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Curcumin Glucuronides: Assessing the Proliferative Activity against Human Cell Lines

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Abstract

A gram scale synthesis of the glucuronide metabolites of curcumin were completed in four steps. The newly synthesized curcumin glucuronide compounds **2** and **3** along with curcumin **1** were tested and their anti-proliferative effects against KBM-5, Jurkat cell, U266, and A549 cell lines were reported. Biological data revealed that as much as 1 μ M curcumin **1** exhibited anticancer activity and almost 100% cell kill was noted at 10 μ M on two out of four cell lines; while curcumin mono-glucuronide **2** as well as diglucuronide **3** displayed no suppression of cell proliferation.

1. Introduction

Curcumin **1** (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione/diferuloyl methane), the main yellow pigment of the *Curcuma longa* L, has been reported to have antioxidant, antiproliferative and various biological properties.¹⁻¹³ Curcumin has also been reported to decrease total cholesterol and LDL cholesterol level in serum and to increase the beneficial HDL cholesterol level.¹⁴ It has been claimed that curcumin inhibits HIV-I integrase protein, thus potentially preventing HIV-I from infecting CD-4 and CD-8 cell.¹⁵ Curcumin is a non-nutritive, non-toxic compound in turmeric, a spice that has been used for centuries in India and elsewhere as a herbal medicinal treatment for wounds, jaundice, and rheumatoid arthritis.¹⁶ It exerts cancer chemo-preventive efficacy in a wide variety of rodent models of carcinogenesis.¹⁷ In common with several other diet-derivative polyphenols, curcumin has very poor bioavailability,¹³ cellular uptake is slow and it gets metabolized fast, once inside the cell, requiring repeated oral doses in order to achieve significant concentration inside the cells for therapeutic activity. This poor bioavailability is attributed to one of the major metabolites, curcumin glucuronide, that is accumulated in the intestine¹⁸ when curcumin is administered orally.¹⁹ Interestingly enough, in the *miliu intrieur* of studies done on curcumin, the critical central question which has remained unanswered or even addressed, is that of whether or not curcumin glucuronides exhibit any biological activity. In an effort to address this central question, curcumin glucuronides were

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Supplementary Material

Supplementary data associated with this article can be found, at <http://>

synthesized and its potency against human cell lines was compared to the inhibitory activities with that of curcumin thereby providing irrefutable evidence demonstrating complete biological inactivity.

While the chemistry and biology of glucuronides is well known and plays a central role in detoxification pathway,²⁰ the synthesis of curcumin glucuronides have been largely overlooked with only one briefly description in the literature via enzymatic synthesis with no chemical characterization.²¹ Thus, to date no chemical synthesis of curcumin glucuronides has not yet been reported nor the products chemically characterized. Herein we report an efficient synthesis of curcumin glucuronides starting from vanillin.

Results and Discussion

The syntheses of curcumin derivatives are shown in schemes 1-3. Conversion of vanillin to its corresponding β -D-glucuronide was performed with acetobromo- α -D-glucuronic acid methyl ester and silver oxide in quinoline to give the key aldehyde β -D-glucopyranosiduronic acid derivative **6** in 70% yield. Keto-enol compounds (**7** and **9**) were synthesized from the corresponding aldehydes with two equivalents of acetylacetone-boron complex in presence of *n*-butylamine (Scheme 1).²² A preliminary purification by flash column over silica gel followed by recrystallization from ethanol and water gave products that were visualized by fluorescence and UV illumination. Compound **8** was synthesized by coupling keto-enol compound **7** and the vanillin-boron complex in the presence of piperidine which was found to give better yields than when *n*-butylamine was used. Subsequent hydrolysis of acetyl and ester groups at the glucuronide moiety **8** was achieved by treatment with aqueous 1 N NaOH in methanol (1:1) followed by acidification with 50% formic acid in ice water to give curcumin mono-glucuronide **2** in 70% yield.

Similarly compound **9** was converted to the curcumin di-glucuronide **10** starting with the treatment of aldehyde **6** using piperidine as condensation reagent as shown in Scheme 2. Compound **8** was also synthesized from the keto-enol compound **9** using vanillin **4** as the aldehyde substrate for the condensation reaction. Hydrolysis of methyl ester and removal of acetyl groups was done using the same method described in scheme 1 affording curcumin di-glucuronide **3** as a yellow solid. In scheme 3, an equivalent molar mixture of **6** and vanillin **4** was reacted with the acetylacetone-boron complex in the same manner as described in Scheme 1 as well as in Scheme 2.²³ After treatment of the reaction mixture with 1 N HCl and work up, the crude mixture was purified by silica gel flash chromatography to give the compounds **8** (25%) and **10** (<5%) along with the curcumin **1** (10%) as yellow products.

Curcumin and curcumin glucuronides were evaluated for their ability to suppress the transcription factor, NK- κ B in human leukemic KBM-5 cells as they expressed TNF receptors and were response to TNF activation. The KBM-5 cells were pre-incubated for 4 h with curcumin **1** and curcumin glucuronides (compound **2** and **3**) and were treated 0.1 nM TNF for 30 min. The nuclear extracts were prepared and assayed for the NK- κ B activation by electrophoretic mobility shift assay (EMSA). Curcumin inhibited TNF-induced NK- κ B

activation at 25 μ M concentration; however curcumin glucuronides had no effects on NK- κ B suppression (Figure. 2).

Because NK- κ B activation is known to regulate proliferation of tumor cells, we examined the effect of curcumin **1**, and compounds **2** and **3** on proliferation of tumor cell lines. The effect of curcumin **1** and compounds **2** and **3** were compared at equimolar concentrations. The anti-proliferative effects (cytotoxicities) against different cell lines are shown in Figure 3.

In all four KBM-5, Jurkat, U266 and A549 cells, curcumin **1** showed anti-proliferative effects with IC₅₀ values of 3.84, 4.29, 7.57 and 17.3 μ M respectively. In contrast to curcumin **1**, compounds **2** and **3** have no suppression against cell proliferation and IC₅₀'s were more than 200 μ M concentrations (Figure 3).

2. Conclusion

Curcumin mono-glucuronide **2** and curcumin-di-glucuronide **3** were synthesized in four steps with overall yields of 61% and 62% respectively. It was also shown that both the acetylated compounds **8** and **10** along with curcumin **1** can be synthesized in one-pot condensation of **6** and **4** in presence of acetylacetone-B₂O₃ complex. The newly synthesized curcumin glucuronide compounds **2** and **3** along with curcumin **1** were tested and their anti-proliferative effects against KBM-5, Jurkat, U266 and A549 cell lines were reported. The biological data revealed that curcumin glucuronides showed very little anti-proliferative activity and no effect on NK- κ B; while, curcumin displayed NK- κ B inhibitory effects at 25 μ M. Thus, confirming that curcumin mono-glucuronide **2** and curcumin-di-glucuronide **3** are non-toxic and non-antiinflammatory.

3. Experimental

4.1 Reagents, Instrumentation and Cell lines

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. Melting points were measured in open capillary tubes on a Hoover capillary apparatus and are uncorrected. ¹H-NMR and ¹³C-NMR spectra were recorded on an IBM-Bruker Avance 300 (300 MHz for ¹H-NMR and 75.48 MHz for ¹³C-NMR), and IBM-Bruker Avance 500 (500 MHz for ¹H-NMR and 125.76 MHz for ¹³C-NMR), spectrometers. Chemical shifts (δ) are determined relative to CDCl₃ (referenced to 7.27 ppm (δ) for ¹H-NMR and 77.0 ppm for ¹³C-NMR) or DMSO-d₆ (referenced to 2.49 ppm (δ) for ¹H-NMR and 39.5 ppm for ¹³C-NMR). Proton-proton coupling constants (J) are given in Hertz and spectral splitting patterns are designated as singlet (s), doublet (d), triplet (t), quadruplet (q), multiplet or overlapped (m), and broad (br). Low resolution mass spectra (ion spray, a variation of electrospray) were acquired on a Perkin-Elmer Sciex API 100 spectrometer or Applied Biosystems Q-trap 2000 LC-MS-MS.

Flash chromatography was performed using Merk silica gel 60 (mesh size 230-400 ASTM) or using an Isco (Lincon, NE) combiFlash Companion or SQ16x flash chromatography system with RediSep columns (normal phase silica gel (mesh size 230-400ASTM) and

Fisher Optima TM grade solvents. Thin-layer chromatography (TLC) was performed on E.Merk (Darmstadt, Germany) silica gel F-254 aluminum-backed plates with visualization under UV (254nm) and by staining with potassium permanganate or ceric ammonium molybdate. Penicillin, streptomycin, RPMI 1640 medium, Dulbecco's modified eagles medium and Iscove's modified Dulbecco's medium, and fetal bovine serum were obtained from Invitrogen. Bacteria-derived human recombinant tumor necrosis factor (TNF), purified to homogeneity with a specific activity of 5×10^7 units/mg, and was kindly provided by Genentech (South San Francisco, CA). Solvents and reagents were purchased from Sigma-Aldrich, Acros organics and Fluka.

The KBM-5 (human chronic myeloid leukemia) cell lines were obtained from Dr. Nicholas J. Donato (University of Michigan Comprehensive Cancer Center), Jurkat (human T cell leukemia), U266 (multiple myeloma) and A549 (lung adenocarcinoma) cells were obtained from American Type Culture Collection (Manassas, VA). The Jurkat and U266 cells were cultured in RPMI 1640 medium, A549 cells were cultured in Dulbecco's modified eagles medium with 10% fetal bovine serum. KBM-5 cells were cultured in Iscove's modified Dulbecco's medium with 15% fetal bovine serum. All culture media were supplemented with 100 units/ml penicillin and 100 µg/mL streptomycin.

4.1.1 β-D-glucopyranosiduronic acid, 4-formy-2-methoxyphenyl, methyl ester, triacetate (6)—Vanillin **4** (0.5 g, 3.3 mmol) and acetobromo-α-D-glucuronic acid methyl ester **5** (2.3 g, 5.9 mmol) were dissolved in distilled quinoline (20 mL) and silver oxide (0.8 g, 4 mmol) was added in portions at 0 °C. The reaction mixture was stirred in the dark at 0 °C for 30 min. and at room temperature for 90 min. After the addition of acetic acid (40 mL), the mixture was poured into water (400 mL) and passed through celite pad and the filtrate was extracted with ethyl acetate (2 × 200 mL), washed with brine and then dried. Purification by flash chromatography using dry-packed silica gel and elution with hexane–EtOAc (70:30, first 25 min, and then 20:80 for 40 min) gave **6** (1.08 g, 70%) as a white solid: m.p. 100–102 °C. ¹H NMR (500 MHz CDCl₃) δ 9.90 (s, 1H), 7.44 (s, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 1H), 5.35 (m, 3H), 5.19 (d, *J* = 6.6 Hz, 1H), 4.17 (d, *J* = 9 Hz, 1H), 3.89 (s, 3H), 3.73 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H); ¹³C NMR (125 MHz CDCl₃) 188.5, 167.7, 166.9, 166.7, 164.4, 148.6, 148.3, 130.6, 123.1, 116.4, 108.2, 97.2, 70.3, 69.1, 68.4, 66.6, 53.6, 50.4, 18.2, 18.2, 18.1; IR (neat) 1752.87, 1686.3, 1594.47; HRMS (C₂₁H₂₄O₁₂) calcd. 468.12 found 486.4 (M+NH₄).

4.1.2 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1,4-hexadien-3-one (7)—2,4-Pentanedione (3.3 mL, 32.07 mmol) and B₂O₃ (2 g, 28.7 mmol) were dissolved in ethyl acetate (30 mL), and the solution was stirred at 80 °C for 30 min. To this mixture was added an ethyl acetate solution of (40 mL) of the vanillin **4** (2.2 g, 14.4 mmol) and (*n*-BuO)₃ (1.6 mL, 5.96 mmol). After stirring for 30 min at 85 °C, *n*-butylamine (0.5 mL, 5.04 mmol) was added drop wise to the mixtures, which was allowed to stir at 105 °C for 1.5 h. Then the reaction mixture was treated with 1 N HCl (10 mL) at 50 °C and stirred at the same temperature for 1 h. Reaction mixture was cooled and then extracted with ethyl acetate, washed with water and dried over Na₂SO₄. Flash chromatography over silica gel (2:1, hexanes/EtOAc) followed by recrystallization from ethanol and water, gave the **7** as yellow

crystals (1.69 g, 50%): mp 143.1- 145.1 °C; ^1H NMR (CDCl_3) δ 15.5 (bs, 1H), 7.52 (d, J = 0.6 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 7.01 (s, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.32 (d, J = 15.6 Hz, 1H), 5.90 (bs, 1H), 5.63 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (CDCl_3) δ 196.9, 178.0, 147.8, 146.8, 140.1, 127.7, 122.7, 120.4, 114.8, 109.6, 100.7, 56.0, 26.8; IR (neat) 1752.87, 1686.3, 1594.47; HRMS ($\text{C}_{13}\text{H}_{14}\text{O}_4$) calcd. 234.08 found 235.2 (M+H).

4.1.3 Curcumin β -D-glucopyranosiduronic acid 2,3,4-Tri-O-acetyl, methyl ester (8)—Compound **7** (1.35 g, 5.7 mmol) and B_2O_3 (600 mg, 8.6 mmol) were dissolved in ethyl acetate (15 mL) at 85 °C. To this mixture was added an ethyl acetate solution (30 mL) of **6** (1.6 g, 3.4 mmol) and (*n*-BuO) $_3\text{B}$ (2.28 mL, 8.49 mmol). After stirring for 1 h, the mixture was treated with piperidine (200 μL , mmol) at 85 °C for 30 min, and then with 0.5 N HCl (20 mL) at 50 °C for 30 min. Reaction mixture was cooled and then extracted with ethyl acetate, washed with water and dried over Na_2SO_4 . Flash chromatography over silica gel (1:2 to 1:9 hexanes/EtOAc) gave **8** as yellow foam (2.17 g, 55%). IR (neat) 1755.68, 1626.44, 1586.48, 1509.51; ^1H NMR (CDCl_3) δ 7.59 (d, J = 15.6 Hz, 1H), 7.56 (d, J = 15.6 Hz, 1H), 7.10 (m, 5H), 6.92 (d, J = 7.8 Hz, 1H), 6.49 (d, J = 16.2 Hz, 1H), 6.48 (d, J = 16.2 Hz, 1H), 5.81 (s, 1H), 5.34 (m, 3H), 5.10 (d, J = 7.2 Hz, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 3.74 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (CDCl_3) δ 184.2, 182.2, 170.1, 169.4, 169.3, 166.9, 150.8, 148.0, 147.3, 147.3, 146.8, 141.0, 139.5, 131.9, 127.6, 123.6, 122.9, 121.7, 121.6, 120.2, 114.9, 111.7, 109.7, 101.4, 100.3, 72.7, 71.8, 71.1, 69.2, 56.1, 55.9, 52.9, 20.6, 20.6, 20.5; HRMS ($\text{C}_{34}\text{H}_{36}\text{O}_{15}$) calcd. 684.2054 found 685.5 (M+H).

4.1.4 β -D-Mono-glucuronidecurcumin (2)—To a cooled (ice-water bath) solution of the glucuronate ester (500 mg, 0.74 mmol) in methanol (14 mL) was added aqueous 1 N NaOH solution (14 mL) drop wise. The resulting solution was then stirred for 3h at 0 °C and then pH of the solution was adjusted to 3-4 by adding 50% aqueous formic acid. The yellow solid was filtered, collected and purified by reversed phase chromatography using water and methyl alcohol as eluent (gradient 20 to 80, 50 min) to give **2** as yellow solid (0.28 g, 70%); IR (Neat): 3400, 1624.4, 1585.9. ^1H NMR ($\text{DMSO}-d_6$) δ 7.56 (d, J = 7.2 Hz, 1H), 7.56 (d, J = 6.6 Hz, 1H), 7.37 (s, 1H), 7.31 (s, 3H), 7.23 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 9.6 Hz), 7.12 (d, J = 8.4 Hz, 1H), 6.84 (m, 2H), 6.75 (d, J = 15.6 Hz, 1H), 6.08 (s, 1H), 5.21 (bs, 1H), 5.10 (bs, 1H), 4.95 (d, J = 7.2 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.4-3.12 (m, 4H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 184.3, 182.9, 172.2, 166.2, 150.2, 149.7, 149.2, 148.5, 141.3, 140.3, 129.1, 126.6, 123.7, 122.9, 121.6, 116.2, 115.8, 111.8, 111.8, 101.5, 100.2, 77.4, 74.1, 73.5, 72.5, 56.3, 56.2; HRMS ($\text{C}_{27}\text{H}_{28}\text{O}_{12}$) calcd. 544.1581 found 545.5 (M+H) and 567.4 (M+Na).

4.1.5 1-[4-(2,3,4-Tri-O-acetyl, methyl ester, β -D-glucuronides of-3-methoxyphenyl)-5-hydroxy-1,4-hexadien-3-one (9)]— ^1H NMR (CDCl_3) δ 7.58 (d, J = 15.6 Hz, 1H), 7.11 (m, 3H), 6.52 (d, J = 15.6 Hz, 1H), 5.33 (m, 3H), 5.1 (d, J = 7.2 Hz, 1H) 3.87 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (CDCl_3) δ 197.7, 190.9, 176.9, 170.1, 169.4, 169.2, 166.9, 150.9, 147.4, 139.1, 131.8, 125.5, 122.2, 121.3, 120.2, 111.6, 111.8, 101.1, 100.3, 72.7, 71.8, 69.2, 60.4, 56.1, 52.9, 20.6, 20.5; HRMS ($\text{C}_{26}\text{H}_{30}\text{O}_{13}$) calcd. 550.16 found 551.5(M+4H) and 568.5(M+NH $_4$).

4.1.6 Bis-[2,3,4-Tri-O-acetyl, β -D-glucopyranosiduronic acid methyl ester]-

curcumin (10)—IR (Neat): 2255, 1756, 1629; ^1H NMR (CDCl_3) δ 7.58 (d, J = 15.6 Hz, 2H), 7.10 (m, 6H), 7.31 (s, 3H), 6.53 (d, J = 15.6 Hz, 2H), 5.83 (bs, 1H), 5.33 (m, 6H), 5.10 (d, J = 7.2 Hz, 2H), 3.86 (s, 6H), 3.74 (s, 6H), 2.08 (s, 6H), 2.06 (s, 6H), 2.04 (s, 6H); ^{13}C NMR (CDCl_3) δ 183.1, 170.1, 169.3, 169.2, 166.9, 150.9, 147.5, 140.0, 131.8, 123.6, 121.7, 120.2, 111.8, 100.3, 100.1, 72.7, 71.8, 71.1, 69.2, 56.2, 52.9, 20.6, 20.5; HRMS ($\text{C}_{47}\text{H}_{52}\text{O}_{24}$) calcd. 1000.9014 found 1001.9 (M+H) and 1018.1 (M+NH₄).

4.1.7 β -D-Di-glucuronidecurcumin (3)—IR (Neat): 2816, 2777, 2682, 1614; ^1H NMR (DMSO-d_6) δ 7.52 (d, J = 15.6 Hz, 2H), 7.30 (s, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 16.2 Hz, 2H), 5 (d, J = 6.6 Hz, 2H), 3.80 (s, 6H), 3.64 (d, J = 9.0 Hz, 2H), 3.34 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 183.9, 173.3, 150.1, 149.1, 141.5, 130.2, 123.8, 123.7, 117.1, 116.5, 112.3, 100.7, 76.8, 76.2, 73.8, 72.6, 56.9; HRMS ($\text{C}_{33}\text{H}_{36}\text{O}_{18}$) calcd. 720.6281 found 721.6 (M+H) and 743.4 (M+Na).

4.2 Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays for NF- κ B

Nuclear extracts were prepared as described previously.²⁴ Briefly, 1.0×10^6 cells were washed with cold PBS and suspended in 0.1 mL hypotonic lysis buffer containing protease inhibitors for 30 min. The cells were then lysed with 5 μL of 10% NP840. The homogenate was centrifuged, and supernatant containing the cytoplasmic extracts was removed and stored frozen at -80°C . The nuclear pellet was resuspended in 20 μL ice-cold nuclear extraction buffer. After 1 h of intermittent mixing, the extract was centrifuged, and supernatants containing nuclear extracts were secured. To determine NK- κ B activation, we did electrophoretic mobility shift assay as described previously, with the following exceptions. Briefly, nuclear extracts were incubated with ^{32}P -end-labeled 45-mer double-stranded NK- κ B oligonucleotide (15 μg protein with 16 fmol DNA) from the HIV long terminal repeat, 5'-

TTGTTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTGG-3' (boldface indicates NK- κ B-binding sites), for 30 min at 37°C , and the DNA-protein complex formed was separated from free oligonucleotide on 6.6% native polyacrylamide gels. The dried gels were visualized with a Storm820 and radioactive bands were quantified using ImageQuant software (GE Healthcare, Piscataway, NJ).

4.2.1 MTT Assay—The cell growth effects of curcumin and curcumin glucuronides were determined by the MTT uptake method as described. Briefly, 5×10^3 cells were seeded in triplicate in 96-well plates and then treated with various concentrations of curcumins for 72 h at 37°C . Thereafter, MTT solution was added to each well. After a 2 h incubation at 37°C , an extraction buffer (20% SDS and 50% dimethylformamide) was added; the cells were then incubated overnight at 37°C , and the absorbance was measured at 570 nm by using a 96-well multiscanner (MRX Revelation, Dynex Technologies, Chantilly, VA).

4.2.2 Statistical analysis—All data values were presented as mean $\pm$ SD. Statistical comparisons were measured using Student's t-test. Differences were considered significant at a $P < 0.05$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Chemical structures of curcumin, curcumin monoglucuronide and curcumin diglucuronide.

Scheme 1.

Reagents and conditions: (a) Ag_2O , quinoline, 0 °C to rt, 1.5 h, 70%; (b) 2,4-pentanedione, B_2O_3 , $(n\text{-BuO})_3\text{B}$, EtOAc, 85 °C, 30 min, $n\text{-BuNH}_2$, 105 °C, 30 min, 1 N HCl, 50%; (c) B_2O_3 , $(n\text{-BuO})_3\text{B}$, EtOAc, 85 °C, 30 min, piperidine, 85 °C, 30 min, 0.5 N HCl, 50 °C, 30 min, 55%; (d) MeOH, 1 N NaOH, 50 °C, 1 h, 50% formic acid, 70 %.

Scheme 2.

Reagents and condition: (a) 2, 4-pentanedione, B_2O_3 , $(n-BuO)_3B$, EtOAc, 85 °C, 30 min, $n-BuNH_2$, 105 °C, 30 min, 1 N HCl, 50%; (b) B_2O_3 , $(n-BuO)_3B$, ethyl acetate, 85 °C, 30 min, piperidine, 85 °C, 30 min, 0.5 N HCl, 50 °C, 30 min, 55% (c) MeOH, 1 N NaOH, 50 °C, 1 h, 50% formic acid, 75 %.

Scheme 3.

Reagents and conditions: (a) 2,4-pentanedione, B_2O_3 , $(n\text{-BuO})_3B$, EtOAc, 85 °C, 30 min, $n\text{-BuNH}_2$, 105 °C, 30 min, 1 N HCl, 25%.

Figure 2.

Evaluation of curcumin **1** and its glucuronide derivatives **2** and **3** on TNF-induced NF-κB DNA binding in KBM-5 human myeloid cells. Cells were pretreated with curcumins for 4 h, then, stimulated with TNF (0.1 nmol/L) for 30 min, and analyzed for NF-κB DNA binding by EMSA.

Figure 3.

Evaluation of curcumin **1** and its glucuronide derivatives **2** and **3** on cell proliferation. Five thousand KBM-5 (**A**) Jurkat (**B**) U266 (**C**) and A549 (**D**) cells per well were seeded in triplicate onto 96-well plates; treated with the each compound at 1, 5, 10, 25, 50, 100 or 200 μM. After 72 the cell viability was measured by the MTT method and presented as % cell viability. The data were expressed as the means ± SD of triplicate samples. *P < 0.05 and **P < 0.01 vs. untreated cells.