Effects of Pyridine Analogs of Curcumin on Growth, Apoptosis and NF-κB Activity in Prostate Cancer PC-3 Cells

XINGCHUAN WEI[1], DAIYING ZHOU[2], HUAQIAN WANG[2], NING DING[3], XIAO-XING CUI[3], HONG WANG[3], MICHAEL VERANO[3], KUN ZHANG[2,4], ALLAN H. CONNEY[2,3], XI ZHENG[2,3] and ZHI-YUN DU[2]

[1]*Department of Chemistry and Chemical Engineering, Guangzhou University, Guangzhou, P.R. China;*
[2]*Allan H. Conney Laboratory for Anticancer Research, Guangdong University of Technology, Guangzhou, P.R. China;*
[3]*Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ, U.S.A.;*
[4]*Wuyi University, Jiangmen City, Guangdong, P.R. China*

Abstract. *Twelve pyridine analogs of curcumin were studied for their effects on growth and apoptosis in human prostate cancer PC-3 cells. The ability of these compounds to inhibit the transcriptional activity of nuclear factor-kappa B (NF-κB) and the level of phosphorylated extracellular signal-regulated kinases (phospho-ERK1/2) in PC-3 cells was also determined. Treatment of PC-3 cells with the pyridine analogs of curcumin resulted in concentration-dependent growth inhibition and apoptosis stimulation. Only pyridine analogs of curcumin with a tetrahydrothiopyrane-4-one linker (FN compounds) exhibited a strong inhibitory effect on growth and a strong stimulatory effect on apoptosis at low concentrations (≤1 μM). Mechanistic studies showed that NF-κB transcriptional activity in PC-3 cells was strongly inhibited by treatment with group FN compounds. Treatment of PC-3 cells with 1 μM FN1 resulted in a decrease of activated ERK1/2. Results from the present study indicate that FN compounds warrant further in vivo studies using suitable animal models of prostate cancer.*

A large number of studies have been published on the beneficial effects of curcumin, a major constituent of the yellow spice turmeric derived from the rhizome of *Curcuma longa* Linn (1-3). Curcumin inhibits cell proliferation and induces apoptosis in a variety of malignant cell types, including breast, colon, gastric, ovarian and prostate cancer cells (4-8). *In vivo* studies demonstrated that curcumin administration reduced tumorigenesis of the stomach (9), colon (10, 11), liver (11), mammary gland (12, 13) and of the skin (9, 14). Extensive studies have revealed that curcumin exerts its wide range of anticancer effects through modulating a diversity of signaling pathways including nuclear factor-kappa B (NF-κB) and other transcription factors (15-18).

Although curcumin has been used in clinical trials for certain types of human cancers (19, 20), the efficacy of curcumin is limited, which is likely a result from its low bioavailability (21). It has been hypothesized that the β-diketone moiety of curcumin may cause its instability and poor metabolic properties. Enhanced stability of curcumin analogs was found by removing this moiety (22). It has been shown that cyclohexanone analogs of curcumin had enhanced stability in biological mediums compared to curcumin (22). The cyclohexanone-containing curcumin analogue 2,6-*bis*[(3-methoxy-4-hydroxyphenyl)methylene]-cyclohexanone was found to be more potent than curcumin in inhibiting human breast cancer cells *in vitro* (23, 24). Replacement of ortho-hydroxylmethoxyl benzene with a pyridine ring, further increased the inhibitory effect of this compound on breast cancer cells (24).

In a recent study, we synthesized a series of 12 pyridine analogs of curcumin with cyclohexanone, cyclopentanone, tetrahydropyran-4-one or tetrahydrothiopyran-4-one as the linker and determined their anticancer activities in cultured human cancer cells (25). We found that these pyridine analogs had stronger inhibitory effects than curcumin on the growth of a number of human cancer cell lines, including human prostate cancer PC-3 cells (25). In the present study, we investigated the effects and mechanisms of these pyridine curcumin analogues on growth and apoptosis in PC-3 cells.

Correspondence to: Xi Zheng, Ph.D. and Zhi-Yun Du, Ph.D., Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854, U.S.A. Tel: +1 7324454940, Fax: +1 7324450687, e-mail: xizheng@pharmacy.rutgers.edu and zhiyundu@yahoo.com.cn

Key Words: Prostate cancer, curcumin analogues, proliferation, apoptosis, PC-3 cells.

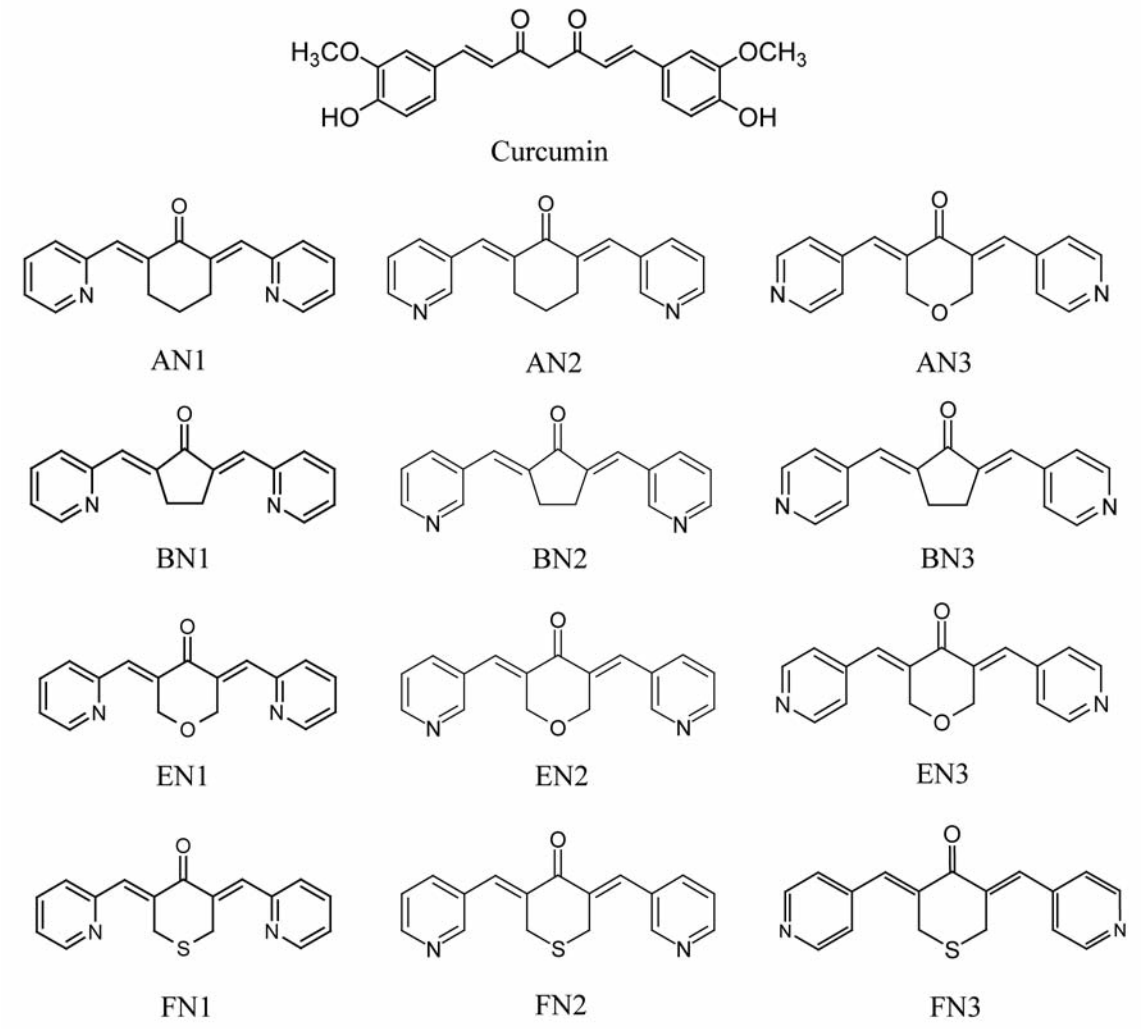


Figure 1. *Structure of curcumin and its pyridine analogs.*

Materials and Methods

Chemistry. A series of pyridine analogs of curcumin were synthesized by coupling the appropriate pyridyl aldehyde with cyclohexanone (group AN), cyclopentanone (group BN), tetrahydropyran-4-one (group EN) or tetrahydrothiopyran-4-one (group FN) (Figure 1), as previously described (25). Characterization of these compounds (AN1, AN2, AN3, BN1, BN2, BN3, EN1, EN2, EN3, FN1, FN2, FN3) was previously described in detail (25).

Cell culture and reagents. PC-3 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Curcumin was obtained from Sigma-Aldrich (St. Louis, MO, USA). RPMI-1640 tissue culture medium, penicillin-streptomycin, L-glutamine and fetal bovine serum (FBS) were from Gibco (Grand Island, NY, USA). PC-3 cells were maintained in RPMI-1640 culture medium containing 10% FBS that was supplemented with penicillin (100 units/ml)-streptomycin (100 μg/ml) and L-glutamine (300 μg/ml). Cultured cells were grown at 37˚C in a humidified atmosphere of 5% CO_2 and were passaged twice a week. PC-3 cells were initially seeded at a density of 0.2×10^5 cells/ml in 35-mm tissue culture dishes (2 ml/dish) for assays of proliferation and apoptosis. Curcumin and its analogs were dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO in all experiments was 0.1%.

Determination of the number of viable cells. The number of viable cells after each treatment was determined using a hemacytometer under a light microscope (Nikon Optiphot, Tokyo, Japan). Cell viability was determined by the trypan blue exclusion assay, which was carried out by mixing 80 μl of cell suspension and 20 μl of 0.4% trypan blue solution for 2 min. Blue cells were counted as dead cells and the cells that did not absorb dye were counted as live cells.

Morphological assessment of apoptotic cells. Cytospin slides were prepared after each experiment and cells were fixed with acetone/methanol (1:1) for 10 min at room temperature, followed by 10 min with propidium iodide staining (1 μg/ml in PBS) and

analyzed using a fluorescence microscope (Nikon Eclipse TE200; Tokyo, Japan). Apoptotic cells were identified by classical morphological features including nuclear condensation, cell shrinkage, and formation of apoptotic bodies (26).

Caspase-3 (active form) immunostaining. An antibody (#AF835) that reacts with the active form of caspase-3 was purchased from R&D Systems (Minneapolis, MN, USA). Cytospin slides were incubated with caspase-3 primary antibody (1:2,000 dilution) for 30 min at room temperature followed by incubation with a biotinylated anti-rabbit secondary antibody for 30 min and an incubation with conjugated-avidin solution (ABC Elite kit purchased from Vector Laboratories, Burlingame, CA, USA) for 30 min. Color development was achieved by incubation with 0.02% 3,3'-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide for 10 min at room temperature. The slides were then counterstained with hematoxylin. A positive reaction was shown as a brown precipitate in the cytoplasm and/or perinuclei of the cells. At least 200 cells were counted in each sample and the percentage of positive cells was determined (27).

Nuclear factor-kappa B (NF-κB)-dependent reporter gene expression assay. An NF-κB luciferase construct (#CLS-013L; SABiosciences, Valencia, CA, USA) was stably transfected into PC-3 cells and a single stable clone, PC-3/N, was obtained and used in the present study. In brief, PC-3/N cells were treated with different curcumin analogs for 24 h, and the NF-κB-luciferase activities were measured using luciferase assay kits from Promega (Madison, WI, USA), as described previously (28).

Western blotting. After treatment, the cells were lysed as described elsewhere (26). Proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 100 V for 110 min on 4% to 12% gradient gels. Separated proteins were transferred to nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA). After blocking non-specific binding sites with a blocking buffer (LI-COR Corp, Lincoln, NE, USA), the membrane was incubated overnight at 4˚C with anti-phosphorylated ERK1/2 (#4376; Cell Signaling Technology, Boston, MA, USA). The β-actin protein was used as a loading control. Following removal of the primary antibody, the membrane was washed three times with TBS (PBS containing 0.05% tween 20) buffer at room temperature and then incubated with fluorochrome-conjugated secondary antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA). Final detection was carried out with an Odyssey infrared imaging system (LI-COR Corp, Lincoln, NE, USA).

Statistical analyses. Analysis of variance (ANOVA) with the Tukey-Kramer test (29) was used for the comparison of viable cells, apoptotic cells and NF-κB activities among the different treatment groups of cultured PC-3 cells.

Results

Inhibitory effect of pyridine analogs of curcumin on the growth of human prostate cancer PC-3 cells. Effects of the 12 pyridine analogs of curcumin on the growth of cultured human prostate cancer PC-3 cells were determined by using the MTT and the trypan blue exclusion assays. In experiments using the MTT assay, we found that curcumin and its pyridine analogs inhibited the growth of PC-3 cells in a concentration-dependent manner (Figure 2A-D). All pyridine analogs of curcumin had a stronger inhibitory effect on the growth of PC-3 cells than did curcumin. Group FN compounds showed stronger inhibitory effects on the growth of PC-3 cells than compounds in the other groups. Group BN compounds had weaker effects for inhibiting PC-3 cells than did compounds in the other groups. In experiments using the trypan blue exclusion assay, we determined the inhibitory effects of different curcumin analogs at a low concentration (1 μM) on the growth of PC-3 cells. We found that group FN compounds strongly reduced the number of viable cells, while group AN and EN compounds only had moderate inhibitory effects on the cells (Figure 2E). Statistical analysis using ANOVA with the Tukey-Kramer test showed that treatment with group AN, EN and FN compounds significantly ($p>0.05$) reduced the number of viable cells (see Figure 2 legend for specific *p*-values). Differences for the number of viable cells between the control group and the curcumin-treated group and between the control group and those treated with BN compounds were not statistically significant ($p>0.05$).

Stimulatory effect of pyridine analogs of curcumin on apoptosis in PC-3 cells. Morphological assessment and the caspase-3 assay were used to determine the effect of pyridine analogs of curcumin on stimulating apoptosis of PC-3 cells. As shown in Figure 3A-D, treatment with the 12 pyridine analogs of curcumin stimulated apoptosis of PC-3 cells in a concentration-dependent manner. Treatment with curcumin caused a small increase in apoptotic cells (Figure 3). We also determined the effect of a low concentration (1 μM) of curcumin and its pyridine analogs on apoptosis of PC-3 cells using the caspase-3 assay. As shown in Figure 3E, treatment of PC-3 cells with group FN curcumin analogs (1 μM) caused strong increases in apoptosis. Treatment with groups of AN, BN and EN compounds (1 μM) resulted in small to moderate increases in apoptosis, while treatment with this low concentration of curcumin did not have any effect on apoptosis (Figure 3E). Statistical analysis using ANOVA with the Tukey-Kramer test showed that apoptosis was significantly ($p>0.01$) increased after PC-3 cells were treated with groups AN, EN and FN compounds (see Figure 3 legend for specific *p*-values). Treatment with curcumin and group BN compounds did not cause a significant increase in apoptosis ($p>0.05$). The number of apoptotic cells was significantly higher in cells treated with the group FN compounds than that in cells treated with compounds from any other group (see Figure 3 legend for p-value).

Effect of pyridine analogs of curcumin on NF-κB activity. We used an NF-κB-luciferase reporter gene expression assay to

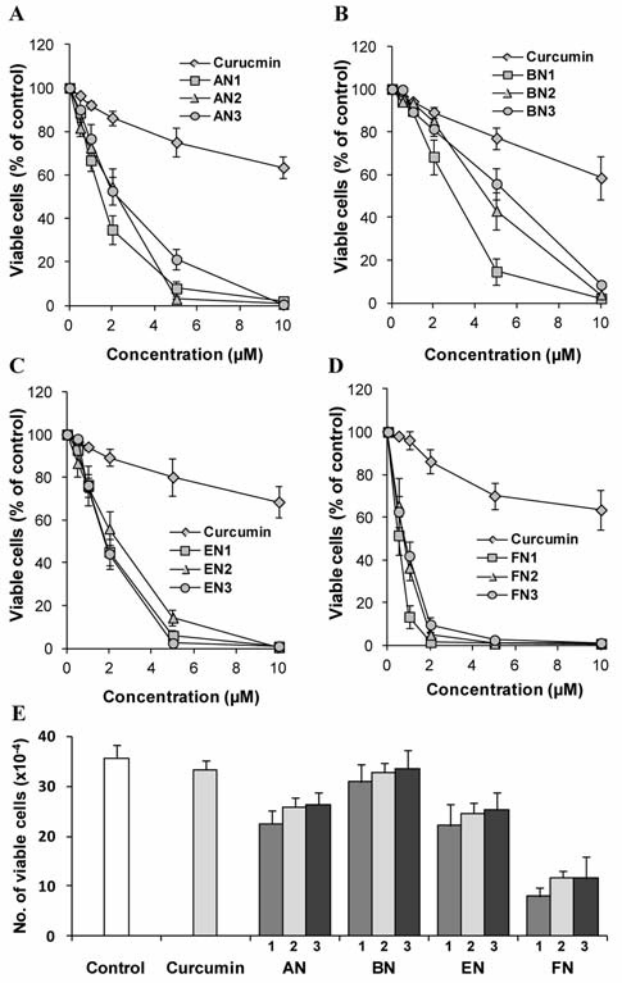


Figure 2. *Inhibitory effect of pyridine analogs of curcumin on the growth of human prostate cancer PC-3 cells. A-D: PC-3 cells were seeded at a density of 0.2×10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 h. The cells were then treated with different concentrations (0.1-10 μM) of the different compounds for 72 h. Effects of the different compounds on the growth of PC-3 cells were determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. E: PC-3 cells were seeded at a density of 0.2×10^5 cells/ml of medium in 35-mm tissue culture dishes and incubated for 24 h. The cells were then treated with 1 μM of the different compounds (AN, BN, EN or FN 1, 2 and 3) for 72 h. Effects of the different compounds on the growth of PC-3 cells were determined by the trypan blue assay. Statistical analysis for the study in (E) used ANOVA with the Tukey-Kramer test. $p<0.001$ for control vs. AN1, EN1, FN1, FN2 and FN3; $p<0.01$ for control vs. EN2 and EN3; $p<0.05$ for control vs. AN2 and AN3; $p<0.001$ for FN group compounds compared to all other compounds, except FN2 vs. AN1 ($p<0.01$), FN2 vs. EN1 ($p<0.01$), FN3 vs. AN1 ($p<0.01$), and FN3 vs. EN1 ($p<0.01$).*

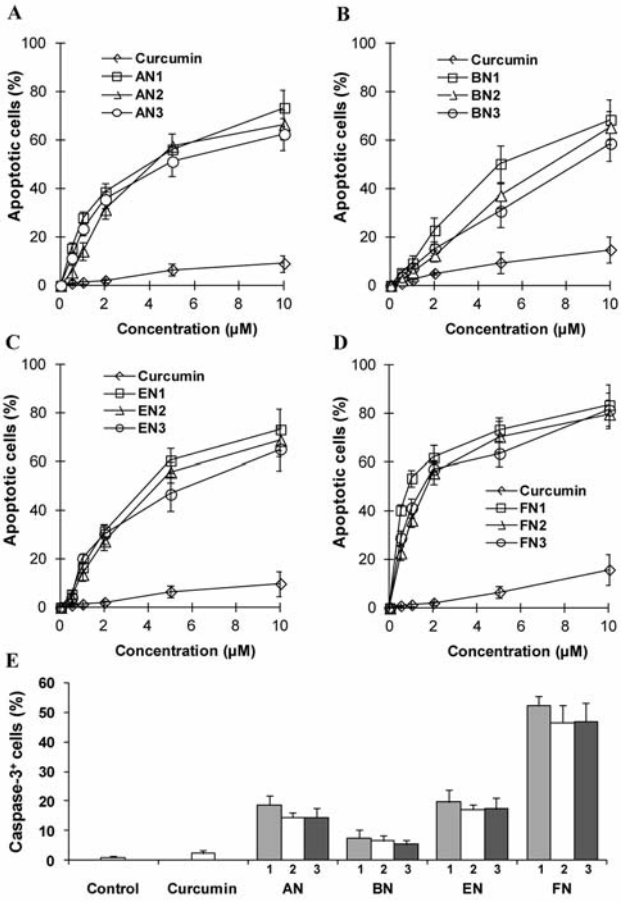


Figure 3. *Effect of curcumin and its pyridine analogs on the stimulation of apoptosis of PC-3 cells. PC-3 cells were seeded at a density of 0.2×10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 h. The cells were then treated with different concentrations (0.1-10 μM) of the compounds for 72 h. Apoptosis was determined by morphological assessment of cells stained with propidium iodide (A-D) and by caspase-3 (active form) immunostatining (E). In (E), 1 μM of AN, BN, EN or FN 1, 2 or 3 was added and incubated for 72 h. Statistical analysis for the study in (E) used ANOVA with the Tukey-Kramer test. $p<0.001$ for control vs. AN1, and EN1-3; $p<0.01$ for control vs. AN2 and AN3; $p<0.001$ for FN group compounds compared to all other compounds.*

determine the effect of the pyridine analogs of curcumin on activation of NF-κB. In these experiments, PC-3/N cells were treated with a low concentration (1 μM) of curcumin and its analogs for 24 h. Treatment of PC-3/N cells with group FN compounds resulted in strong decreases in NF-κB transcriptional activity (Figure 4). Group AN and EN compounds had moderate effects for reducing the NF-κB transcriptional activity, while curcumin and group BN compounds had no effect (Figure 4). Statistical analysis using ANOVA with the Tukey-Kramer test showed that NF-κB activity was significantly lower in cells treated with group FN compounds than in cells treated with curcumin or other pyridine analogs of curcumin (see Figure 4 legend for *p*-values).

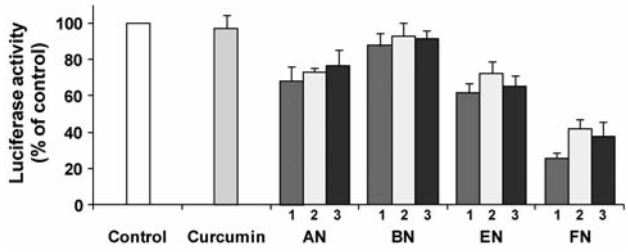
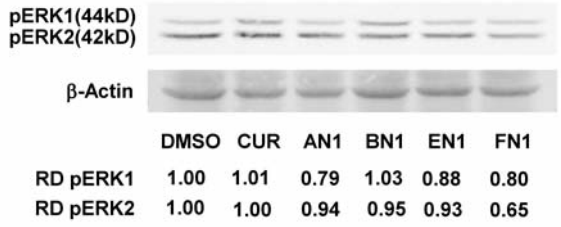


Figure 4. *Inhibitory effects of curcumin and its pyridine analogs on nuclear factor-kappa B (NF-κB) transcriptional activity in PC-3/N cells. PC-3/N cells were seeded at a density of 0.2×10^5 cells/ml of medium in 24-well plates (1.0 ml/well) and incubated for 24 h. The cells were then treated with 1 μM of the different compounds for 24 h. The NF-κB transcriptional activity was determined by the luciferase reporter assay. Statistical analysis used ANOVA with the Tukey-Kramer test. $p<0.001$ for FN group compounds compared to all other compounds except FN3 vs. EN1 ($p<0.01$), FN2 vs. EN3 ($p<0.01$) and FN2 vs. EN1 ($p<0.05$).*



	DMSO	CUR	AN1	BN1	EN1	FN1
RD pERK1	1.00	1.01	0.79	1.03	0.88	0.80
RD pERK2	1.00	1.00	0.94	0.95	0.93	0.65

Figure 5. *Inhibitory effects of curcumin and its pyridine analogs on the activation of extracellular signal-regulated kinases (ERK)1/2. PC-3 cells were seeded at a density of 1×10^5 cells/ml of medium and incubated for 24 h. The cells were then treated with 0.1% dimethyl sulfoxide (DMSO) (control), curcumin, AN1, BN1, EN1 or FN1 (all at 1 μM) for 24 h. Activation of ERK1/2 was determined using western blot analysis with a phospho-ERK1/2 antibody. β-Actin was used as a loading control. The intensity of the pERK1/2 bands relative to that of β-actin was determined and expressed as relative density (RD). A representative blot from three experiments is shown.*

Effect of pyridine analogs of curcumin on activated ERK1/2. In additional experiments, we determined the effect of AN1, BN1, EN1 and FN1 on the level of ERK1/2 activation in PC-3 cells. In these experiments, PC-3 cells were treated with a low concentration (1 μM) of curcumin, AN1, BN1, EN1 and FN1 for 24 h. The levels of phospho-ERK1/2 in the cells were determined by western blot analysis. The levels of phospho-ERK1/2 on western blots were analyzed by absorbance measurements and were normalized for actin. We found that treatment of PC-3 cells with curcumin or BN1 had no effect on the level of phospho-ERK1, and treatment with AN1, EN1 and FN1 had small to moderate inhibitory effects (Figure 5). Treatment with FN1 caused a 35% decrease in the level of phospho-ERK2 while curcumin, AN1, BN1 and EN1 had little or no effect (Figure 5).

Analysis of structure–activity relationship. Twelve pyridine analogs of curcumin with a five-carbon linker and a cyclohexanone, cyclopentanone, tetrahydropyran-4-one or tetrahydrothiopyran-4-one structure were studied for their anticancer activities towards human prostate cancer PC-3 cells. A 2-μM concentration of these curcumin analogs had stronger inhibitory effects on growth and stronger stimulatory effects on apoptosis of PC-3 cells than did curcumin. Among the four groups of these curcumin analogs, compounds with a tetrahydrothiopyran-4-one linker had stronger activities than any of the other compounds tested. Compounds with a cyclopentanone linker had weaker activities than compounds with a cyclohexanone, tetrahydropyran-4-one or tetrahydrothiopyran-4-one linker. These results indicate that compounds with cyclopentanone as a linker had weaker anticancer activity than the other compounds tested. Among compounds with the same linker, a nitrogen heteroatom in the *ortho*-position of the pyridine ring had stronger activities than those with a nitrogen in the *meta*- or *para*-position in the pyridine ring.

Discussion

A large number of curcumin analogs that inhibit the growth of cultured cancer cells have been synthesized, but most studies utilize relatively high concentrations, and there is only limited information on the *in vivo* effects of the analogs. In the present study, we first determined the effects of 12 pyridine analogs of curcumin on the growth of human prostate cancer PC-3 cells using the MTT assay. In agreement with the observations we reported earlier (25), we found that all 12 pyridine analogs of curcumin at concentrations of 2-10 μM had stronger inhibitory effects on the growth of PC-3 cells than did curcumin. However, only the FN group of curcumin analogs retained strong activity at low concentrations (≤1 μM). Because the achievable concentrations of curcumin analogs *in vivo* may be low, we subsequently determined the effects of all compounds at 1 μM on growth and apoptosis in PC-3 cells by using the trypan blue exclusion assay and the caspase-3 assay. Our results indicate that group FN curcumin analogs had strong effects on growth inhibition and apoptosis stimulation. Group AN and EN compounds had moderate activities, while group BN compounds and curcumin had no effect on growth and apoptosis at this low concentration. Further studies are needed to determine the plasma levels of these curcumin analogs and their possible inhibitory effects on tumor growth in animal models.

During the past decade, many curcumin analogs have been synthesized in order to improve the stability and anticancer activity of curcumin. Studies from our laboratory and other

investigators indicate that cyclohexanone-containing analogs of curcumin had more potent anticancer activities than did curcumin (25, 30, 31). Further modification of cyclohexanone curcumin analogs by replacing the *ortho*-hydroxylmethoxyl benzene with a pyridine ring, strongly enhanced the anticancer activities of these curcumin analogs (23, 25). In the present study, pyridine analogs of curcumin that contain a five-carbon linker with a cyclohexanone, cyclopentanone, tetrahydropyran-4-one or tetrahydrothiopyran-4-one moiety were synthesized and evaluated for their effects on growth inhibition and apoptosis in PC-3 cells. We found that analogs with tetrahydrothiopyran-4-one as the linker had more potent effects than analogs with tetrahydropyran-4-one, cyclohexanone or cyclopentanone as the linker. Earlier studies showed that the six-membered cyclohexanone ring system is in general superior to the five-membered cyclopentanone system for inhibiting the growth of several cancer cell lines (24, 25). Our results, indicating that pyridine cyclohexanone curcumin analogs (AN1-3) had stronger activities than pyridine cyclopentanone curcumin analogs (BN1-3), further confirm this structure–activity relationship. Our studies also showed that replacement of the cyclohexanone core with tetrahydrothiopyran-4-one further increased the anticancer activity while replacement of cyclohexanone with tetrahydropyran-4-one did not enhance the activities of these curcumin analogs. This result indicates that introduction of sulfur in the linker may enhance the anticancer activities of pyridine curcumin analogs. In addition, we found that compounds with a nitrogen heteroatom in the *ortho*-position of the pyridine ring usually had stronger activities than compounds with a nitrogen heteroatom in the *meta*- or *para*-position of the pyridine ring.

To elucidate the mechanisms by which the pyridine analogs of curcumin inhibit the growth and induce apoptosis in PC-3 cells, the effects of these compounds on activation of NF-κB were determined. Earlier studies showed that curcumin exerts its wide range of antitumor effects through modulating a diversity of signaling pathways including effects on the transcription factor NF-κB (14, 16, 32). NF-κB is an important cellular regulator of growth and apoptosis in a variety of cells including prostate cancer cells (33-35). Activated NF-κB is absent from normal prostate epithelial and prostatic intra-epithelial neoplastic lesions, whereas it is commonly present in invasive prostate cancer (36-38) and is related to prostate cancer progression due to transcriptional regulation of NF-κB-responsive genes (38). Moreover, NF-κB activation predicts a high risk of relapse in patients with localized disease (37, 39). NF-κB may, thus, serve as a therapeutic target for the treatment of prostate cancer. Although curcumin was shown to inhibit activation of NF-κB at a high concentration (≥20 μM; 16, 32), in the present study, we found that curcumin had no effect on NF-κB activation at 1 μM. In contrast, group FN curcumin analogs had a strong inhibitory effect on activated NF-κB at 1 μM.

Since we found that compounds with a nitrogen heteroatom in the ortho-position of the pyridine ring, in general, had stronger activities than those with a nitrogen heteroatom in the meta- or para-position of the pyridine ring, we further determined the effects of AN1, BN1, EN1 and FN1 on the level of activation of ERK1/2. The results of our study showed that FN1 at 1 μM reduced the level of phosphorylated ERK1 and ERK2. Constitutive activation of ERK has been observed in prostate cancer (40, 41). ERK was shown to regulate gene expression through the activation of transcription factors, such as NF-κB (42, 43). Recent studies showed that curcumin at a concentration of 10 μM inhibited ERK and NF-κB activations in oral cancer YD-10B cells (44) and at concentrations of ≥20 μM inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-mediated activation of ERK and NF-κB in leukemia U937 cells (45). In the present study, we found that curcumin at 1 μM had no effect on the level of activated ERK1/2, while the pyridine curcumin analog FN1 at the same concentration, reduced the level of activated ERK1/2.

In summary, the present study demonstrated that pyridine analogs of curcumin with a five-carbon linker with a tetrahydrothiopyran-4-one moiety at ≤1 μM had potent anticancer activities in human prostate cancer PC-3 cells. Mechanistic studies indicate that the anticancer activities of the curcumin analogs tested are associated with inhibition of NF-κB transcriptional activity and a reduced level of activated ERK1/2. Further studies are needed to determine the blood levels of these compounds and the *in vivo* effects of these compounds on tumor growth using suitable animal models.

Acknowledgements

The present study was supported by the 2011 Guangdong Province Leadership Grant, Chinese National Science Foundation Grants 81272452 and 21272043, and by departmental funds from the Department of Chemical Biology in the Ernest Mario School of Pharmacy at Rutgers University. The Authors thank Ms. Annette Dionisio for her excellent help in the preparation of this manuscript.

References

1 Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL and Brenner DE: Dose escalation of a curcuminoid formulation. BMC Complement Altern Med *6*: 10-14, 2006.

2 Goel A, Kunnumakkara AB and Aggarwal BB: Curcumin as "Curecumin": from kitchen to clinic. Biochem Pharmacol *75*: 787-809, 2008.

3 Aggarwal BB and Sung B: Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends Pharmacol Sci *30*: 85-94, 2009.

4 Sa G and Das T: Anti cancer effects of curcumin: cycle of life and death. Cell Div *3*: 14-28, 2008.

5 Moragoda L, Jaszewski R and Majumdar AP: Curcumin induced modulation of cell cycle and apoptosis in gastric and colon cancer cells. Anticancer Res *21*: 873-878, 2001.

6 Squires MS, Hudson EA, Howells L, Sale S, Houghton CE, Jones JL, Fox LH, Dickens M, Prigent SA and Manson MM: Relevance of mitogen-activated protein kinase (MAPK) and phosphotidylinositol-3-kinase/protein kinase B (PI3K/PKB) pathways to induction of apoptosis by curcumin in breast cells. Biochem Pharmacol *65*: 361-376, 2003.

7 Hanif R, Qiao L, Shiff SJ and Rigas B: Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. J Lab Clin Med *130*: 576-584, 1997.

8 Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P and Aggarwal BB: Curcumin down-regulates cell survival mechanisms in human prostate cancer cell lines. Oncogene *20*: 7597-7609, 2001.

9 Azuine MA and Bhide SV: Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer *17*: 77-83, 1992.

10 Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR and Conney AH: Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res *54*: 5841-5847, 1994.

11 Rao CV, Rivenson A, Simi B and Reddy BS: Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res *55*: 259-266, 1995.

12 Inano H and Onoda M: Prevention of radiation-induced mammary tumors. Int J Radiat Oncol Biol Phys *52*: 212-223, 2002.

13 Pereira MA, Grubbs CJ, Barnes LH, Li H, Olson GR, Eto I, Juliana M, Whitaker LM, Kelloff GJ, Steele VE and Lubet RA: Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[a] anthracene-induced mammary cancer in rats. Carcinogenesis *17*: 1305-1311, 1996.

14 Huang MT, Smart RC, Wong CQ and Conney AH: Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-O-tetradecanoylphorbol-13-acetate. Cancer Res *48*: 5941-5946, 1988.

15 Lin YG, Kunnumakkara AB, Nair A, Merritt WM, Han LY, Armaiz-Pena GN, Kamat AA, Spannuth WA, Gershenson DM, Lutgendorf SK, Aggarwal BB and Sood AK: Curcumin inhibits tumor growth and angiogenesis in ovarian carcinoma by targeting the nuclear factor-kappaB pathway. Clin Cancer Res *13*: 3423-3430, 2007.

16 Kunnumakkara AB, Guha S, Krishnan S, Diagaradjane P, Gelovani J and Aggarwal BB: Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. Cancer Res *67*: 3853-3861, 2007.

17 Sun ZJ, Chen G, Zhang W, Hu X, Liu Y, Zhou Q, Zhu LX and Zhao YF: Curcumin dually inhibits both mammalian target of rapamycin and nuclear factor-κB pathways through a crossed phosphatidylinositol 3-kinase/Akt/IκB kinase complex signaling axis in adenoid cystic carcinoma. Mol Pharmacol 79: 106-118, 2011. erratum in: Mol Pharmacol *79*: 628-630, 2011.

18 Jutooru I, Chadalapaka G, Lei P and Safe S: Inhibition of NF kappa B and pancreatic cancer cell and tumor growth by curcumin is dependent on specificity protein down-regulation. J Biol Chem *285*: 25332-25344, 2010.

19 Anand P, Sundaram C, Jhurani S, Kunnumakkara AB and Aggarwal BB: Curcumin and cancer: an old-age disease with an age-old solution. Cancer Lett *267*: 133-164, 2008.

20 Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL, Ng CS, Badmaev V and Kurzrock R: Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res *14*: 4491-4499, 2008.

21 Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC and Hsieh CY: Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res *21*: 2895-2900, 2001.

22 Liang G, Shao L, Wang Y, Zhao C, Chu Y, Xiao J, Zhao Y, Li X and Yang S: Exploration and synthesis of curcumin analogues with improved structural stability both *in vitro* and *in vivo* as cytotoxic agents. Bioorg Med Chem *17*: 2623-2631, 2009.

23 Yadav B, Taurin S, Rosengren RJ, Schumacher M, Diederich M, Somers-Edgar TJ and Larsen L: Synthesis and cytotoxic potential of heterocyclic cyclohexanone analogues of curcumin. Bioorg Med Chem *18*: 6701-6707, 2010.

24 Somers-Edgar TJ, Taurin S, Larsen L, Chandramouli A, Nelson MA and Rosengren RJ: Mechanisms for the activity of heterocyclic cyclohexanone curcumin derivatives in estrogen receptor negative human breast cancer cell lines. Invest New Drugs *29*: 87-97, 2011.

25 Wei X, Du ZY, Zheng X, Cui XX, Conney AH and Zhang K: Synthesis and evaluation of curcumin-related compounds for anticancer activity. Eur J Med Chem *53*: 235-245, 2012.

26 Zheng X, Cui XX, Avila GE, Huang MT, Liu Y, Patel J, Kong AN, Paulino R, Shih WJ, Lin Y, Rabson AB, Reddy BS and Conney AH: Atorvastatin and celecoxib inhibit prostate PC-3 tumors in immunodeficient mice. Clin Cancer Res *13*: 5480-5487, 2007.

27 Zheng X, Cui XX, Huang MT, Liu Y, Shih WJ, Lin Y, Lu YP, Wagner GC and Conney AH: Inhibitory effect of voluntary running wheel exercise on the growth of human pancreatic Panc-1 and prostate PC-3 xenograft tumors in immunodeficient mice. Oncol Rep *19*: 1583-1588, 2008.

28 Zheng X, Chang RL, Cui XX, Avila G, Huang MT, Liu Y, Kong AN, Rabson AB and Conney AH: Inhibition of NF-kappaB by(E)3-[(4-methylphenyl)-sulfonyl]-2-propenenitrile (BAY11-7082; BAY) is associated with enhanced 12-O-tetradecanoylphorbol-13-acetate-induced growth suppression and apoptosis in human prostate cancer PC-3 cells. Int J Oncol *32*: 257-264, 2008.

29 Hsu JC. Multiple Comparisons: Theory and Methods. NewYork: Chapman and Hall, 1996.

30 Markaverich BM, Schauweker TH, Gregory RR, Varma M, Kittrell FS, Medina D and Varma RS: Nuclear type II sites and malignant cell proliferation: Inhibition by 2,6-bis-benzylidenecyclohexanones. Cancer Res *52*: 2482-2488, 1992.

31 Adams BK, Ferstl EM, Davis MC, Herold M, Kurtkaya S, Camalier RF, Hollingshead MG, Kaur G, Sausville EA, Rickles FR, Snyder JP, Liotta DC and Shoji M: Synthesis and biological evaluation of novel curcumin analogs as anticancer and anti-angiogenesis agents. Bioorg Med Chem *12*: 3871-3883, 2004.

32 Aggarwal BB, Surh YJ and Shishodia S: The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Adv Exp Med Bio Vol 595. Springer, New York, 2007.

33 Connors SK, Chornokur G and Kumar NB: New insights into the mechanisms of green tea catechins in the chemoprevention of prostate cancer. Nutr Cancer *64*: 4-22, 2012.

34 DiDonato JA, Mercurio F and Karin M: NF-κB and the link between inflammation and cancer. Immunol Rev *246*: 379-400, 2012.

35 Paule B, Terry S, Kheuang L, Soyeux P, Vacherot F and de la Taille A: The NF-kappa B/IL-6 pathway in metastatic androgen-independent prostate cancer: New therapeutic approaches? World J Urol *25*: 477-489, 2007.

36 Lessard L, Mes-Masson AM, Lamarre L, Wall L, Lattouf JB and Saad F: NF-κB nuclear localization and its prognostic significance in prostate cancer. BJU Int *91*: 417-420, 2003.

37 Ross JS, Kallakury BV, Sheehan CE: Fisher HA, Kaufman RP Jr, Kaur P, Gray K and Stringer B: Expression of nuclear factor-κB and IκB proteins in prostatic adenocarcinomas: correlation of nuclear factor-κB immunoreactivity with disease recurrence. Clin Cancer Res *10*: 2466–2472, 2004.

38 Shukla S, MacLennan GT, Fu P, Patel J, Marengo SR, Resnick MI and Gupta S: Nuclear factor-kappa B/p65 (Rel A) is constitutively activated in human prostate adenocarcinoma and correlates with disease progression. Neoplasia *6*: 390-400, 2004.

39 Fradet V, Lessard L, Begin LR, Karakiewicz P, Masson AM and Saad F: Nuclear factor-κB nuclear localization is predictive of biochemical recurrence in patients with positive margin prostate cancer. Clin Cancer Res *10*: 8460-8464, 2004.

40 Gioeli D, Mandell JW, Petroni GR, Frierson HF and Weber MJ: Activation of mitogen-activated protein kinase associated with prostate cancer progression. Cancer Res *59*: 279-284, 1999.

41 Uzgare AR and Isaacs JT: Prostate cancer: Potential targets of anti-proliferative and apoptotic signaling pathways. Int J Biochem Cell Biol *37*: 707-714, 2005.

42 Raman M, Chen W and Cobb MH: Differential regulation and properties of MAPKs. Oncogene *26*: 3100-3112, 2007.

43 Kook SH, Jang YS and Lee JC: Involvement of JNK-AP-1 and ERK-NF-κB signaling in tension-stimulated expression of type I collagen and MMP-1 in human periodontal ligament fibroblasts. J Appl Physiol *111*: 1575-1583, 2011.

44 Shin HK, Kim J, Lee EJ and Kim SH: Inhibitory effect of curcumin on motility of human oral squamous carcinoma YD-10B cells *via* suppression of ERK and NF-κB activations. Phytother Res *24*: 577-582, 2010.

45 Lim JH and Kwon TK: Curcumin inhibits phorbol myristate acetate (PMA)-induced MCP-1 expression by inhibiting ERK and NF-κB transcriptional activity. Food Chem Toxicol *48*: 47-52, 2010.

Received January 18, 2013
Revised February 22, 2013
Accepted February 22, 2013