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Research Article

Synthesis and Cytotoxic Evaluation of Monocarbonyl Analogs of Curcumin as Potential Anti-Tumor Agents

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ABSTRACT A series of mono-carbonyl curcumin analogs with different substituents at the 4/4′-position of the phenyl group were synthesized and screened for in vitro cytotoxicity against a panel of human cancer cell lines using a methyl thiazolyl tetrazolium assay. Several of the curcumin analogs, especially B114, exhibited a wide-spectrum of anti-tumor properties in all tested cell lines, indicating their potential in as anti-cancer lead compounds. Further toxicity testing in the NRK-52E kidney cell line revealed that the analogs A111, A113, and B114 had comparable or higher safety than curcumin. These data suggested that the introduction of appropriate substituents in the 4/4′-positions could be a promising approach for curcumin-based drug design. Drug Dev Res 77 : 43–49, 2016.

Key words: monocarbonyl curcumin analogs; synthesis; anti-tumor activity

INTRODUCTION

Curcumin is a polyphenolic natural product isolated from the rhizome of *Curcuma longa Linn* (Turmeric). It has multiple biological properties including antioxidant, anti-inflammatory, anti-infective, anti-cancer, and wound healing activities [Wilken et al., 2011; Prasad et al., 2014; Rainey et al., 2015]. In regard to its anti-cancer properties, curcumin can inhibit cell growth and induce apoptosis in a variety of cancer cell lines by modulating the activities of numerous transcription factors, growth regulators, adhesion molecules, apoptotic genes, and cellular signaling pathways [Chen et al., 2014; Wang et al., 2015]. Due to its anti-tumor properties and extremely low toxicity, curcumin is regarded as an ideal candidate for cancer therapy [Hatcher et al., 2008]. However, its clinical utility is limited by its chemical instability in vitro and poor metabolic properties in vivo [Pan et al., 1999; Rosemond et al., 2004]. This prompted the chemical modification and analog design of curcumin to identify more stable entities that may improve the in vivo metabolic profile and enhance anti-proliferative activity against cancer cells.

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The instability and metabolic defects of curcumin may result from the high reactivity of the β-diketone group in the curcumin structure. Deletion of the β-diketone moiety increases the stability and improves the bioavailability of curcumin analogs in rat [Liang et al., 2009; Zhang et al., 2014]. Mono-carbonyl analogs of curcumin (MACs) with better pharmacokinetic properties and bioactivities than curcumin may represent novel therapeutic entities for the treatment of tumor and inflammatory diseases [Zhao et al., 2013].

Substituents on the 4/4′-position of curcumin may represent an important pharmacophore for biological activity [Ohtsu et al., 2002; Lin et al., 2006a,b; Quincoces Suarez et al., 2010]. The curcumin analog ASC-J9, which has a methoxy group at the 4/4′-position had enhanced anti-androgenic activity and cytotoxicity against prostate cancer cell lines [Lin et al., 2006a,b; Shi et al., 2009].

In the present study, a variety of functional groups was used to replace the 4/4′-OH groups in MACs to develop potent and selective anticancer agents\(Figure.1). Three series of compounds with different 5-carbon linkers (series A: cyclopentanone, series B: acetone and series C: cyclopentanone) and various substituents on the 4/4′-position of benzene rings were designed and synthesized. The cytotoxicity of these MACs was screened in the non-small cell lung cancer cell line H460 using a methyl thiazolyl tetrazolium (MTT) assay. Further, an anti-tumor evaluation in a panel of tumor cell lines showed that some analogs may possess improved anti-cancer activities as compared to curcumin.

METHODS AND MATERIALS

Chemistry

Melting points were determined on a SGW X-4 melting point apparatus and are uncorrected. Electron-spray ionization-mass spectra in positive mode (ESI-MS) data were obtained with a Bruker Esquire 3000^+ spectrometer. ^{1}H-NMR spectra were recorded on Bruker 600 MHz instrument, and chemical shifts presented as parts per million with TMS as the internal reference. Solvents were distilled and dried by standard methods. Tetrahydrofuran (THF) was prepared by drying over 4Å molecular sieves overnight. Various alkyl halide and anhydride reagent were purchased from Aladdin and Sigma-Aldrich. Other chemicals were obtained from local suppliers and were used without further purification. (2E,5E)−2,5-bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone (**4a**), (1E,4E)−1,5-bis (4-hydroxy-3-methoxyphenyl)penta-1,4-dien-3-one (**4b**) and (2E,5E)−2,5-bis(4-hydroxy-3-methoxybenzylidene) cyclohexanone (**4c**) were prepared as described in the literature. [Liang et al., 2008].

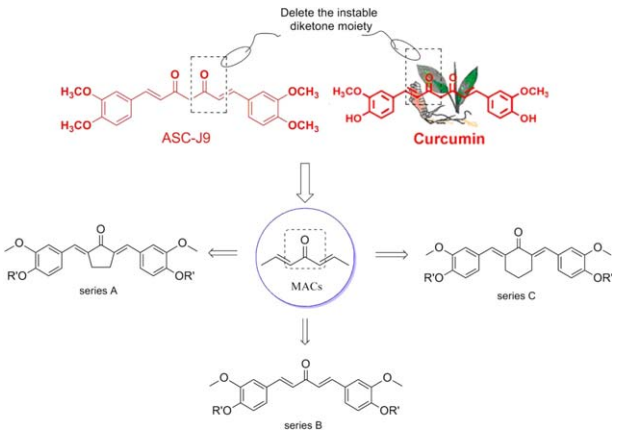


Fig. 1. Design of mono-carbonyl curcumin analogs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

General Procedure (A)

Compounds **4a**, **4b,** or **4c** (5 mmol) were dissolved in dry acetone (10 mL) and anhydrous K_2CO_3 (7.5 mmol) and various alkyl halide (10 mmol) were added. The mixture was refluxed until TLC analysis indicated the reaction was complete; water was added and the mixture extracted with ethyl acetate (50 mL×3). The combined organic phase was washed with H_2O, dried over anhydrous $MgSO_4$ and the solvent removed under vacuum. The products were separated by column chromatography using petroleum ether and ethyl acetate as eluent to yield compounds **A111, A112, A115, A116, B111, B115, B116, C111, C112, C115,** and **C116**, respectively.

General Procedure (B)

Ac_2O or other anhydride (10 mmol) was added to a solution of 4a, 4b, or 4c (5 mmol) in THF (10 mL) in the presence of triethylamine (2–3 drops). The reaction mixture was stirred at room

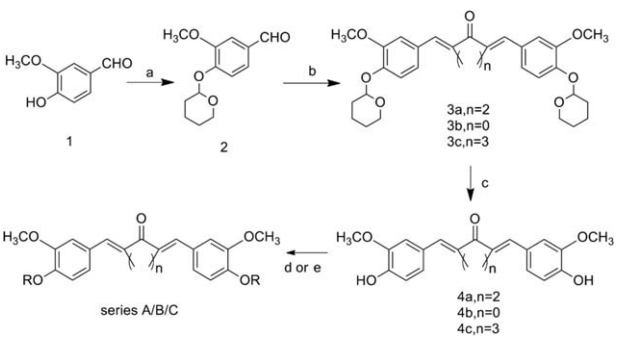


Fig. 2. Synthesis of target compounds. Reagents and conditions: (**a**) pyridine-PTSA, CH_2Cl_2, rt.; (**b**) acetone or cyclopentanone, NaOH, EtOH, rt; (**c**) HCl, CH_2Cl_2, rt. (**d**) K_2CO_3, acetone, reflux; (**e**) TEA, THF, rt.

temperature and the mixture extracted with ethyl acetate (50 mL×3) and the organic phase was washed with H_2O, dried over anhydrous $MgSO_4$ and the solvent evaporated to dryness. The crude product was purified by column chromatography eluting with petroleum ether and ethyl acetate to afford **A110, A113, B110, B113,** and **B114**.

A110 - ((1E,1′E)-(2-Oxocyclopentane-1,3-diylidene)bis(methanylylidene))bis(2-methoxy-4,1-phenylene) dibenzoate

Yellow powder, 64.5% yield, mp 193.7–194.9°C. ^{1}H-NMR ($CDCl_3$) δ: 3.163 (4H, s, CH_2—CH_2), 3.855 (6H, s, —OCH_3), 7.243 (2H, d, J = 9.0 HZ, Ar—H^5), 7.25 (2H, s, —CH=C), 7.282 (2H, d, J = 8.4 HZ, Ar-H^6), 7.483–7.540 (4H, m, Ar—$H^{3',5'}$), 7.609 (2H, s, Ar—H^2), 7.659(2H, d, J = 7.2 HZ, Ar—$H^{4'}$), 8.212-8.236 (4H, m, Ar—$H^{2',6'}$). ESI-MS m/z: 561.1$(M+H)^+$, calculated for $C_{35}H_{28}O_7$: 560.18.

A-111 - (2E,5E)-2,5-bis(4-(2-Hydroxyethoxy)-3-methoxybenzylidene)cyclopentanone

Yellow powder, 73.6% yield, mp 185.3–187.8°C. ^{1}H-NMR ($CDCl_3$) δ: 3.108 (4H, s, CH_2—CH_2), 3.917 (6H, s, —OCH_3×2), 3.991 (4H, t, —OCH_2 ×2), 4.185 (4H, t, —OCH_2 ×2), 6.974 (2H, d, J = 7.8 HZ, Ar—H^5×2), 7.133(2H, s, Ar—H^2 ×2), 7.208(2H, d, J = 7.8 HZ, Ar—H^6×2), 7.535 (2H, s, Ar—CH=×2). ESI-MS m/z: 441.0$(M+H)^+$, calculated for $C_{25}H_{28}O_7$: 440.18.

A-112 - (2E,5E)-2,5-bis(4-(Cyclopentyloxy)-3-methoxybenzylidene)cyclopentanone

Yellow powder, 45.3% yield, mp 114.0–116.2°C. ^{1}H-NMR ($CDCl_3$) δ: 1.58 (8H, m, $H^{3',4'}$), 2.23 (8H, m, $H^{2',5'}$), 3.81 (2H, s, $H^{1'}$), 3.91 (6H, s, —OCH_3×2), 3.10 (4H, s, CH_2—CH_2), 6.96 (2H, d, J = 7.8 HZ, Ar—H^5), 7.20 (2H, d, J = 7.8 HZ, Ar—H^6), 7.27 (2H, s, Ar—H^2), 7.53 (2H, s, Ar—CH=]). ESI-MS m/z: 489.1 $(M+H)^+$, calculated for $C_{31}H_{36}O_5$: 488.26.

A-113- (1E,1′E)-(2-Oxocyclopentane-1,3-diylidene)bis(methanylylidene))bis (2-methoxy-4,1-phenylene) diacetate

Yellow powder, 92.0% yield, mp 200.6-201.8°C. ^{1}H-NMR ($CDCl_3$) δ: 2.337 (6H, s, CO—CH_3×2), 3.110 (4H, s, CH_2—CH_2), 3.884 (6H, s, —OCH_3×2), 7.110 (2H, d, J = 7.8 HZ, Ar—H^5×2), 7.173(2H, s, Ar—H^2×2), 7.214 (2H, d, J = 8.4 HZ, Ar—H^6×2), 7.557 (2H, s, Ar—CH= ×2). ESI-MS m/z: 437.2 $(M+H)^+$, 459.2 $(M+Na)^+$, calculated for $C_{25}H_{24}O_7$: 436.15.

A-115 - (2E,5E)-2,5-bis(3-Methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzylidene) cyclopentanone

Orange powder, 98.9% yield, mp 148.2-148.6°C. ^{1}H-NMR ($CDCl_3$) δ:1.754-1.785(12H, m, —CH_3×4), 3.113 (4H, s, CH_2—CH_2), 3.917 (6H, s, —OCH_3×2), 4.645 (4H, d, J = 6.6 HZ, —OCH_2 ×2), 5.522 (2H, t, —CH=C ×2), 6.935 (2H, d, J = 8.4 HZ, Ar—H^5×2), 7.135 (2H, s, Ar—H^2×2), 7.205 (2H, d, J = 8.4 HZ, Ar—H^6×2), 7.542(2H, s, Ar—CH=×2). ESI-MS m/z: 489.1$(M+H)^+$, calculated for $C_{31}H_{36}O_5$: 488.26.

A-116 - (2E,5E)-2,5-bis(3-Methoxy-4-propoxybenzylidene)cyclopentanone

Yellow powder, 68.5% yield, mp 112.2-114.2°C. ^{1}H-NMR ($CDCl_3$) δ: 1.043 (6H, t, —CH_3×2), 1.877-1.913 (4H, m, —CH_2—×2), 3.112 (4H, s, CH_2—CH_2), 3.865 (6H, s, —OCH_3×2), 4.038 (4H, t, —OCH_2×2), 6.933 (2H, d, J = 8.4 HZ, Ar—H^5×2), 7.140 (2H, s, Ar—H^2×2), 7.211(2H, d, J = 8.4 HZ, Ar—H^6×2), 7.541 (2H, s, Ar—CH= ×2). ESI-MS m/z: 437.1$(M+H)^+$, calculated for $C_{27}H_{32}O_5$: 436.22.

B-110 - ((1E,4E)-3-Oxopenta-1,4-diene-1,5-diyl)bis(2-methoxy-4,1-phenylene)dibenzoate

Yellow powder, 56.2% yield, mp 234.0–235.7°C. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (6H, s, —OCH_3×2), 7.02 (2H, d, J = 15.6 Hz, CO—CH=), 7.23 (2H, d, Ar—H^5), 7.28 (2H, d, Ar—H^6), 7.53 (4H, t, J = 7.8 HZ, Ar—$H^{3',5'}$), 7.61 (2H, s, Ar—H^2), 7.65 (2H, t, J = 7.8 HZ, Ar—$H^{4'}$), 7.71 (2H, d, J = 15.6 HZ, Ar—CH=), 8.23 (4H, d, Ar—$H^{2',6'}$). ESI-MS m/z: 535.0$(M+H)^+$, calculated for $C_{33}H_{26}O_7$: 534.17.

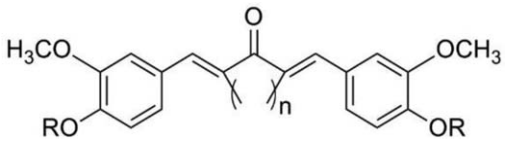
B-111 - (1E,4E)-1,5-bis(4-(2-Hydroxyethoxy)-3-methoxyphenyl)penta-1,4-dien-3-on

Yellow powder, 75.4% yield, mp 91.2–93.6°C. ^{1}H-NMR (DMSO) δ: 3.344 (6H, s, —OCH_3×2), 3.387 (2H, s, —OH), 3.73 (4H, t, J = 5.4 HZ, CH_2—CH_2), 4.03 (4H, t, J = 4.8 HZ, —OCH_2), 7.03 (2H, d, J = 15.6 HZ, CO—CH=), 7.17 (2H, d, Ar—H^5), 7.29 (2H, d, Ar—H^6), 7.49 (2H, s, Ar—H^2), 7.71 (2H, d, J = 15.6 HZ, Ar—CH=). ESI-MS m/z: 437.0$(M+Na)^+$, calculated for $C_{23}H_{26}O_7$: 414.17.

B-113 (1E,4E)-3-Oxopenta-1,4-diene-1,5-diyl)bis(2-methoxy-4,1-phenylene)diacetate

Yellow powder, 60.0% yield, mp 72.8–75.2°C . ^{1}H-NMR ($CDCl_3$) δ: 2.33 (6H, s, —$COCH_3$×2), 3.88 (6H, s, —OCH_3 ×2), 7.03 (2H, d, J = 15.6 HZ, —COCH= ×2), 7.11 (2H, d, J = 7.8 HZ, Ar—H^5×2), 7.22 (2H, d, J = 8.4 HZ, Ar—H^6 ×2), 7.27 (2H, s,

TABLE 1. The Structures of Synthesized Compounds



Compd.	n	R	Compd.	N	R	Compd.	n	R
A110	2	-acetophenone	B110	0	-acetophenone			
A111	2	$-CH_2CH_2OH$	B111	0	$-CH_2CH_2OH$	C111	3	$-CH_2CH_2OH$
A112	2	-cyclopentane	B113	0	$-OCOCH_3$	C112	3	-cyclopentane
A113	2	$-OCOCH_3$	B114	0	$-COCH_2CH_3$	C115	3	$-CH_2CH=C(CH_3)_2$
A115	2	$-CH_2CH=C(CH_3)_2$	B115	0	$-CH_2CH=C(CH_3)_2$	C116	3	$-(CH_2)_2CH_3$
A116	2	$-(CH_2)_2CH_3$	B116	0	$-(CH_2)_2CH_3$			

Ar—H^2 ×2), 7.71 (2H, d, $J = 15.6$ HZ, Ar—CH=×2). ESI-MS m/z: 432.1 $(M+Na)^+$, calculated for $C_{23}H_{22}O_7$: 410.14.

B-114 - ((1E,4E)-3-Oxopenta-1,4-diene-1,5-diyl)bis(2-methoxy-4,1-phenylene)dipropionate

Yellow powder, 47.9% yield, mp 147.4–148.7°C .^{1}H-NMR ($CDCl_3$) δ: 1.247–1.298 (6H, m, $-CH_3$×2), 2.614–2.652 (4H, m, $-CH_2$×2), 3.885 (6H, s, $-OCH_3$×2), 7.010(2H, d, $J = 15.6$ Hz, CO—CH=×2), 7.078 (2H, d, $J = 7.8$Hz, Ar—H^5×2), 7.181 (2H, s, Ar—H^2 ×2), 7.219 (2H, d, $J = 7.8$Hz, Ar—H^6 ×2), 7.810(2H, d, $J = 15.6$ HZ, Ar—CH=×2). ESI-MS m/z: 438.9$(M+H)^+$, calculated for $C_{25}H_{26}O_7$: 438.17.

B-115 -(1E,4E)-1,5-bis(3-Methoxy-4-((3-methylbut-2-en-1-yl)oxy)phenyl)penta-1,4-dien-3-one

Brick red powder, 58.6% yield, mp 93.9–95.2°C. ^{1}H-NMR ($CDCl_3$) δ: 1.755–1.780 (12H, m, $-CH_3$×4), 3.910 (6H, s, $-OCH_3$×2), 4.65 (4H, d, $J = 6.6$ HZ, $-OCH_2$×2), 5.520 (2H, t, —CH=C×2), 6.9400 (2H, d, $J = 8.4$ HZ, Ar—H^5×2), 7.02 (2H, d, $J = 15.6$ Hz, —CH=×2), 7.190 (2H, d, $J = 8.4$ HZ, Ar—H^6×2), 7.27 (2H, s, Ar—H^2×2), 7.71(2H, d, $J = 15.6$ HZ, Ar—CH=×2). ESI-MS m/z: 463.1$(M+H)^+$, calculated for $C_{29}H_{34}O_5$: 462.24.

B-116 - (1E,4E)-1,5-bis(3-Methoxy-4-propoxyphenyl)penta-1,4-dien-3-one

Yellow powder, 42.2% yield, mp 134.5–136.9°C. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (6H, t, $-CH_3$×2), 1.83 (4H, m, $-CH_2$×2), 3.86 (6H, s, $-OCH_3$×2), 4.05 (4H, t, $-OCH_2$×2), 6.94 (2H, d, Ar—H^5×2), 7.14 (2H, d, Ar—H^6×2), 7.21 (2H, s, Ar—H^2×2), 7.54 (2H, s, Ar—CH=×2), 7.71 (2H, d, $J = 15.6$ HZ, Ar—CH=×2). ESI-MS m/z: 411.1$(M+H)^+$, calculated for $C_{25}H_{30}O_5$: 410.42.

C-111-(2E,6E)-2,6-bis(4-(2-Hydroxyethoxy)-3-methoxybenzylidene)cyclohexanone

Yellow powder, 72.1% yield, mp 136.3–138.4°C . ^{1}H-NMR ($CDCl_3$) δ: 1.23–1.25 (2H, m, $-CH_2-$), 2.93 (4H, d, $J = 6.0$ HZ, $-CH_2-$), 3.81 (4H, t, $-CH_2-$×2), 3.91 (6H, s, $-OCH_3$×2), 4.19 (4H, t, $-OCH_2$×2), 6.96 (2H, d, $J = 7.8$ HZ, Ar—H^5×2), 7.20 (2H, d, $J = 7.8$ HZ, Ar—H^6×2), 7.27 (2H, s, Ar—H^2×2), 7.53 (2H, s, Ar—CH=×2). ESI-MS m/z: 455.2(M+1)+, 477.1(M+Na)+, calculated for $C_{26}H_{30}O_7$: 454.20.

C-112 (2E,6E)−2,6-bis(4-(Cyclopentyloxy)-3-methoxybenzylidene)cyclohexanone

Yellow powder, 42.3% yield, mp 96.2–99.6°C . ^{1}H-NMR ($CDCl_3$) δ: 1.24–1.25 (2H, m, $-CH_2-$), 1.57–1.59 (8H, m, $-CH_2-$), 2.22–2.23 (8H, m, $-CH_2-$), 2.93 (4H, t, $-CH_2-$), 3.81 (2H, s,

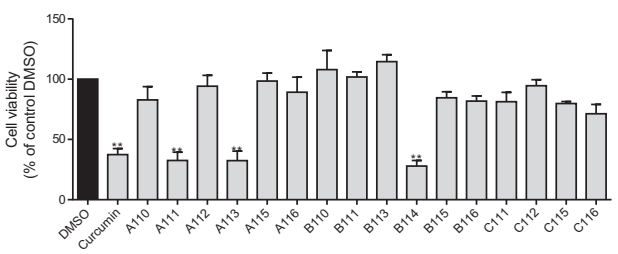


Fig. 3. Evaluation of the anti-proliferative activities of synthesized compounds. U2-OS cells were seeded in 96 well plate and incubated with different compounds with the concentration of 20 μM for 24 hours. Cell viability was assessed using an MTT assay. Data are expressed as fold change relative to control values (samples treated with DMSO alone), mean ± SEM. $n \geq 3$. $^{**}P < 0.01$ vs. DMSO group.

TABLE 2. Cytotoxic Test of A111, A113, B114 (IC_{50}) Against Different Cancer Cell Lines

Comp. cell line	IC50 (M)			
	A111	A113	B114	Curcumin
U2-OS	2.84	6.91	7.24	9.94
OS-732	8.17	8.15	9.23	11.6
A549	>20	>20	10.86	13.97
HepG2	>20	>20	15.32	>20
P815	3.26	>20	0.84	10.33
PC 3	2.23	>20	4.12	15
Hela	>20	>20	3.23	17.5

—CH—), 3.91 (6H, s, —OCH_3×2), 6.96 (2H, d, $J = 7.8$ HZ, Ar—H^5×2), 7.20 (2H, d, $J = 7.8$ HZ, Ar—H^6×2), 7.27 (2H, s, Ar—H^2×2), 7.53 (2H, s, Ar—CH=×2). ESI-MS *m/z*: 503.2$(M+1)^+$, calculated for $C_{32}H_{38}O_5$: 502.27.

C-115 (2E,6E)-2,6-bis(3-Methoxy-4-((3-methylbut-2-en-1-yl)oxy)benzylidene)*cyclohexanone*

Brick red powder, 56.7% yield, mp 89.2–91.6°C . ^{1}H-NMR ($CDCl_3$) δ: 1.25-1.26 (2H, m, —CH_2—), 2.93 (4H, t, —CH_2—), 3.81 (4H, t, —CH_2—×2), 3.91 (6H, s, —OCH_3×2), 4.19 (4H, t, —OCH_2×2), 6.96 (2H, d, $J = 7.8$ HZ, Ar—H^5×2), 7.12 (2H, d, $J = 15.6$ Hz, —CH—×2), 7.20 (2H, d, $J = 7.8$ HZ, Ar—H^6×2), 7.27 (2H, s, Ar—H^2×2), 7.53 (2H, s, Ar—CH=×2). ESI-MS m/z: 455.2$(M+1)^+$, 477.1$(M+Na)^+$, calculated for $C_{26}H_{30}O_7$: 454.20.

C-116 - (2E,6E)-2,6-bis(3-Methoxy-4-propoxybenzylidene)*cyclohexanone*

Yellow powder, 45.3% yield, mp 136.3–138.4°C. ^{1}H-NMR ($CDCl_3$) δ: 1.039 (6H, t, —CH_3×2), 1.24–1.26 (2H, m, —CH_2—), 1.81 (4H, m, —CH_2—×2), 2.93 (4H, t, —CH_2—), 3.89 (6H, s, —OCH_3×2), 4.03 (4H, t, —OCH_2×2), 6.91 (2H, d, $J = 8.4$ HZ, Ar—H^5×2), 7.09 (2H, d, $J = 8.4$ HZ, Ar—H^6×2), 7.27 (2H, s, Ar—H^2×2), 7.74 (2H, s, Ar—CH=×2). ESI-MS m/z: 451.1$(M+1)^+$, calculated for $C_{28}H_{34}O_5$: 450.57.

Cell Culture and Methyl Thiazolyl Tetrazolium Assay

U2-OS, OS-732, HepG2, A549, P815, PC3, HeLa and normal renal NRK-52E cell lines were obtained from the American Type Culture Collection (ATCC, USA). All cell lines were cultured according to ATCC recommendations. Antiproliferative activity was determined using a MTT assay. Briefly, cells were seeded into 96-well plates at a density of 3000–5000 cells per well in 1640 medium, supplemented with 5% heat-inactivated serum, 100 U/ml penicillin, and 100 μg/mL streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were carried out 24 h after cells were seeded. Tested compounds were dissolved in DMSO, and diluted with 1640 medium to the different concentrations of each compound. The tumor cells were incubated with test compounds for 72 h before the MTT assay. A fresh solution of MTT (5 mg/mL) prepared in NaCl solution (0.9%) was added to each single well of the 96-well plate. Plates were then incubated in a CO_2 incubator for 3 h, cells dissolved with 150 μL DMSO, and then analyzed in a multi-well-plate reader at 490 nm. All results were representative from three or more independent experiments.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the target MACs is shown in Figure 2. As the method reported in literature, the synthesis of compounds **4a, 4b** and **4c** started from the protection of commercially available vanillin with tetrahydropyran-2-yl to afford protected compound **2**. 3-methoxy-4-(tetrahydro-2H-pyran-2-yloxy)benzaldehyde **2** was obtained by reacting vanillin **1** with 3,4-2H-dihydropyran in the presence of pyridinium p-toluenesulfonate. The aldol condensation of **2** with cyclopentanone or acetone afforded compound 3a, 3b and 3c, respectively. Hydroxylated analogs **4a, 4b** and **4c** were then obtained by deprotection with diluted hydrochloric acid as catalyst.

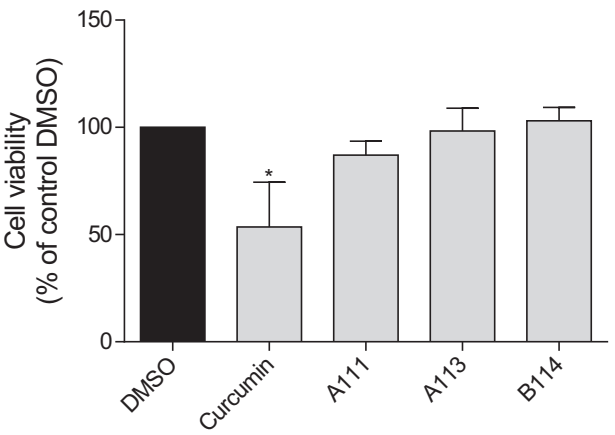


Fig. 4. Evaluation of the cytotoxicity of active compounds in NRK-52E cells. NRK-52E cells were seeded in 96 well plate and incubated with active compounds at a concentration of 20 μM for 24 hours. Cell viability was assessed using MTT assay. Data are expressed as fold change relative to control values (samples treated with DMSO alone), mean ± SEM. $n \geq 3$. *$P < 0.05$ vs. DMSO group.

Finally, the intermediate compounds **4a, 4b** and **4c** were reacted with different alkyl halides in the presence of anhydrous potassium carbonate to yield the target compounds **A111, 112, A115, A116, B111, B115, B116, C111, C112, C115,** and **C116**. The 4′-OH of **4a, 4b,** and **4c** was also substituted by various alkyl esters through reacted with anhydride in the presence of TEA as catalyst. In this way, **A110, A113, B110, B113,** and **B114** were obtained in good yield. The structures of all compounds are listed in Table 1.

Antiproliferative Activity

The in vitro anti-tumor activities of synthesized compounds were first evaluated in the human osteosarcoma cell line U2-OS using MTT assay at a concentration of 20 μM. The results presented in Figure 3 showed that these synthetic curcumin analogues exhibited different cytotoxic activities against U2-OS cells. Most compounds displayed lower activity compared with curcumin except compounds **A111, A113,** and **B114**. These showed more pronounced anti-tumor activity than curcumin in U2-OS cells. As curcumin has a wide-spectrum of anti-tumor properties, the IC_{50} values for **A111, A113,** and **B114** were determined in other cancer cell lines. The antiproliferative activity of active compounds **A111**, **A113**, and **B114** on human osteosarcoma OS-732, human hepatic cancer HepG2, lung cancer A549, prostate cancer PC-3, HeLa and mouse leukemia P815 cell lines were evaluated in the MTT assay. The results shown in Table 2 suggest that **B114** had wide-spectrum anti-proliferative properties, especially against P815 ($IC_{50} = 840$ nM). **A111** had selective, potent cytotoxicity against U2-OS ($IC_{50} = 2.84$ μM), OS-732 ($IC_{50} = 8.17$ μM), P815 ($IC_{50} = 3.26$ μM) and PC-3 ($IC_{50} = 2.23$ μM) cell lines, while **A113** was only cytotoxic in U2-OS ($IC_{50} = 7.24$ μM) and OS-732 ($IC_{50} = 9.23$ μM) cells. Further studies were carried out to observe the preliminary safety of these compounds in the normal rat renal epithelial cell line NRK-52E by MTT assay. The results shown in Figure 4 indicated that the cell viability was reduced by approximately 50% when cells were incubated with 20 μM curcumin. However, growth of NRK-52E cells was only minimally affected by **A111**, while **A113** and **B114** had no inhibitory effect on renal cell growth suggesting that these three compounds have comparable or improved safety over curcumin at the same concentration.

CONCLUSIONS

In summary, we designed and synthesized a series of monocarbonyl curcumin analogs, some of which inhibited tumor cell proliferation. In particular, **B114** had higher activity than curcumin against the tested tumor cell lines. Meanwhile, **A111** showed potent cytotoxicity against human osteosarcoma cell line U2-OS, prostate cancer cell line PC-3, human osteosarcoma cell line OS-732, and mouse mastocytoma cell line P815, implying their specific potential in the chemotherapy of cancer. Toxicity testing in vitro showed that **A111, A113**, and **B114** did not affect growth of normal renal cells. This study presents a series of novel curcumin derivatives as potential anti-tumor candidates.

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