) with a DD2 console at 500 MHz for^{1}H-NMR and 125 MHz for^{13}C-NMR, using tetramethylsilane as the internal standard and $CDCl_3$ as the solvent for all compounds; and high-resolution mass spectra (HR-MS) were recorded with an ESI-TOF LCT Premier XE mass spectrometer (Waters Corp., USA).

Synthesis of asymmetrical monocarbonyl analogs of curcumin, (2E,6E)-2-({4-hydroxy-3-methoxyphenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one (1)

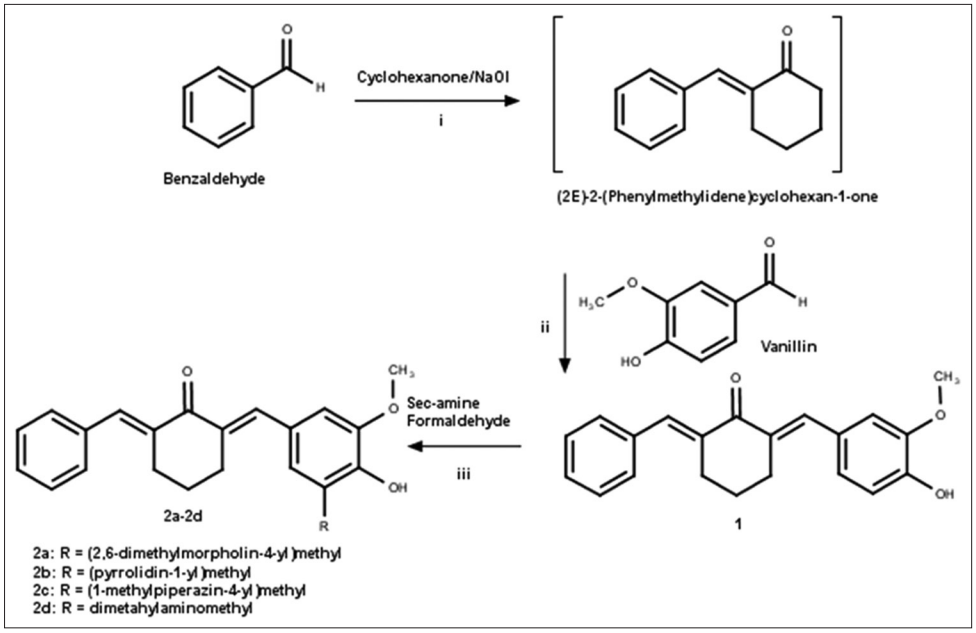


Fig. 1: Synthesis scheme for the Mannich base derivatives of (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]-6-(phenylmethylidene)cyclohexan-1-one

AMAC compound 1 was synthesized using a previously reported procedure (Fig. 1) [11].

General synthesis of Mannich bases of (2E,6E)-2-({4-hydroxy-3-methoxyphenyl} methylidene)-6-(phenylmethylidene)cycloh exan-1-one derivatives (2a-d)

The Mannich base derivatives of (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl) methylidene]-6-(phenylmethylidene)cyclohexan-1-one (2a–d) were prepared via Mannich reactions of compound 1 using the previously reported synthesis method for morpholine Mannich base AMAC derivatives [9]. Compound 1 (2 mmol) was dissolved in ethanol (5 ml) and cooled in an ice bath. Then, we added a corresponding secondary amine (4 mmol) and 37% (v/v) formaldehyde solution (4 mmol) dropwise. The mixture was stirred for 1 h at room temperature (r.t.) and refluxed for 1–10 h until the reaction was deemed complete based on monitoring with TLC. The solvent was evaporated, and the residue obtained was dissolved in methanol (50 ml) and subsequently evaporated. The resulting residue was dissolved in cooled methanol (50 ml) by adding cooled distilled water dropwise. The colored precipitate obtained was filtered, washed with cold methanol, dried at r.t., and purified by column chromatography to obtain pure compounds 2a–d.

(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(2,6-dimethylmorpholin-4-yl)methyl]phenyl}me-thylidene)-6-(phenylmethylidene) cyclohexan-1-one (2a)

Yellow powder, 82.4% yield, mp 108–110°C. FT-IR (KBr) cm^{-1}: 2852–2933 (H-C-aliphatic), 1662 (C=O), 1573 and 1446 (C=C-aromatic), 1595 (C=C), 1145 (C-N), 1084 (C-O).^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.17 and 1.25 (two d peaks, 6H, CH_3-CH two isomers of 2,6-di-CH_3 morpholine) [12,13], 2.71, 3.62, and 3.85 (three m peaks, 2H, -CH-O-CH-two isomers of 2,6-di-CH_3 morpholine) [12,13], 1.90 (p, CH_2-CH_2-CH_2 cyclohexanone), 1.81 and 2.85 (two s and d peaks, 4H, CH_2-N-CH_2 two isomers of 2,6-di-CH_3 morpholine) [12,13], 2.93 (t, 4H, =C-CH_2-CH_2 cyclohexanone), 3.90 (s, 3H, CH_3-O-Ar), 3.72 (s, 2H, Ar-CH_2-N), 4.09 (b, 1H, OH), 6.82 (1H, s, H-Ar), 6.99 (s, 1H, H-Ar), 7.44 (t, 2H, J=7.4 Hz, H-Ar), 7.32 (t, 1H, J=8.3 Hz, H-Ar), 7.45 (d, 2H, J=8.7 Hz, H-Ar), 7.72 and 7.79 (s, 1H, and s, 1H, Ar-CH=C-ethylenic). ^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 19.07 (2C, 2,6-d-CH_3-morpholine), 23.15 (1C, CH_2-CH_2-CH_2-cyclohexanone), 28.47 and 28.81 (2C, =C-CH_2-cyclohexanone), 58.47 (2C, CH_2-N-CH_2-morpholine), 61.61 (1C, Ar-CH_2-N), 56.69 (1C, CH_3-O), 71.78 (2C, CH_2-O-CH_2-morpholine), 113.74, 120.77, 124.10, 127.30, 128.59, and 133.82 (6C, C_{Ar}), 128.47, and 130.42 (4C, C_{Ar}), 136.19, 136.39, 136.59, and 137.59 (4C, C=C-ethylenic), 147.84 (1C, CAr-O), 148.31 (1C, CAr-O), 190.18 (1C, C=O). HR ESI-MS (*m*/z) was 448.2421 (M+H)+, calculated mass for $C_{28}H_{33}NO_4$: 448.2409 (error: 2.67 ppm).

(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(pyrrolidin-1-yl)methyl] phenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one (2b)

Dark red powder, 62.6% yield, mp 120–122°C. FT-IR (KBr) cm^{-1}: 2835–2951 (H-C-aliphatic), 1716 (C=O), 1662 (C=C), 1575 and 1489 (C=C-aromatic), 1269 (C-N), 1155 (C-O). ^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.79 (m, 2H, -CH_2-CH_2-CH_2-cyclohexanone), 1.86 (4H, -CH_2-CH_2-pyrroldine), 2.69 (s, 4H, -CH_2-N-CH_2-pyrroldine), 2.92 (t, 4H, =C-CH_2-cyclohexanone), 3.89 (s, 3H, CH_3-O-Ar), 3.88 (s, 2H, Ar-CH_2-N), 6.83 (1H, s, H-Ar), 6.97 (s, 1H, H-Ar), 7.31 (t, 2H, J=7.4 Hz, H-Ar), 7.39 (t, 2H, J=8.7 Hz, H-Ar), 7.45 (d, 1H, J=8.3 Hz, H-Ar), 7.72 and 7.78 (s, 1H, and s, 1H, Ar-CH=C-ethylenic).^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 23.14 (1C, CH_2-CH_2-CH_2-cyclohexanone), 23.72 (2C, -CH_2-CH_2-pyrrolidine), 28.45 and 28.78 (2C, =C-CH_2-cyclohexanone), 53.49 (2C, -CH_2-N-CH_2-pyrrolidine), 56.09 (1C, Ar-CH_2-N), 58.37 (1C, CH_3-O), 113.59, 122.17, 123.75, 126.74, 128.44, and 137.92 (6C, CAr), 128.53, and 130.39 (4C, CAr), 133.46, 136.20, 136.42, and 137.46 (4C, C=C-ethylenic), 147.80 (1C, CAr-O), 149.05 (1C, CAr-O), 190.21 (1C, C=O). HR ESI-MS (m/z) was 404.2224 (M+H)+, calculated mass for $C_{26}H_{30}NO_3$: 404.2147 (error: 0.5 ppm).

(2E,6E)-2-({4-hydroxy-3-methoxy-5-[(1-methylpiperazin-4-yl) methyl]phenyl}methyl-idene)-6-(phenylmethylidene)cyclohexan-1-one (2c)

Orange powder, 79.5% yield, mp 128–130°C. FT-IR (KBr) cm^{-1}: 2750–2931 (H-C-aliphatic), 1670 (C=O), 1531 and 1468 (C=C-aromatic), 1591 (C=C), 1261 (C-N), 1153 (C-O).^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.79 (m, 2H, -CH_2-CH_2-CH_2-cyclohexanone), 2.28 (s, 3H, CH3-N-piperazine), 2.58 (m, 8H, -CH_2-N-CH_2-piperazine), 2.92 (t, 4H, =C-CH_2-cyclohexanone), 3.89 (s, 3H, CH_3-O-Ar), 3.75 (s, 2H, Ar-CH_2-N), 6.81 (1H, s, H-Ar), 6.97 (s, 1H, H-Ar), 7.31 (t, 2H, J=7.4 Hz, H-Ar), 7.38 (d, 2H, J=8.7 Hz, H-Ar), 7.44 (t, 1H, J=8.3 Hz, H-Ar), 7.70 and 7.77 (s, 1H, and s, 1H, Ar-CH=C-ethylenic).^{13}C-NMR (100 MHz, $CDCl_3$) δ/

ppm: 23.13 (1C, -CH_2-CH_2-CH_2-cyclohexanone), 28.45 and 28.79 (2C, =C-CH_2-CH_2-cyclohexanone), 45.94 (1C, CH_3-N-piperazine), 52.50 and 54.92 (4C, CH_2-N-CH_2-piperazine), 56.08 (1C, Ar-CH_2-N), 61.24 (1C, CH_3-O), 113.75, 121.11, 123.99, 127.10, 128.44, and 137.69 (6C, CAr), 128.6, and 130.43 (4C, CAr), 133.68, 136.19, 136.40, and 136.48 (4C, C=C-ethylenic), 147.87 (1C, CAr-O), 148.22 (1C, CAr-O), 190.20 (1C, C=O). HR ESI-MS (m/z) was 433.2473 (M+H)+, calculated mass for $C_{27}H_{33}N_2O_3$: 433.2413 (error: 4.2 ppm).

(2E,6E)-2-({4-hydroxy-3-methoxy-5-(dimethylaminomethyl) phenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one (2d)

Brownish-orange powder, 32.49% yield, mp 102–104°C. FT-IR (KBr) cm^{-1}: 2930–2992 (H-C-aliphatic), 1661 (C=O), 1559 and 1485 (C=C-aromatic), 1586 (C=C), 1258 (C-N), 1159 (C-O).^{1}H-NMR (500 MHz, $CDCl_3$), δ/ppm: 1.82 (m, 2H, -CH_2-CH_2-CH_2-cyclohexanone), 2.36 (s, 6H, CH_3-N-CH_3), 2.93 (t, 4H, =C-CH_2-cyclohexanone), 3.91 (s, 3H, CH_3-O-Ar), 3.69 (s, 2H, Ar-CH_2-N), 6.82 (1H, s, H-Ar), 6.98 (s, 1H, H-Ar), 7.39 (t, 2H, J=7.4 Hz, H-Ar), 7.45 (d, 2H, J=8.7 Hz, H-Ar), 7.33 (t, 1H, J=8.3 Hz, H-Ar), 7.72 and 7.78 (s, 1H, and s, 1H, Ar-CH=C-ethylenic).^{13}C-NMR (100 MHz, $CDCl_3$) δ/ppm: 23.16 (1C, CH_2-CH2-CH_2-cyclohexanone), 28.47 and 28.81 (2C, =C-CH_2-cyclohexanone), 44.55 (2C, CH_3-N-CH_3), 56.13 (1C, Ar-CH_2-N), 61.72 (1C, CH_3-O), 113.77, 121.89, 124.01, 126.91, 128.49, and 137.87 (6C, CAr), 128.56, and 128.46 (4C, CAr), 133.54, 130.41, 136.22, and 136.46 (4C, C=C-ethylenic), 147.83 (1C, CAr-O), 149.02 (1C, CAr-O), 190.24 (1C, C=O). HR ESI-MS (m/z) was 378.2067 (M−H)+, calculated mass for $C_{24}H_{28}NO_3$: 378.1991 (error: 0.5 ppm).

Anti-inflammatory activity

The synthesized compounds (2a–d) were screened for anti-inflammatory activity via the inhibition of heat-induced albumin denaturation method, previously reported by our research group, using diclofenac sodium and curcumin as the standards [9]. The reaction mixtures consisted of 0.5 ml solutions of the standard or test compounds in methanol, in various concentrations, combined with 4.5 ml of bovine serum albumin (BSA) solution (0.5% w/v, pH 6.3) prepared in Tris-buffered saline. These mixtures were heated for 10 min in a water bath at 70°C ± 2. After cooling to r.t., the mixtures' turbidity was measured in triplicate at 660 nm using a UV-Vis spectrophotometer (1601, Shimadzu, Japan). The control was prepared as above but without the test compounds. The percentage (%) of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test compound})}{\text{Absorbance of control}} \times 100$$

The test compound's capacity to inhibit denaturation was expressed as an IC_{50} value which was calculated by plotting the percentage inhibition against the tested compound concentration.

Antioxidant activity

The synthesized compounds (2a–d) were evaluated for antioxidant activity using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method, according to a procedure reported previously, with slight modifications [8,14]. Quercetin and curcumin were used as the standards. A series of solutions with various concentrations of the test compounds or standards was prepared in methanol, and 0.5 ml of each solution was added to 0.5 ml of 50 µg/ml DPPH dissolved in methanol and then incubated at r.t. in the darkroom for 30 min. The test solution absorbance was measured at 517 nm. The series of solutions of the test compounds (no DPPH added) served as blanks because they showed slight absorbance at 517 nm. This experiment was performed in triplicate. The percentage (%) of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance before reaction} \quad \text{Absorbance after reaction})}{\text{Absorbance before reaction}} \times 100$$

The free radical scavenging capacity of each test compound was expressed as an IC_{50} value which was calculated by plotting the percentage inhibition against the concentration of the test compound.

RESULTS AND DISCUSSION

Chemistry

Various MACs have been synthesized in an attempt to improve the biological activity and other properties [3-8]. The recent study reported that AMACs containing a morpholine Mannich base substituted into the phenolic ring and various substituents at para position on another phenyl ring exhibited low antioxidant activity, whereas the compounds containing methoxy or fluoro substituents at the para position on another phenyl ring exhibited potent anti-inflammatory activity that was almost comparable to that of the standard, diclofenac sodium [9]. However, the synthesis and biological activity of various Mannich base AMAC derivatives have not yet been reported. In this study, a series of four novel Mannich base derivatives of (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]-6-(phenylmethylidene)cyclohexan-1-one (2a–d) was synthesized. The title compounds (2a–d) were synthesized stepwise using the methods summarized in Fig. 1. The synthesized compounds' structures were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HR-MS.

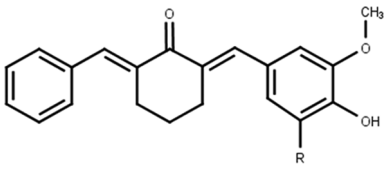
The IR spectra of these compounds showed C-H aliphatic bands at 2750–2992 cm^{-1}. The bands representing the α,β-unsaturated carbonyl groups, C=C aromatic or ethylenic groups, and the C-O-C or C-N-C moieties appeared between 1661–1716, 1446–1662, and 1084–1269 cm^{-1}, respectively. The^{1}H-NMR spectra of these compounds exhibited two singlet peaks for the two ethenyl chain protons at 7.70–72 and 7.77–7.79 ppm (1H), respectively, indicating the compounds' asymmetrical structures. The two methylene protons connecting the nitrogen of the corresponding amine group to the phenyl ring (2H, Ar-CH_2-N-) and the three protons of the methoxy groups (3H, Ar-OCH_3) were observed as singlets at 3.69–3.88 and 3.89–3.91 ppm, respectively. The molecular structures were further supported by the ^{13}C-NMR spectra, which provided the number and types of carbons in the compounds, and the HR-MS spectra, which provided the compounds' molecular masses. These values showed complete agreement with the assigned molecular structures [12,13,15].

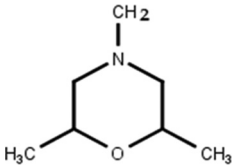
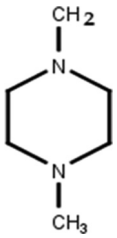
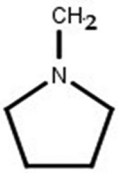
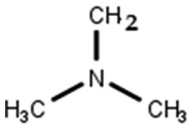
Anti-inflammatory and antioxidant activity

The synthesized compounds (2a–2d) were evaluated for anti-inflammatory activity using the inhibition of heat-induced albumin denaturation method. The decline in the test compounds' absorbance, with respect to the control, indicated protein stabilization. Denaturation of protein *in vivo* is one cause of inflammation; indeed, denaturation in certain rheumatic and arthritic diseases stimulates autoantigen production, which, in turn, drives inflammation [16]. Several anti-inflammatory drugs can inhibit heat-induced albumin denaturation. Therefore, agents inhibiting protein denaturation are worthwhile candidates for anti-inflammatory drugs [17,18]. The compounds showed moderate-to-high inhibition of heat-induced albumin denaturation, which was expressed as IC_{50} values in the range of 1.93–32.75 µM (Table 1 and Fig. 2). In this series, compound 2d, containing the dimethylamine Mannich base moiety, exhibited the most potent activity (IC_{50}=1.93 µM). 2d's activity was comparable to diclofenac sodium (IC_{50}=1.53 µM), four-fold higher than curcumin (IC_{50}=8.43 µM), and 29-fold higher than the parent compound, (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]-6-(phenylmethylidene) cyclohexan-1-one (1) (IC_{50}=56.29 µM). A previous study reported that the morpholine Mannich base derivative of AMAC compound 1 exhibited anti-inflammatory activity with IC_{50}=41.11 µM [9]. This result is consistent with the results of another study on the introduction of Mannich bases into ibuprofen [19]. Compound 2d should be considered for further study to investigate its action mechanism and toxicity.

The synthesized compounds' (2a–d) antioxidant activities evaluated with the DPPH free radical scavenging assay. This method is fast and

Table 1: Anti-inflammatory activity and antioxidant activity of the synthesized compounds (2a–d)



Compounds	Substituent (*R*)	IC_{50} (µM)±SD	
		Anti-inflammatory[a]	Antioxidant[b]
1	H	56.29±0.87	144.22±0.78
2a		10. 67±0.01	229.62±0.01
2b		10.72±0.09	57.29±1.14
2c		32.75±1.58	280.43±0.50
2d		1.93±0.07	219.22±4.34
Curcumin		8.43±0.12	26.45±0.03
Diclofenac sodium		1.52±0.01	-
Quercetin		-	27.28±0.48

[a]Evaluated with the inhibition of heat-induced albumin denaturation method, (n=3), [b]Evaluated with the DPPH free radical scavenging assay, (n=3). SD: Standard deviation, DPPH: 2,2-diphenyl-2-picrylhydrazyl

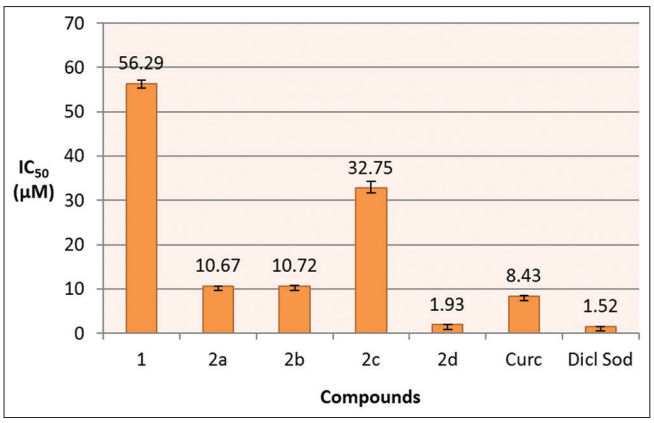


Fig. 2: The inhibition of heat-induced bovine serum albumin denaturation (IC_{50}) by the Mannich base derivatives of (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]-6-(phenylmethylidene)cyclohexan-1-one (compounds 2a–d). Data represented as mean ± standard deviation (n=3)

uncomplicated, ensuring a reliable result. Furthermore, the free radical scavenging approach is suitable with the test compounds' antioxidant mechanisms because they are phenolic compounds [20]. The compounds showed low-to-moderate DPPH free radical scavenging activity, which was expressed as IC_{50} values in the range of 57.29–280.43 µM (Table 1 and Fig. 3). In this series, the compound 2b, containing the pyrrolidine Mannich base moiety, was found to have the highest activity compared to the other three test compounds. It showed moderate activity (IC_{50}=57.29 µM) compared to that of quercetin (IC_{50}=27.28 µM) and curcumin (IC_{50}=26.45 µM). A previous study reported that the morpholine Mannich base derivative of AMAC (compound 1) exhibited antioxidant activity with IC_{50}=229.20 µM [9]. In our study, the other three Mannich base derivatives of compound 1 exhibited lower antioxidant activity than that of the parent compound (AMAC, 1) (IC_{50}=144.22 µM). This result is inconsistent with our previous study on the Mannich base derivatives of dehydrozingerone (DHZ), which indicated that most of the compounds' Mannich bases exhibited higher free radical scavenging activity than DHZ [21]. In curcumin analogs, electron-donating substituents at ortho positions relative to the phenol groups enhance antioxidant activity, while bulky alkyl substituents inhibit it [22,23]. Mannich bases can also enhance or lower cyclovalone's free radical activity: the higher the basicity of

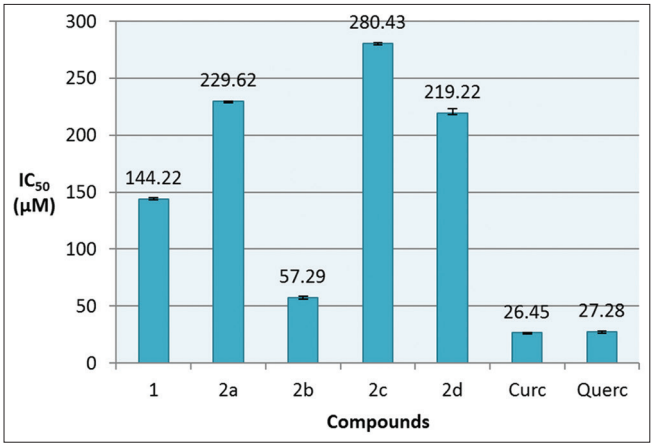


Fig. 3: The free radical scavenging activity (IC_{50}) of the Mannich base derivatives of (2*E*,6*E*)-2-[(4-hydroxy-3-methoxyphenyl) methylidene]-6-(phenylmethylidene)cyclohexan-1-one (compounds 2a–d). Data represented as mean ± standard deviation (n=3)

the nitrogen atom of the Mannich base derivative of cyclovalone, the higher the DPPH free radical scavenging activity of the compound [14]. However, this relationship was not observed for compounds 2a–d.

CONCLUSION

A series of four novel Mannich bases derivatives (compounds **2a–d**) of one AMAC (2*E*,6*E*)-2-({4-hydroxy-3-methoxyphenyl}methylidene)-6-(phenylmethylidene)cyclohexan-1-one, was successfully synthesized. The compounds' anti-inflammatory and antioxidant activities were evaluated using the inhibition of heat-induced albumin denaturation method and the DPPH free radical scavenging assay, respectively. All synthesized compounds (2a–d) showed moderate-to-high anti-inflammatory activity and low-to-moderate antioxidant activity. Compound 2d, containing the dimethylamine Mannich base moiety, showed the highest anti-inflammatory activity, comparable to that of diclofenac sodium. This compound should be studied further to investigate its action mechanism and its toxicity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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