Synthesis and biological evaluation of curcumin analogues having a piperidone core as potential antioxidant agents

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A series of new unsymmetrical curcumin derivatives were designed and synthesised to which the indole fragment was introduced according to the principle of association. These compounds were characterised by ^{1}H NMR, IR, ESI-MS and elemental analysis and their antioxidant activity was investigated using the 1,1-diphenyl-2-picrylhydrazyl method. The results indicated that curcumin analogues having a piperidone structure exhibit varying degrees of antioxidant activity.

Keywords: unsymmetrical curcumin analogue, piperidone, antioxidant activity

Curcumin, which is extracted from *Curcuma longa*, a medicinal plant widely cultivated in tropical regions of Asia, has antioxidant,[1] anti-inflammatory,[2] anticancer,[3] antirheumatic,[4] antibacteral,[5] antihepatotoxic,[6] anti-Alzheimer's disease[7–9] and anti-HIV integrase activity.[10] Efforts to improve the biological activity of curcumin have led to the development of analogues by appropriate structural modification of curcumin.[11,12]

In the past, large numbers of curcumin analogues, containing *N*-substituted piperidone groups have been synthesised as potential antitumour agents.[13,14] Subbagh synthesised a series of curcumin analogues; compound **1** (Fig. 1) showed moderate activity against human immunodeficiency virus-1 (HIV-1).[15] The other synthetic curcumin analogues showed potent α-glucosidase inhibitory effects with IC_{50} of 2.8, 2.6, 1.6, and 8.2 μM, respectively.[16] EF24,[17] which has lower toxicity than curcumin can inhibit a variety of tumour cells, while anti-tumour tests showed that the IC_{50} was 7.5% that of curcumin, and is about to enter clinical trials. The interesting discovery of the anti-tumour activity of *N*-substituted or *N*-unsubstituted piperidone compounds as curcumin analogues prompted us to study a series of curcumin analogues.

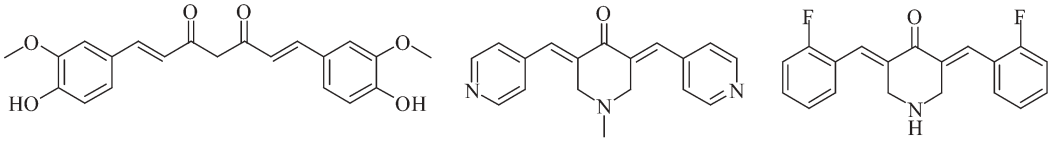
In the work described here, we designed and synthesised a series of new compounds in order to introduce the indole group to *N*-substituted piperidone analogues of curcumin via sequential Knoevenagel reactions (Scheme 1). It was hoped that these novel curcumin analogues would exhibit improved physicochemical and biological properties. The study of their other biological activity is in progress.

Results and discussion

As shown in Scheme 1, compounds **4** and **5a–i** were obtained via the Knoevenagel reaction between the appropriate aldehyde and 1-methyl-4-piperidone in the presence of piperidine, sodium hydroxide or glacial acetic acid saturated with anhydrous hydrogen chloride. From the reaction between the *N*-unsubstituted indole-3-carbaldehyde **2** and 1-methyl-4-piperidone, we could only isolate the starting materials and the yield of **4** was very low even when using a stronger base, such as sodium hydroxide in methanol. The intermediate **4** was easily converted to **5a–i**, and Method B could efficiently enhance the yield of **5g–i** which contained hydroxyl groups. However, when **3** was used for condensation, the reaction proceeded with cleavage of the *N*-acyl group by piperidine during the prolonged stirring process (15 h). In this way, the intermediate **4** could be obtained easily with the yield up to 58%. The results showed that it was necessary to use **3** rather than indole-3-carbaldehyde itself. According to the principle of vinylogy, the electron-donating effect of the NH group in the indole core can be transmitted through the conjugated carbon–carbon double bond to the carbonyl group which makes it less electrophilic. Decreasing the electron density of the carbonyl group of **3** by use of the strong electron-drawing *N*-benzoyl group makes the aldehyde **3** more reactive than **2**.

The antioxidant properties of the new compounds were determined by the free radical scavenging reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH). The method is simple and straightforward and does not require the use of any biological media. In its radical form DPPH absorbs at 515 nm, but upon reduction by an antioxidant or radical species its absorption decreases. A volume of 2.0 mL of a 1.2×10^{-4} M solution DPPH in methanol was used. The reaction was started by the addition of 2.0 mL of 6.4×10^{-5} M methanol solutions of the samples. The bleaching of DPPH was followed at 515 nm at 0 min, 5 min, and every 5 minutes thereafter until the reaction reached a steady state. This plateau was attained within 60 minutes (Fig. 2).

Determination of the antioxidant activity revealed promising activity. Amongst the new compounds, **5h** showed high free radical scavenger activity with inhibition values of 58.87%. This study suggested that curcumin analogues would be lead compounds suitable for designing new free radical inhibitors of this kind. Depending on the rate of the oxygen free radical scavenger activity of the tested compounds, the following structural requirements for high antioxidant activity were identified: (a) a phenolic hydroxy group at the *para*-position is the most essential component in the antioxidant structure; (b) electron-donating substituents on the benzene ring *ortho* to the phenolic hydroxy group effectively enhance the free radical scavenging rate.



Curcumin **1** EF24

Fig. 1 The structures of curcumin, compound **1** and EF24.

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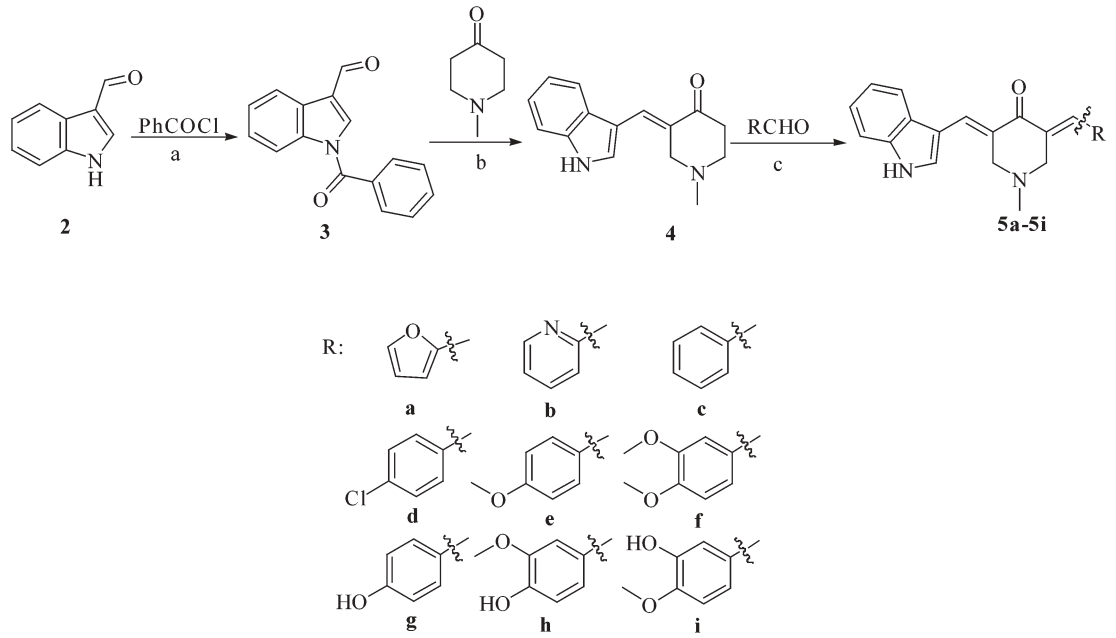

Scheme 1 Synthesis of curcumin analogues. Reagents and conditions: (a) Et_3N, r.t., 4 h; (b) piperidine, 50 °C, 6 h; (c) Method A (for **5a–f**): NaOH (aq), 35–40 °C, 4 h; or method B (for **5g–i**): AcOH, HCl (g), 25–30 °C, 24 h.

Experimental

DPPH was purchased from Sigma–Aldrich (USA). The other starting materials came from commercial sources. The melting points are uncorrected. UV-Vis spectra were obtained on a Shimadzu UV-2450 spectrophotometer. The IR spectra were obtained for KBr pellets using a Prestige-21 Shimadzu IR spectrophotometer; ν_{max} is expressed in cm^{-1}. The ^1H NMR spectra, were recorded for solutions in $CDCl_3$ or DMSO-d_6 with TMS as an internal standard, unless otherwise stated on a Bruker-400 spectrometer. Chemical shifts are reported in δ (ppm) and *J* in Hz. Multiplicities were recorded as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). MS (ESI) spectra were obtained using an Agilent LC-MS 6310 EV instrument. Elemental analyses were carried out with a Flash EA 1112 elemental analyser. The DPPH radical scavenging activity was evaluated as reported previously.[18]

*1-Benzoyl-1*H*-indole-3-carbaldehyde* (**3**): Indole-3-carbaldehyde **2** (10 mmol) was dissolved in dry THF to which was added triethylamine (10 mmol) and benzoyl chloride (15 mmol). The mixture was stirred at room temperature for 4–5 h (TLC) and after removal of the solvent under reduced pressure, adjusted to pH 8 (sat.aq.$NaHCO_3$) and then filtered. The crude product was recrystallised from dichloromethane to give **3** as a white solid (yield 95%). m.p. 84 °C (lit.[20] 83.5 °C). ^1H NMR ($CDCl_3$, 400 MHz) *δ*: 10.07 (1H, s), 8.33 (2H, dd, *J* = 7.0 Hz, 2.0 Hz), 8.34 (2H, m), 7.96 (1H, s), 7.78 (2H, d, *J* = 7.2 Hz), 7.44–7.72 (5H, m). IR (KBr) ν (cm^{-1}): 1669 (CO). MS (*m/z*) 250 (M^+). Anal. Calcd for $C_{16}H_{11}NO_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 76.81; H, 4.45; N, 5.62%.

*(*E*)-3-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**4**):[19] A solution of **3** (10 mmol) in methanol (100 mL) was treated with 1-methyl-4-piperidinone (15 mmol) and piperidine (10 mmol). The mixture was stirred for 6 h at 50 °C and for 15 h at room temperature. After removal of the methanol under reduced pressure, the residue was purified by column chromatography using ethyl acetate–methanol (8:1) as eluent to afford **4** as a yellow solid (yield 58%). m.p. 147–149 °C. ^1H NMR ($CDCl_3$, 400 MHz) *δ*: 8.97 (1H, s), 8.09 (1H, s), 7.89 (2H, d, *J* = 7.8 Hz), 7.43 (1H, d, *J* = 7.8 Hz), 7.37 (1H, s), 7.32–7.24 (2H, m), 3.66 (2H, s), 2.86 (2H, t, *J* = 6.2 Hz), 2.70 (2H, t, *J* = 6.0 Hz), 2.54 (3H, s). IR (KBr) ν (cm^{-1}): 1652(CO). MS (*m/z*) 241 (M^+). Anal. Calcd for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.66. Found: C, 74.85; H, 6.74; N, 11.61%.

Preparation of **5a–i***; general procedure*

Method A (for **5a–f**): To a mechanically stirred solution of **4** (10 mmol) in methanol (100 mL) was added sodium hydroxide (10 mL; 10%) keeping the temperature at 35–40 °C, followed by addition of the appropriate aldehyde (15 mmol). The mixture was stirred for 4–5 h (TLC). After cooling the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using ethyl acetate-methanol (20:1) as eluent to afford **5a–f**.

Method B (for **5g–i**): A mixture of the **2a** and the appropriate aldehyde was dissolved in glacial acetic acid saturated with anhydrous hydrogen chloride and warmed in a water bath at 25–30 °C for 2 h. After standing for 2 days, the mixture was treated with cold water and filtered. The solid obtained was then washed and dried. The crude product was purified by column chromatography using ethyl acetate-methanol (20:1) as eluent to afford **5g–i**.

*(3*E*,5*E*)-3-(Furan-2-ylmethylene)-5-[(1*H*-indol-3-yl)methylene])-1-methylpiperidin-4-one* (**5a**): Yield 65%; red solid; m.p. 223–226 °C. UV-Vis: λ_{max}(MeOH) 418.8 nm. ^1H NMR (DMSO-d_6, 400 MHz) *δ*: 11.97 (1H, s), 7.96 (2H, d, *J* = 3.2 Hz), 7.79 (1H, d, *J* = 7.7 Hz), 7.72(1H, s), 7.50 (1H, d, *J* = 7.9 Hz), 7.35 (1H, s), 7.25–7.16 (2H, m), 6.95 (1H, d, *J* = 3.5 Hz), 6.70 (1H, dd, *J* = 2.9 Hz, 1.6 Hz), 3.81 (2H, s), 3.70 (2H, s), 2.49 (3H, s). IR (KBr) ν (cm^{-1}): 3182 (NH), 2935 (CH_2), 2770 (CH_2), 1655 (CO). MS (*m/z*) 319 (M^+). Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.29; H, 5.72; N, 8.79%.

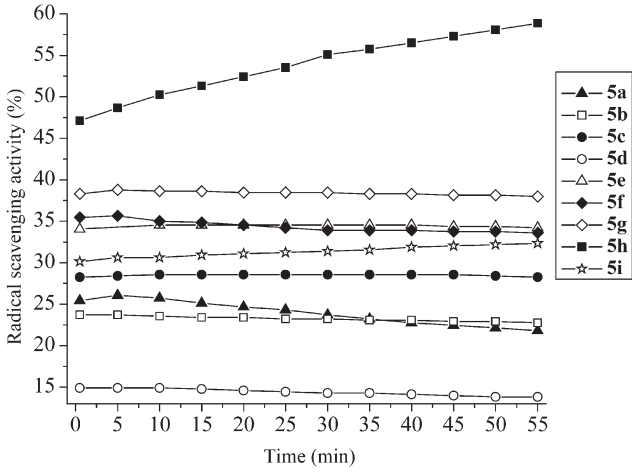

Fig. 2 Free radical scavenging rate of **5a–i**.

*(3*E*,5*E*)-3-[(1*H*-Indol-3-yl)methylene]-1-methyl-5-(pyridin-2-ylmethylene)piperidin-4-one* (**5b**): Yield 72%; red solid; m.p. 177–179 °C. UV-Vis: λ_{max}(MeOH) 420.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.01 (1H, s), 8.74 (1H, s), 7.98 (1H, s), 7.88 (1H, td, J = 7.6 Hz, 2.0 Hz), 7.80 (1H, d, J = 7.7 Hz), 7.75 (1H, s), 7.68 (1H, d, J = 7.9 Hz), 7.50 (2H, d, J = 8.8 Hz), 7.36 (1H, dd, J = 7.5 Hz, 4.8 Hz), 7.26–7.17 (2H, m), 3.70 (2H, d, J = 1.9 Hz), 3.18(2H, d, J = 4.9 Hz), 2.48 (3H, s). IR (KBr) ν (cm^{-1}): 3264 (NH), 2937 (CH_2), 2757 (CH_2), 1653 (CO). MS (*m/z*) 330 (M^+). Anal. Calcd for $C_{21}H_{19}N_3O$: C, 76.57; H, 5.81; N, 12.76. Found: C, 76.12; H, 5.77; N, 12.81%.

*(3*E*,5*E*)-3-Benzylidene-5-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5c**): Yield 71%; yellow solid; m.p. 100–104 °C. UV-Vis: λ_{max}(MeOH) 204.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.00 (1H, s), 7.98 (1H, s), 7.80 (1H, d, J = 7.7 Hz), 7.74 (1H, s), 7.63 (1H, s), 7.45–7.51 (5H, m), 7.42 (1H, dd, J = 8.3 Hz, 4.0 Hz), 7.26–7.17 (2H, m), 3.71 (4H, s), 2.45 (3H, s). IR (KBr) ν (cm^{-1}):3213 (NH), 2933 (CH_2), 1622 (CO). MS (*m/z*) 329 (M^+). Anal. Calcd for $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53. Found: C, 80.20; H, 6.17; N, 8.55%.

*(3*E*,5*E*)-3-(4-Chlorobenzylidene)-5-[(1H-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5d**): Yield 77%; yellow solid; m.p. 216–217 °C. UV-Vis: λ_{max}(MeOH) 222.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.03 (1H, s), 7.98 (1H, s), 7.80 (1H, d, J = 7.8 Hz), 7.74 (1H, s), 7.59 (1H, s), 7.49–7.54 (5H, m), 7.26–7.17 (2H, m), 3.69 (4H, d, J = 4.16 Hz), 2.45 (3H, s). IR (KBr) ν (cm^{-1}): 3242 (NH), 2941 (CH_2), 1651 (CO). MS (*m/z*) 363 (M^+). Anal. Calcd for $C_{22}H_{19}ClN_2O$: C, 72.82; H, 5.28; N, 7.72. Found: C, 72.50; H, 5.35; N, 7.68%.

*(3*E*,5*E*)-3-[(1*H*-Indol-3-yl)methylene]-5-(4-methoxybenzylidene)-1-methylpiperidin-4-one* (**5e**): Yield 85%; yellow solid; m.p. 237–239 °C. UV-Vis: λ_{max}(MeOH) 412.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.96 (1H, s), 7.95 (1H, s), 7.78 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 2.7 Hz), 7.58 (1H, s), 7.49 (1H, d, J = 7.8 Hz), 7.44 (2H, d, J = 8.7 Hz), 7.25–7.16 (2H, m), 7.03 (2H, d, J = 8.7 Hz), 3.80 (3H, s), 3.70 (4H, d, J = 4.8 Hz), 2.50 (3H, s). IR (KBr) ν (cm^{-1}): 3177 (NH), 2931 (CH_2), 1634 (CO). MS (*m/z*) 359 (M^+). Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 76.80; H, 6.17; N, 7.81%.

*(3*E*,5*E*)-3-(3,4-dimethoxybenzylidene)-5-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5f**): Yield 82%; yellow solid; m.p. 205–208 °C. UV-Vis: λ_{max}(MeOH) 422.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.96 (1H, s), 7.96 (1H, s), 7.80 (1H, d, J = 7.8 Hz), 7.72 (1H, d, J = 2.7 Hz), 7.60 (1H, s), 7.50 (1H, d, J = 7.9 Hz), 7.25–7.16 (2H, m), 7.09 (1H, s), 7.05 (2H, s), 3.81 (3H, s), 3.80 (3H, s), 3.74 (2H, s), 3.70 (2H, s), 2.50 (3H, s). IR (KBr) ν (cm^{-1}): 3172 (NH), 2938 (CH_2), 1653 (CO). MS (*m/z*) 389.2 (M^+). Anal. Calcd for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 73.29; H, 6.31; N, 7.18%.

*(3*E*,5*E*)-3-(4-Hydroxybenzylidene)-5-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5g**): Yield 66%; yellow solid; m.p. 268–270 °C. UV-Vis: λ_{max}(MeOH) 371.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.94 (1H, s), 9.98 (1H, s), 7.94 (1H, s), 7.78 (1H, d, J = 7.8 Hz), 7.69 (1H, d, J = 2.7 Hz), 7.54 (1H, s), 7.48 (1H, d, J = 8.0 Hz), 7.34 (2H, d, J = 8.5 Hz), 7.25–7.16 (2H, m), 6.86 (2H, d, J = 8.2 Hz), 3.70 (4H, s), 2.46 (3H, s). IR (KBr) ν (cm^{-1}): 3401 (OH), 3169 (NH), 2936 (CH_2), 1649 (CO). MS (*m/z*) 345.1 (M^+). Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 75.91; H, 5.69; N, 8.14%.

*(3*E*,5*E*)-3-(4-Hydroxy-3-methoxybenzylidene)-5-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5h**): Yield 50%; yellow solid; m.p. 227–230 °C. UV-Vis: λ_{max}(MeOH) 419.0 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.96 (1H, s), 9.59 (1H, s), 7.95 (1H, s), 7.78 (1H, d, J = 7.7 Hz), 7.71 (1H, d, J = 2.7 Hz), 7.57 (1H, s), 7.49 (2H, d, J = 7.9 Hz), 7.25–7.16 (2H, m), 6.94 (1H, dd, J = 8.5 Hz, 1.7 Hz), 6.87 (2H, d, J = 8.1 Hz), 3.83 (3H, s), 3.73 (2H, s), 3.70 (2H, s), 2.47 (3H, s). IR (KBr) ν (cm^{-1}): 3443 (OH), 3177 (NH), 2930 (CH_2), 1617 (CO). MS (*m/z*) 375 (M^+). Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.27; H, 6.01; N, 7.56%.

*(3*E*,5*E*)-3-(3-Hydroxy-4-methoxybenzylidene)-5-[(1*H*-indol-3-yl)methylene]-1-methylpiperidin-4-one* (**5i**): Yield 52%; yellow solid; m.p. 207–208 °C. UV-Vis: λ_{max}(MeOH) 415.6 nm. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.96 (1H, s), 9.28 (1H, s), 7.94 (1H, s), 7.78 (1H, d, J = 2.7 Hz), 7.71 (1H, d, J = 2.7 Hz), 7.49 (2H, d, J = 8.2 Hz), 7.25–7.16 (2H, m), 7.01 (1H, d, J = 8.6 Hz), 6.92 (2H, dd, J = 9.1 Hz, 1.7 Hz), 3.80 (3H, s), 3.69 (4H, s), 2.50 (3H, s). IR (KBr) ν (cm^{-1}): 3429 (OH), 3296 (NH), 2949 (CH_2), 2785 (CH_2), 1651 (CO). MS (*m/z*) 375.2 (M^+). Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.57; H, 5.77; N, 7.63%.

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