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Research Article

Synthesis and Characterization of New Curcumin Derivatives as Potential Chemotherapeutic and Antioxidant Agents

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The purpose of this work was to synthesize a series of symmetrical analogs (**CA2–CA7**) of curcumin and determine their efficacy as antioxidant and anticancer agents in vitro. The six analogs were successfully synthesized and characterized, one of which, **CA6**, had not been previously reported in the literature. With the exception of **CA2**, the analogs had lower predicted aqueous solubilities and higher partition coefficients than curcumin. Two analogs, **CA2** and **CA3**, had lower potencies as anticancer agents compared with curcumin, while **CA6** had a slightly higher IC_{50} value. Two different trends in the antioxidant capabilities of curcumin and its analogs were determined when assessed in vitro or in cell culture. The in vitro DPPH assay clearly showed curcumin as the strongest antioxidant as compared with the analogs when tested at the same concentration or at their IC_{50} value. The cell culture-based reactive oxygen species/reactive nitrogen species assay indicated that **CA3** and **CA6** were equal to curcumin in their free radical scavenging ability at the same concentration, but when curcumin and its analogs were tested at their respective IC_{50} values, **CA4** and **CA5** showed excellent antioxidant capacities. These results indicate that in cell culture, the ability of these analogs to produce antioxidant effects may be tied to their downstream effects. Drug Dev Res 75 : 88–96, 2014.

Key words: curcumin analogs; antioxidant; chemotherapeutics; chemoprevention

INTRODUCTION

Curcumin [Ammon and Wahl, 1991], also known as diferuloyl methane, is a member of the curcuminoid family isolated from the spice turmeric of the herb *Curcuma longa*. Turmeric has been used for centuries as a dye, food additive, dietary spice, and therapeutic in the treatment of wounds, cuts, jaundice, and rheumatoid arthritis [Ammon and Wahl, 1991; Shobana and Naidu, 2000; Naik et al., 2003; Rao, 2003]. Curcumin, the major bioactive component, possesses numerous interesting biological activities, such as antioxidant [Toda et al., 1985; Jayaprakasha et al., 2006], anti-inflammatory [Nurfina et al., 1997], antimalarial [Mishra et al., 2008], and anticarcinogenic [Agrawal and Mishra, 2010]. Studies on the potential role of curcumin in the treatment of Alzheimer's disease have also been reported [Park and Kim, 2002].

Chemically, curcumin contains three important functional regions: α,β-unsaturated β-diketo group; an olefinic linker; and an orthomethoxy phenolic hydroxy group. As shown in Figure 1, curcumin is conjugated and symmetric with two aromatic ring systems connected by a bis-α,β-unsaturated β-diketone. Therefore,

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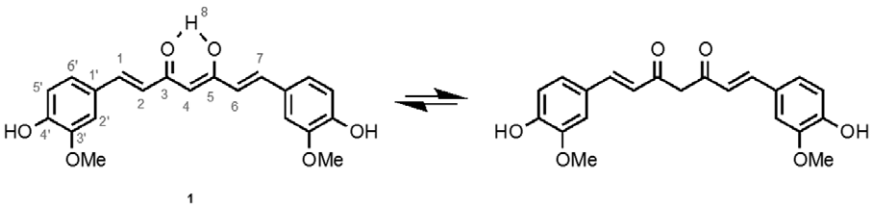

Fig. 1. Curcumin in its keto–enol equilibrium.

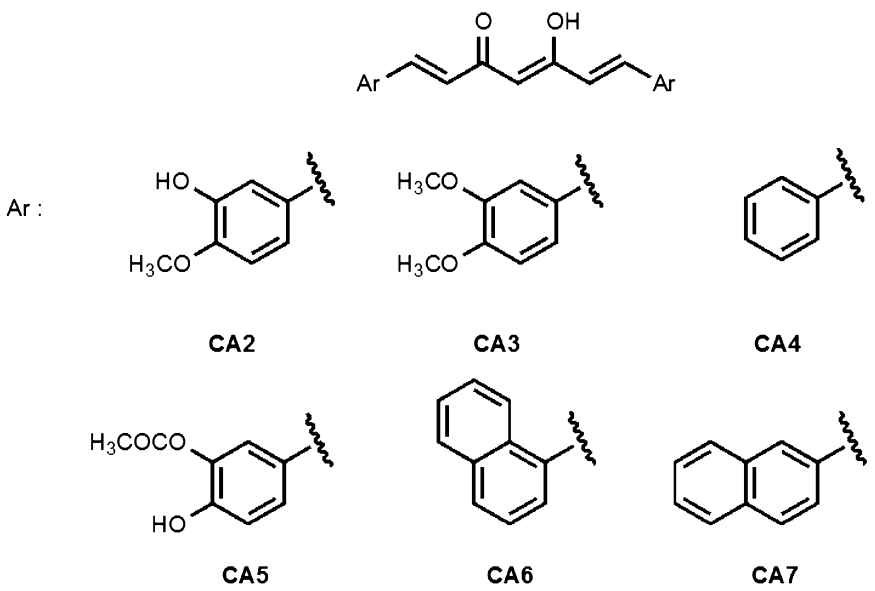

Fig. 2. Structures of curcumin analogs and their abbreviations, CA2–CA7.

it can undergo keto–enol tautomerism, where the enol is the predominant form in solution as a result of conjugation and intramolecular hydrogen bonding that increase the stability of the enol form. Considerable structure activity-based research has shown that the ortho-methoxy phenolic OH group is required for antioxidant activity and the α,β-unsaturated β-diketo group is essential for anticancer activity [Priyadarsini et al., 2003; Chen et al., 2006]. Moreover, the length of the olefinic linker is linked to the prevention of protein aggregation in Alzheimer's disease models [Huang et al., 2005; Anand et al., 2007].

Though curcumin has been extensively studied and used in various herbal preparations, its efficacy in vivo is limited by low aqueous solubility and oral low bioavailability [Anand et al., 2008]. Curcumin has an intrinsic aqueous solubility of 0.6 μg/mL [Kurien et al., 2007], with the oral bioavailability of curcumin in human being less than 1% [Anand et al., 2007]. The only clinically beneficial in vivo effects demonstrated with curcumin have been in preventative clinical trials in colorectal cancer where the high local concentration of the compound may induce a protective effect [Carroll et al., 2011]. Therefore, there is a need to develop curcumin formulations or analogs to better address the solubility and the bioavailability issues related to curcumin dosing. This will also aid in advancing the use of curcumin in various disease states where it has shown great promise in vitro.

Therefore, the objective of this work was to synthesize a series of symmetrical analogs (**CA2–CA7**) of curcumin (Fig. 2) with varying solubilities and partition coefficients, and determine their efficacy as antioxidant and anticancer agents in vitro. As in curcumin, all these compounds maintain the 7-carbon dienone spacer between the rings, but have various substituents on the ring, including nonphenolic groups. These analogs were characterized and evaluated for their antioxidant and antitumor activities in vitro to determine if a correlation exists between the structural modifications and the biological activities.

MATERIALS AND METHODS

Cell Titer Blue® was purchased from Promega (Madison, WI, USA). OxiSelect™ in vitro reactive

oxygen species/reactive nitrogen species (ROS/RNS) assay kit (Green Fluorescence) was purchased from Cell Biolabs, Inc (San Diego, CA, USA). Human ovarian adenocarcinoma cells, SKOV-3, were obtained from American Type Culture Collection (Manassas, VA, USA). Cell culture supplies, including RPMI 1640 1X, fetal bovine serum, antibiotics penicillin/streptomycin, and phosphate-buffered saline (PBS) were all purchased through VWR (Radnor, PA, USA). All solvents used were of HPLC grade and were obtained from VWR.

Synthesis of Curcumin Analogs

An aliquot of 0.050 mol of appropriate aldehyde was added to 25.0 mL of ethyl acetate. To this mixture, 0.100 mol of tributyl borate was added. Separately, a solution of 0.025 mol of acetyl acetone and 0.0180 mol of boric anhydride was prepared and added to the reaction mixture. While stirring, 0.125 mL of n-butylamine was added dropwise every 10 min for 40 min. The reaction was stirred for an additional 4 h and allowed to stand overnight. The following day, 37.5 mL of 0.4 M HCl was heated to 60°C, added to the reaction mixture, and stirred for 1 h. The layers were separated and the water layer washed three times with 12.5 mL of ethyl acetate. The organic layers were combined, washed with brine, and dried over $MgSO_4$. Using a rotary evaporator, the solution was allowed to evaporate to a volume of about 20 mL. Then 12.5 mL of methanol was added and the reaction mixture was allowed to sit for 2 h in an ice bath. Solid was collected via vacuum filtration and recrystallized from CH_2Cl_2/methanol mixture.

Characterization of Curcumin Analogs

Analogs were characterized by their melting points, IR, UV, and NMR spectra. Melting points were recorded on an Electrothermal Mel-Temp melting point apparatus (Bibby Scientific, Staffordshire, UK) and are presented uncorrected. The IR spectra were recorded on a Thermo Scientific Nicolet iS10 FT-IR Spectrometer (Waltham, MA), UV Spectra were recorded on a Shimadzu UV-1601 Spectrophotometer (Columbia, MD). H-NMR and C-NMR were recorded on Bruker Avance 3000 Ultra Shield 300 MHz NMR Spectrometer (Billerica, MA) . Chemical shifts are in ppm (δ) relative to TMS. The partition coefficients and the aqueous solubilities of curcumin and the analogs were predicted using ACD Labs Precepta 14.0.0 (Build 1996) (Toronto, Ontario, Canada) software with Consensus Log P module and solubility in pure water module.

DPPH Radical Scavenging Activity

The free radical scavenging activity of curcumin (compound **1**) and its derivatives was measured by the scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical by modifying a previously reported method [Sreejayan and Rao, 1996]. Two assays were performed. In the first study, DPPH (3.0 mL, 0.5 mM) was incubated for 30 min with curcumin (**1**; Curumin has been identified as compound **1** in the DPPH free radical scavenging activity and the same nomenclature is continued in Table 1) or curcumin derivatives (**CA2–CA7**) or trolox (9.0 mL, 0.3 mM) at final concentrations of 225 μM. In the second study, DPPH (3.0 mL, 0.5 mM) was incubated for 30 min with curcumin (**1**) or curcumin derivatives (**CA2–CA6**) or trolox (9.0 mL, various concentrations) to have final concentrations corresponding to the IC_{50} value for each compound except for **CA7**. Trolox could also not be solubilized at its IC_{50} value and the highest concentration that could be solubilized was 324 μM. The solvent used in preparing all solutions was dichloromethane. The absorbance was read after 30 min at 517 nm. In order to determine the DPPH scavenging activity of each compound, the following equation was used:

TABLE 1. Yields and Melting Points of Curcumin Analogs

Analog	Chemical name	Yield (%)	Melting point (°C)	Referenced melting point (°C)
CA2	(1E,4Z,6E)-5-hydroxy-1,7-bis(3-hydroxy-4-methoxyphenyl) hepta-1,4,6-trien-3-one	63	185–187 (from MeOH/CH_2Cl_2)	190–192 (20)
CA3	(1E,4Z,6E)-1,7-bis(3,4-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one	38	130–131 (from MeOH)	128–130 (7)
CA4	(1E,4Z,6E)-5-hydroxy-1,7-diphenylhepta-1,4,6-trien-3-one	10	140–142 (from Et_2O/pet ether)	140.5 (19)
CA5	dimethyl 5,5'-(1E,3Z,6E)-3-hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-hydroxybenzoate)	51	211–214 (from MeOH/CH_2Cl_2)	210–212 (21)
CA6	(1E,4Z,6E)-5-hydroxy-1,7-di(naphthalen-1-yl)hepta-1,4,6-trien-3-one	54	167–170 (from MeOH/CH_2Cl_2)	None available
CA7	(1E,4Z,6E)-5-hydroxy-1,7-di(naphthalen-2-yl)hepta-1,4,6-trien-3-one	50	256–257 (from MeOH/CH_2Cl_2)	None available

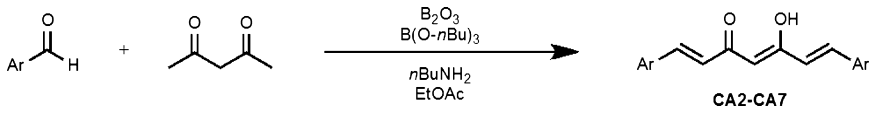

Fig. 3. General synthetic scheme for curcumin analogs, CA2–CA7.

$$\text{Scavenging Activity}\,(\%) = \left(1 - \left(A_s/A_c\right)\right) \times 100$$

where, A_c is the absorbance of DPPH (control) and A_S is the absorbance of DPPH in the presence of the test compound. Each compound was run in triplicate and the data have been presented as mean scavenging effect (%) ± SD. A student's *t*-test was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com) to compare the DPPH radical scavenging effect of each of the treatments to curcumin.

Determination of Cytotoxicity of Curcumin and Its Analogs in SKOV-3 Cells

SKOV-3 cells were maintained in RPMI 1640, 1× with 10% fetal bovine serum and 1% penicillin streptomycin at 37°C with 5% CO_2 and 95% humidity. SKOV-3 cells were seeded in 96-well plates at a concentration of 5000 cells per well and incubated for 24 h for attachment. The cells were treated with curcumin (**1**) or its analogs (**CA2–CA5**) in the concentration range of 0.01–270 μM. Due to the limited solubility of **CA6** in DMSO, treatments were in the concentration range of 0.01–17 μM. Due to the insolubility of **CA7** in DMSO, no further cell culture-based analysis was possible with this analog. The final concentration of DMSO in all the wells was 1%. Posttreatment, the cells were incubated for 48 h and treated with 20 μL of Cell Titer Blue® for 2 h to assess cell viability. The cells were analyzed by fluorescence at excitation wavelength of 560 nm and emission wavelength of 590 nm. All experiments were performed in quadruplicate and data are presented as Mean IC_{50} ± SD.

Quantification of ROS and RNS in SKOV-3 Cells

SKOV-3 cells were seeded in 6-well plates at a cell density of 4×10^5 cells/well. The cells were allowed to attach overnight at 37°C, 5% CO_2, and 95% H_2O. At 24 h, the cells were treated with 10 μM or IC_{50} concentrations of curcumin (**1**), or its analogs (**CA2–CA6**), or trolox and incubated for an additional 24 h. The plates were then centrifuged at 4000 rpm for 5 min, media were aspirated, and cells were washed twice with PBS. The cells were lysed with Triton X–100 cold lysis buffer, scraped and collected into a microcentrifuge tube, and centrifuged at 10,000 rpm for 20 min at 4°C. Supernatant was collected and assayed per manufacturer instructions. Briefly, 50 μL of supernatant was added to a 96-well plate, with each sample run in duplicate. Catalyst (50 μL) was added, mixed, and allowed to incubate for 5 min at room temperature followed by the addition of 100 μL of DCFH (2', 7'-dichlorodihydrofluorescein) solution to each well. The samples were covered to protect from light, allowed to incubate for 45 min, and the fluorescence read (excitation wavelength = 480 nm, emission wavelength = 530 nm). One-way ANOVA with Dunnett's posttest was performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, http://www.graphpad.com) to compare the antioxidant capacity. All experiments were performed in triplicate and data were presented as the mean ROS/RNS Activity ± SD.

RESULTS

Synthesis of Curcumin Analogs

Curcumin derivatives were prepared by the condensation of the appropriate aldehydes with acetyl acetone-boric oxide complex in ethyl acetate (Fig. 3) in the presence of tributyl borate and n-butylamine, as reported earlier [Pabon, 1964]. Upon recrystallization, yellow to orange powders were obtained in 10–63% yields (unoptimized yields). The analog, **CA6** thus far, has not been reported in literature. All curcumin analogs were characterized by interpretation of spectral data (IR, UV, ^{1}H-NMR, and ^{13}C-NMR) and compared with the reported data (Table 1).

Compound **CA6** has been synthesized for the first time, and its structure has been established from a comparison of the observed IR, UV, ^{1}H-NMR, and ^{13}C-NMR spectral data. The IR spectrum (Fig. 4a) shows a broad shallow band around 3000/cm, which corresponds to the enolic O-H bond. The 1614/cm peak indicates carbonyl C = O group involved in intramolecular hydrogen bonding. The UV spectrum (Fig. 4b) in 10^{-5} M DMSO solution shows a strong λ_{max} at 422 nm corresponding to n→π* transition, and a much weaker λ_{max} at 268 nm corresponding to π→π* transition. The ^{1}H-NMR spectrum (Fig. 4c) shows a singlet at δ = 5.97 ppm indicating the presence of the methine proton on C4 with no keto–enol equilibrium observed. The chemical shift of the hydrogen-bonded hydroxyl

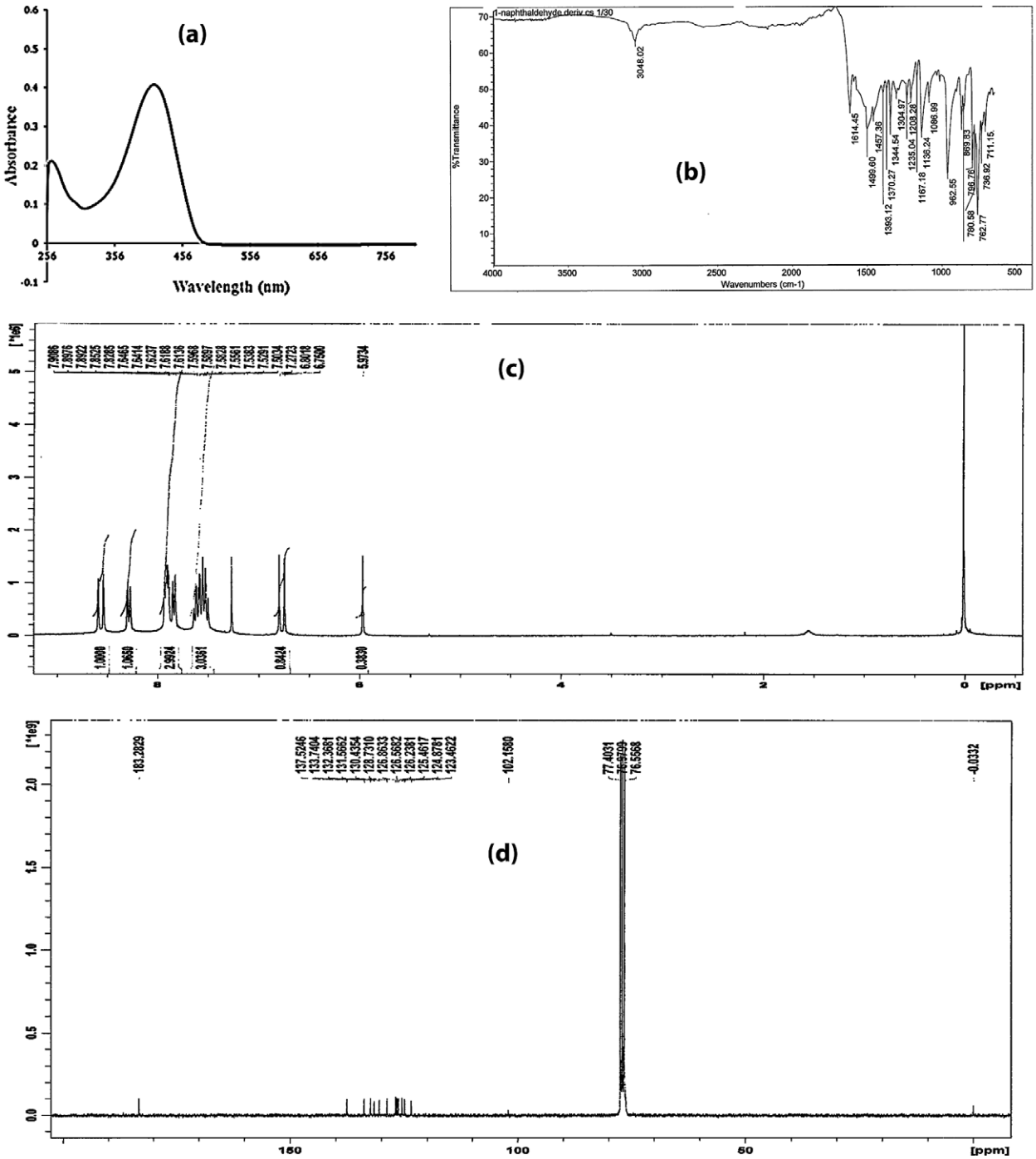

Fig. 4. CA6 spectra—IR (a), UV (b), ^{1}H-NMR (c), and ^{13}C-NMR (d).

proton (H8) is δ 16.04 ppm, exceptionally downfield as with most enol tautomers of 1,3-diketones. The alkenyl protons correspond to the chemical shift at 8.56 ppm (H1 and H7) and 6.78 ppm (H2 and H6), respectively. The trans geometry was identified from the observed J value (Jd = 15.8 Hz).

(1*E*,4*Z*,6*E*)-5-hydroxy-1,7-di(naphthalen-1-yl) hepta-1,4,6-trien-3-one (CA6)

Yield 54%, mp 167–170°C (from MeOH/CH_2Cl_2). Found: 85.82; H, 5.42. Calc. for $C_{27}H_{20}O_2$: C, 86.14; H, 5.36. ^{1}H-NMR: δ ($CDCl_3$) 16.51 (s, 1H), 8.56 (d, 2H, *J* = 15.6 Hz), 8.28 (d, 2H, *J* = 8.3 Hz), 7.90 (m, 4H),

TABLE 2. Predicted Solubilities and Log Distribution Coefficients for Curcumin and its Analogs, CA2–CA6

Compound	Solubility in pure water (mg/mL)	Log partition coefficient
Curcumin (**1**)	0.12	2.64
CA2	0.12	2.56
CA3	0.069	3.22
CA4	0.0041	3.91
CA5	0.016	4.81
CA6	$4.8 \times 10{-}5$	6.05
CA7	$6.5 \times 10{-}6$	6.04

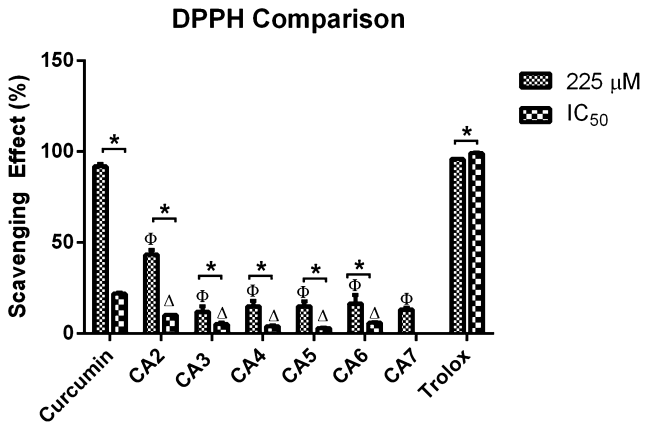

Fig. 5. DPPH scavenging by curcumin, its analogs (**CA2–CA7**) and trolox at 225 μM or at their respective IC_{50} values (except CA7). Data are presented as mean scavenging effect (%) ± SD. $n = 3$; * indicates statistically significant difference in effect at the different concentrations ($P < 0.05$); ϕ indicates statistically significant difference as compared with curcumin at 225 μM ($P < 0.05$); Δ indicates statistically significant difference of the analogs at their IC_{50} values as compared with curcumin at its IC_{50} values ($P < 0.05$).

7.83 (d, 2H, J = 7.2 Hz), 7.59 (m, 4H), 7.53 (d, 2H, J = 8.0 Hz), 6.78 (d, 2H, J = 15.5 Hz), 5.97 (s, 1H); ^{13}C-NMR: δ ($CDCl_3$) 183.3, 137.5, 133.7, 132.4, 131.6, 130.4, 128.7, 126.9, 126.6, 126.2, 125.5, 124.9, 123.5, 102.2.

The solubilities and log partition coefficients for curcumin and its analogs, **CA2–CA7** structures, were predicted using ACD Labs Prospecta software (Toronto, Ontario, Canada) with the Consensus Log P Module and the Solubility in Pure Water Module (Table 2).

DPPH Radical Scavenging Activity

The DPPH radical scavenging effect (Fig. 5) indicates that curcumin and trolox have very similar scavenging effects at 225 μM, but at the IC_{50} concentration of curcumin compared with trolox at 324 μM, trolox is a far more potent antioxidant. However, none of the other analogs matched the ability of curcumin to scavenge DPPH radicals at 225 μM or at their respective IC_{50} values. Statistical analysis indicates that the analogs tested had significantly lower free radical scavenging capability compared with curcumin. The trends in the antioxidant capacity as compared to curcumin were similar at both 225 μM and at the respective IC_{50} values of all the compounds.

TABLE 3. The IC_{50} Values of Curcumin and its Analogues, CA2–CA7, and Trolox in SKOV-3 Cells Post 48 hr Treatment

Compound	Mean (μM)	SD (μM)
Curcumin	6.70	2.44
CA2	5.58	2.00
CA3	3.51	0.74
CA4	75.15	1.19
CA5	72.70	3.84
CA6	14.47	3.87
CA7	ND	ND
Trolox	1607.89	66.37

SD, standard deviation; ND, not determined.

Determination of Cytotoxicity of Curcumin and Its Analogs in SKOV-3 Cells

Data for the cytotoxicity of curcumin and its analogs as Mean IC_{50} ± SD are presented in Table 3. This shows that **CA3** is the most potent analog in comparison with curcumin. Both, **CA2** and **CA3** had cytotoxicities similar to curcumin.

Quantification of ROS and RNS in SKOV-3 Cells

The ROS/RNS activity of curcumin, its analogs and trolox in SKOV-3 cells at 10 μM, and their respective IC_{50} values, were compared with control ROS/RNS (Fig. 6). Based on the data, curcumin, **CA3**, and **CA6** were significantly more potent at scavenging these ROS/RNS species in SKOV-3 at 10 μM. Further analysis comparing curcumin to **CA3** or **CA6** at 10 μM was not significant, indicating that these analogs are as potent at scavenging ROS/RNS species as curcumin at this concentration. At the IC_{50} values, curcumin still demonstrated significant ROS/RNS scavenging ability. **CA4** and **CA5** were the only curcumin analogs that showed significant antioxidant effects at their IC_{50} values. While **CA4** is not significantly different as compared with curcumin in its antioxidant effect, **CA5** showed a significantly higher antioxidant effect as compared with curcumin at its IC_{50} value.

DISCUSSION

Based on the solubility predictions, the structural modifications on the rings in all but **CA2** have lower

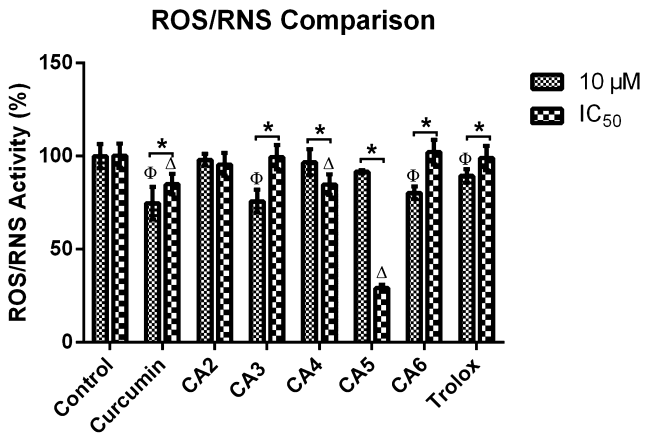

Fig. 6. The ROS/RNS activities of trolox, curcumin (**1**), and curcumin analogs **CA2–CA6** in SKOV-3 cells at 10 μM or at their respective IC_{50} values (except CA7). Data are presented as Mean ROS/RNS Activity ± SD. $n = 3$; * indicates statistically significant difference in effect at the different concentrations ($P < 0.05$); ϕ indicates statistically significant difference as compared with curcumin at 10 μM ($P < 0.05$); Δ indicates statistically significant difference of the analogs at their IC_{50} values as compared with curcumin at its IC_{50} values ($P < 0.05$).

predicted aqueous solubilities than curcumin itself. In addition, **CA6** and **CA7**, derivatives of naphthalene, had far lower solubilities in the μg/mL range, compared with the other ring substituents, consistent with the ring structure. The predicted log partition coefficients for all analogs except **CA2** were higher than curcumin, which correlates with their lower solubility. The naphthalene derivatives have the highest partition coefficients and resulted in far lower aqueous solubilities. Therefore, **CA2** is the only structural derivative of curcumin that has a similar solubility with a lower partition coefficient.

Prior research has demonstrated that curcumin is a more potent free radical scavenger than vitamin E [Zhao et al., 1989]. It has been suggested that the methoxy group on the phenol ring [Sreejayan and Rao, 1996; Priyadarsini et al., 2003] as well as the β-diketone moiety [Jovanovic et al., 2001] in curcumin are the key functional groups responsible for the antioxidant and free radical scavenging properties. The present results indicate that the position of the hydroxyl and methoxy groups is important, as noted from the scavenging activity of **CA2**, where the OH group is on C3' and OMe group is on C4'. All other compounds showed very weak scavenging effects.

CA2 and **CA3**, where the position of the methoxy is reversed (**CA2**) or the methoxy is removed (**CA3**), appear to indicate that this has little effect on cytotoxicity, supporting prior findings that the α,β-unsaturated β-diketo group is essential for anticancer activity [Priyadarsini et al., 2003; Chen et al., 2006] and not the methoxy group. **CA6** also showed efficacy as a cytotoxic agent. The unsubstituted naphthalene derivative did decrease the potency of the analog in comparison with curcumin, possibly due to steric hindrance. **CA7** could not be tested due to its insolubility in DMSO; however, given its structural similarity to **CA6**, it is possible that the same steric hindrance would be an issue. The analogs showing the least potency were **CA4** and **CA5**. For **CA4**, the lack of any substituents might be responsible for the decreased efficacy, while in the case of **CA5**, shifting of the methyl group away from the ring may decrease cytotoxicity. So, while the methoxy group itself might not be the most important factor in cytotoxicity, substitution on the ring at the 5', 4', and 3' position appears to have significant effects on the cytotoxicity in ovarian cancer cells. Curcumin exerts its anticancer activities by altering deregulated cell cycle via cyclin- and p53-dependent and independent pathways [Sa and Das, 2008]. Therefore, interaction of curcumin or its analogs with these pathways is critical to their functionality. The downstream effects of these analogs need to be further investigated to determine the precise role of the substituents in eliciting cell death.

Interestingly, neither **CA3** nor **CA6** contain the *ortho*-methoxy phenolic OH group previously cited as necessary for the antioxidant effect [Priyadarsini et al., 2003; Chen et al., 2006]. This discrepancy may be due to the fact that most antioxidant studies employed in structure activity relationship determination are not based in cell culture or in in vivo models, where the interaction of the molecules with a dynamic redundant system may produce different effects. The trends in the DPPH assay for antioxidant effects remain consistent when tested at the same concentration or at the respective IC_{50} values of curcumin, its analogs (**CA2–CA6**), and trolox. However, in the case of trolox, solubility was limited at 324 μM, which is a quarter of its IC_{50} concentration. In the DPPH assay, there is a concentration-dependent correlation with the ability of the molecule in scavenging DPPH radicals, which may be anticipated, based on the assay principle. DPPH is a free stable radical accepting a hydrogen radical or an electron. DPPH in the free radical form has an absorbance at $\lambda_{max} = 517$ nm, which disappears after accepting the hydrogen radical from an antioxidant. Curcumin and its analogs provide these hydrogen radicals, so by decreasing the concentration of these compounds, we expected an increased in the absorbance of DPPH at 517 nm, thus a weaker scavenging affect. The IC_{50} concentrations are all much lower than 225 μM, and according to our predictions, all scavenging effects are lower.

In comparing the antioxidant activity of curcumin and its analogs in the DPPH assay and in cell culture as assessed by the ROS/RNS assay, it is evident that the

data show different trends in activity. This can be expected as the DPPH assay directly assesses the ability of a compound to scavenge free radicals, while a cell culture-based system like SKOV-3 involves multiple cellular processes that can be affected by antioxidants like curcumin. Curcumin can induce antioxidant and detoxifying enzymes via Nrf2/EpRE signaling pathways [Erlank et al., 2011]. Therefore, in the cell culture treatment, we may be witnessing the combination of direct free radical scavenging ability of the compound and its downstream effects in triggering inherent detoxifying enzymes within the cell. In this context, in cell culture, **CA3** and **CA6** have an equivalent ability to curcumin in decreasing the total free radicals present at 10 μM. However, at their respective IC_{50} values, **CA3** and **CA6** are unable to scavenge any ROS/RNS species. Whether **CA3** and **CA6** work through the same mechanism remains to be elucidated. Interestingly, the positive control, trolox, had ROS/RNS scavenging effects at 10 μM, but at its IC_{50} value no antioxidant effects were observed. Interestingly, at their respective IC_{50} concentrations, only curcumin, **CA4**, and **CA5** show antioxidant effects. **CA4** and **CA5** have much higher IC_{50} (~70 μM) values compared with curcumin (6.5 μM). Therefore, the antioxidant effect for **CA4** and **CA5** may be concentration dependent, and by modulating the concentration between 10 μM and 75 μM we may be able to use these analogs, especially **CA5**, which has a pronounced antioxidant effect at its IC_{50} value. Based on our results, we can classify these analogs as potential antioxidants or chemotherapeutics. **CA2**, **CA3**, and **CA6** may prove to have chemotherapeutic potential, especially if combined with other agents, while **CA4** and **CA5**, with additional studies, might be more useful as chemopreventative based on their low cytotoxicity and strong antioxidants effects.

Trolox is a water-soluble analog of vitamin E and therefore may not have had sufficient permeability across the cell membrane in the time allotted to produce a significant effect in cell lines, limiting its ability to scavenge free radicals in this type of an experimental setup. Therefore, additional studies must be conducted to properly utilize trolox as an antioxidant benchmark for natural compounds in cell culture models or other compounds might have to be used as positive controls in cell culture models.

In conclusion, six analogs of curcumin, one never reported in literature, with differing solubilities and partition coefficients, have been synthesized and assessed for their activity as free radical scavengers and potential chemotherapeutic effects. Based on the cytotoxicity analysis in ovarian cancer cells, we have demonstrated that **CA2**, **CA3**, and **CA6** have lower or similar potencies to curcumin and potentially can be used as chemotherapeutics, while **CA4** and **CA5** have excellent potential to be chemopreventative as antioxidants due to their low cytotoxic potential and strong free radical scavenging ability in cell culture. We have also shown that two different in vitro methods of assessing antioxidant activity can have demonstrably different trends in antioxidant effect. However, we believe that this may be due to the activation of downstream effectors in the in vitro cell culture model and further study is needed to understand these processes.

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