Abstract: Curcumin analogs and methods of use thereof are provided.
Curcumin Analogs and Methods of Use Thereof

This application claims priority under 35 U.S.C. §119 (e) to U.S. Provisional Patent Application No. 61/372,773, filed on August 11, 2010. The foregoing application is incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates to the field of oncology. Specifically, curcumin analogs and methods of use thereof are disclosed.

BACKGROUND OF THE INVENTION

Several publications and patent documents are cited throughout the specification in order to describe the state of the art to which this invention pertains. Each of these citations is incorporated herein by reference as though set forth in full.

Curcumin, a well-known diarylheptanoid, has been identified as a major constituent in turmeric. It is used as a spice to give a specific flavor and yellow color to curry, which is consumed daily by millions of people. Curcumin has been used in traditional medicine for liver disease (jaundice), indigestion, urinary tract diseases, rheumatoid arthritis, and insect bites (Aggarwal et al. (2009) Trends Pharmacol. Sci., 30:85-94). Numerous studies demonstrated the anticancer activity of curcumin in animal models (Aggarwal et al. (2009) Trends Pharmacol. Sci., 30:85-94; Kuttan et al. (1985) Cancer Lett., 29:197-202; Huang et al. (1988)
Cancer Res., 48:5941-5946; Huang et al. (1992)

The weakness of the pharmacokinetic profile of curcumin in vivo significantly inhibits its clinical application. Accordingly, superior analogs of curcumin are greatly desired.

SUMMARY OF THE INVENTION

In accordance with the present invention, curcumin analogs are provided. Compositions comprising the curcumin analog and at least one pharmaceutically acceptable carrier are also provided.

In accordance with another aspect of the instant invention for inhibiting, treating, and/or preventing cancer in a subject are provided. The method comprises administering at least one curcumin analog of the instant invention to the subject.

In accordance with another aspect of the present invention, methods for inhibiting, treating, and/or preventing inflammation or an inflammation disease or disorder in a subject are provided. The method comprises administering at least one curcumin analog of the instant invention to the subject. In a particular embodiment, the inflammation disorder is a neurodegenerative disease such as Alzheimer's disease or Parkinson's disease.

BRIEF DESCRIPTIONS OF THE DRAWING

Figure 1 provides a schematic of the synthesis of group A to group F compounds.

Figure 2 provides a schematic of the synthesis of group AN, BN, EN and FN compounds.

Figure 3 provides a schematic of the synthesis of the AS, BS, ES and FS compounds.
Figure 4 provides structures of curcumin and certain highly active anticancer analogs of curcumin. Figure 5 provides a graph showing the stimulatory effect of E10, F10, FN1 and FN2 on apoptosis in cultured PC-3 cells. Various concentrations of E10, F10, FN1 and FN2 were incubated with PC-3 cells for 96 hours. Apoptosis was determined by morphology. Figure 6 provides the structures of certain anticancer analogs of curcumin.

DETAILED DESCRIPTION OF THE INVENTION

much as 60-fold more active than curcumin in certain cancer cell lines (Ohori et al. (2006) Mol. Cancer Ther., 5:2563-2571).

The instant invention encompasses curcumin analogs. Curcumin analogs of the instant invention include the compounds set forth hereinbelow (e.g., compounds of formulas I-IV) and pharmaceutically acceptable salts thereof, with the proviso that the curcumin analog is not curcumin or a curcumin analog described in Du et al. (Eur. J. Med. Chem. (2006) 41:213) (particularly, A₁, A₃, A₅, A₆, B₁, B₃, B₅, C₁, C₂, C₃, C₅, C₆, D₁) or provided in Figure 6. The curcumin analogs may be isolated.

In a particular embodiment, the curcumin analog has the structure of formula I:

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Y
\[ \text{O} \]
\[ \text{Z} \]
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wherein \( n = 0, 1, 2, 3, 4, 5, 6, 7, \) or 8 (optionally comprising at least one heteroatom), particularly \( n = 1, 2, 3, 4, 5, 6, 7, \) or, 8, and wherein \( Y \) and \( Z \) are each independently an aryl (e.g., a heteroaryl). In a particular embodiment, \( n \) is 2 or 3. In a particular embodiment, when \( n = 3 \), the carbon chain optionally comprises one heteroatom (e.g., O, S, or NH). \( Y \) and \( Z \) can be the same or different. In a particular embodiment, \( Y \) and/or \( Z \) is a pyridyl (e.g., 2-pyridyl, 3-pyridyl, 4-pyridyl), a pyrrole, a furan, a thiophene, a phenyl, a quinoline, an isoquinoline, an indole, a benzofuran, or a benzothiophene, wherein each of these groups is optionally substituted at available carbons with H, OH, halo, OCH₃, alkyl, lower alkyl, O-lower alkyl, or O-alkyl, particularly, H, OH, F, OCH₃, OCF₃, O(C=O)CH₃, or OCH₂CH₃. In a particular embodiment, \( Y \)
and/or Z is selected from the group consisting

wherein \( R_p \) is absent, \( \text{H}, \text{CH}_3, \text{alkyl}, \) or lower alkyl, particularly absent, \( \text{H}, \text{CH}_3, \text{or } \text{C}_2\text{H}_5; \) wherein \( X' \) is 0, \( \text{S, NH, or } \text{NR}_n\text{R}_m, \) wherein \( \text{R}_n \) and \( \text{R}_m \) are each independently \( \text{H}, \text{CH}_3, \text{alkyl, or lower alkyl, particularly } \text{H}, \text{CH}_3, \text{C}_2\text{H}_5, \text{C}_3\text{H}_7, \text{C}_4\text{H}_9, \text{C}_5\text{H}_{11}, \text{or } \text{C}_6\text{H}_{13}; \) and wherein \( \text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \) and \( \text{R}_5 \) are each independently \( \text{H, OH, halo, OCH}_3, \) or O-alkyl, particularly, \( \text{H, OH, F, OCH}_3, \text{OCF}_3, \text{O(C=O)}\text{CH}_3, \) or \( \text{OCH}_2\text{CH}_3. \)

In a particular embodiment, \( \text{R}_1 \) and \( \text{R}_5 \) are \( \text{H}. \)

In a particular embodiment, the curcumin analog has the structure of formula II:

\[
\text{Y} - \text{O} - \text{Z}
\]

wherein \( \text{Y} \) and \( \text{Z} \) are as defined above for formula I and wherein \( X \) is 0, \( \text{S, NH, or } \text{NR}_n\text{R}_m, \) wherein \( \text{R}_n \) and \( \text{R}_m \) are each independently \( \text{H}, \text{CH}_3, \text{alkyl, or lower alkyl, particularly } \text{H}, \text{CH}_3, \text{C}_2\text{H}_5, \text{C}_3\text{H}_7, \text{C}_4\text{H}_9, \text{C}_5\text{H}_n, \) or \( \text{C}_6\text{H}_{13}. \)
In a particular embodiment, the curcumin analog has the structure of formula III:

(III)

wherein \( n \) is as defined above for formula I and wherein \( R_1', R_2', R_3', R_4', R_5', R_i, R_2, R_3, R_4, \text{ and } R_5 \) are each independently \( \text{H}, \text{OH}, \) halo, \( 0\text{CH}_3 \), or \( 0\text{-alkyl}, \) particularly, \( \text{H}, \text{OH}, \text{F}, 0\text{CH}_3, \text{OCF}_3, 0(\text{C}=0)\text{CH}_3, \) or, \( 0\text{CH}_2\text{CH}_3 \). In a particular embodiment, \( R_1, R_5, R_1', \text{ and } R_5' \) are \( \text{H} \).

In a particular embodiment, the curcumin analog has the structure of formula IV:

(IV)

wherein \( X \) is as defined above for formula II and wherein \( R_1', R_2', R_3', R_4', R_5', R_i, R_2, R_3, R_4, \text{ and } R_5 \) are as defined above form formula III.

In a particular embodiment, the curcumin analog is selected from the group consisting of:
In a particular embodiment, the curcumin analog is selected from the group consisting of:
Compositions comprising the curcumin analogs of the instant invention are also encompassed herein. In a particular embodiment, the composition comprises at least one curcumin analog of the instant invention (e.g., pharmaceutically acceptable salts thereof) and at least one pharmaceutically acceptable carrier. The composition may further comprise at least one other chemotherapeutic agent. The composition may further comprise at least one other anti-inflammatory agent (e.g., steroids (e.g., glucocorticoids) or NSAIDs). The composition may further comprise curcumin or at least one other curcumin analog (e.g., those described in Du et al. (2006) Eur. J. Med. Chem., 41:213). The
compositions may also comprise an enhancer compound which works synergistically with the curcumin analog. Enhancer compounds which work synergistically with the curcumin analogs include, without limitation, all-trans retinoic acid, 1,25-dihydroxyvitamin D3, sodium butyrate, and 12-0-tetradecanoylphorbol-13-acetate.

The compositions comprising the curcumin analogs of the instant invention may also be separate from compositions comprising the agents described above (e.g., chemotherapeutic agent, anti-inflammatory, enhancer, and/or curcumin or other curcumin analog) and a pharmaceutically acceptable carrier. For example, the instant invention also encompasses kits comprising a first composition comprising at least one curcumin analog of the instant invention and at least one pharmaceutically acceptable carrier and at least one second composition comprising a chemotherapeutic agent, enhancer, and/or curcumin or other curcumin analog and a pharmaceutically acceptable carrier.

The curcumin analogs of the instant invention can be administered to a patient in need thereof, as described hereinbelow. The curcumin analogs may be delivered to a subject to inhibit or treat cancer. Cancers that may be treated using the present protocol include, but are not limited to: cancers of the prostate, colorectum, pancreas, cervix, stomach, endometrium, brain, liver, bladder, ovary, testis, head, neck, skin (including melanoma and basal carcinoma), mesothelial lining, white blood cell (including lymphoma and leukemia) esophagus, breast, muscle, connective tissue, lung (including small-cell lung carcinoma and non-small-cell carcinoma), adrenal gland, thyroid, kidney, or bone; glioblastoma, mesothelioma, renal cell carcinoma, gastric carcinoma, sarcoma, choriocarcinoma,
cutaneous basocellular carcinoma, skin squamous cell carcinomas, and testicular seminoma. In a particular embodiment, the cancer is prostate, pancreatic, or colon cancer. In a particular embodiment, the curcumin analogs are co-administered with chemotherapy (e.g., radiation). In a particular embodiment, the curcumin analog of the instant invention is administered with at least one chemotherapeutic agent, enhancer, and/or curcumin or other curcumin analog. The curcumin analog of the instant invention may be administered before, after, and/or simultaneously with the other agents or therapy.

The curcumin analogs of the instant invention may also be delivered to a subject to inhibit or treat inflammation in a patient, particularly an inflammation related neurodegenerative disease or neuroinflammatory diseases, such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Parkinson's disease. The curcumin analogs of the instant invention may be administered with at least one anti-inflammatory or other neurodegenerative disease therapies or therapeutic agents. In a particular embodiment, the curcumin analog of the instant invention is administered with at least one anti-inflammatory, enhancer and/or curcumin or other curcumin analog. The curcumin analog of the instant invention may be administered before, after, and/or simultaneously with the other agents or therapy.

The curcumin analog as described herein will generally be administered to a patient as a pharmaceutical preparation. The term "patient" as used herein refers to human or animal subjects. These curcumin analogs may be employed therapeutically, under the guidance of a physician for the treatment of
malignant tumors, metastatic disease, and inflammatory-
disorders.

The pharmaceutical preparation comprising at least one curcumin analog of the invention may be conveniently formulated for administration with at least one pharmaceutically acceptable carrier, such as water, buffered saline, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), dimethyl sulfoxide (DMSO), oils, detergents, suspending agents or suitable mixtures thereof. The concentration of curcumin analog in the chosen medium will depend on the hydrophobic or hydrophilic nature of the medium, as well as the size and other properties of the curcumin analog. Solubility limits may be easily determined by one skilled in the art.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media and the like which may be appropriate for the desired route of administration of the pharmaceutical preparation, as exemplified in the preceding paragraph. The use of such media for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the curcumin analog to be administered, its use in the pharmaceutical preparation is contemplated.

The dose and dosage regimen of a curcumin analog according to the invention that is suitable for administration to a particular patient may be determined by a physician considering the patient's age, sex, weight, general medical condition, and the specific condition and severity thereof for which the antibody is being administered. The physician may also consider the route of administration of the curcumin analog, the
Selection of a suitable pharmaceutical preparation depends upon the method of administration chosen. For example, the curcumin analog of the invention may be administered by direct injection into any cancerous tissue or into the area surrounding the cancer or to the site of inflammation/neurodegeneration. In this instance, a pharmaceutical preparation comprises the antibody molecules dispersed in a medium that is compatible with the cancerous tissue or the damaged nerve tissue. The curcumin analog may also be administered orally.

The curcumin analogs may also be administered parenterally by intravenous injection into the bloodstream, or by subcutaneous, intramuscular, intrathecal, or intraperitoneal injection. Pharmaceutical preparations for parenteral injection are known in the art. If parenteral injection is selected as a method for administering the curcumin analogs, steps should be taken to ensure that sufficient amounts of the molecules reach their target cells to exert a biological effect. The lipophilicity of the curcumin analog, or the pharmaceutical preparation in which they are delivered, may have to be increased so that the molecules can arrive at their target locations. Furthermore, the curcumin analogs may be delivered in a cell-targeting carrier so that sufficient numbers of molecules will reach the target cells. Methods for increasing the lipophilicity of a molecule are known in the art.

Pharmaceutical compositions containing a compound of the present invention as the active ingredient in intimate admixture with a pharmaceutical carrier can be prepared according to conventional pharmaceutical
compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral or parenteral. In preparing the antibody in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. For parenterals, the carrier will usually comprise sterile water, though other ingredients, for example, to aid solubility or for preservative purposes, may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

A pharmaceutical preparation of the invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to a physically discrete unit of the pharmaceutical preparation appropriate for the patient undergoing treatment. Each dosage should contain a quantity of active ingredient calculated to produce the desired effect in association with the selected pharmaceutical carrier. Procedures for
determining the appropriate dosage unit are well known to those skilled in the art.

Dosage units may be proportionately increased or decreased based on the weight of the patient.

Appropriate concentrations for alleviation of a particular pathological condition may be determined by dosage concentration curve calculations, as known in the art.

In accordance with the present invention, the appropriate dosage unit for the administration of curcumin analogs may be determined by evaluating the toxicity of the curcumin analogs in animal models. Various concentrations of antibody pharmaceutical preparations may be administered to mice with transplanted human tumors, and the minimal and maximal dosages may be determined based on the results of significant reduction of tumor size or inflammation and side effects as a result of the treatment. Appropriate dosage unit may also be determined by assessing the efficacy of the treatment in combination with other standard anti-cancer or anti-inflammation drugs.

The pharmaceutical preparation comprising the curcumin analogs may be administered at appropriate intervals, for example, at least twice a day or more until the pathological symptoms are reduced or alleviated, after which the dosage may be reduced to a maintenance level. The appropriate interval in a particular case would normally depend on the condition of the patient.

Definitions

The following definitions are provided to facilitate an understanding of the present invention:
The term "isolated" is not meant to exclude artificial or synthetic mixtures with other compounds or materials, or the presence of impurities that do not interfere with the fundamental activity, and that may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into, for example, pharmaceutically acceptable preparations.

Chemotherapeutic agents are compounds that exhibit anticancer activity and/or are detrimental to a cell (e.g., a toxin). Suitable chemotherapeutic agents include, but are not limited to: toxins (e.g., saporin, ricin, abrin, ethidium bromide, diptheria toxin, Pseudomonas exotoxin, and others listed above; thereby generating an immunotoxin when conjugated or fused to an antibody); alkylating agents (e.g., nitrogen mustards such as chlorambucil, cyclophosphamide, isofamide, mechlorethamine, melphalan, and uracil mustard; aziridines such as thiotepa; methanesulphonate esters such as busulfan; nitroso ureas such as carmustine, lomustine, and streptozocin; platinum complexes such as cisplatin and carboplatin; bioreductive alkylators such as mitomycin, procarbazine, dacarbazine and altretamine); DNA strand-breakage agents (e.g., bleomycin); topoisomerase II inhibitors (e.g., amsacrine, dactinomycin, daunorubicin, idarubicin, mitoxantrone, doxorubicin, etoposide, and teniposide); DNA minor groove binding agents (e.g., plicamycin); antimetabolites (e.g., folate antagonists such as methotrexate and trimetrexate; pyrimidine antagonists such as fluorouracil, fluorodeoxyuridine, CB3717, azacitidine, cytarabine, and flouxuridine; purine antagonists such as mercaptopurine, 6-thioguanine, fludarabine, pentostatin; asparginase; and ribonucleotide reductase inhibitors such as
hydroxyurea); tubulin interactive agents (e.g., vincristine, vinblastine, and paclitaxel (Taxol));
hormonal agents (e.g., estrogens; conjugated estrogens; ethinyl estradiol; diethylstilbestrol; chlortrianisen;
idenestrol; progestins such as hydroxyprogesterone caproate, medroxyprogesterone, and megestrol; and androgens such as testosterone, testosterone propionate, fluoxymesterone, and methyl testosterone); adrenal corticosteroids (e.g., prednisone, dexamethasone, methylprednisolone, and prednisolone); leutinizing hormone releasing agents or gonadotropin-releasing hormone antagonists (e.g., leuprolide acetate and goserelin acetate); and antihormonal antigens (e.g., tamoxifen, antiandrogen agents such as flutamide; and antiadrenal agents such as mitotane and aminoglutethimide).

The term "alkyl," as employed herein, includes both straight and branched chain hydrocarbons containing about 1 to 20 carbons or about 1 to 10 carbons in the normal chain. The hydrocarbon chain of the alkyl groups may be interrupted with at least one heteroatom (e.g., oxygen, nitrogen, or sulfur atoms). Examples of alkyl groups include, without limitation, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4 dimethylpentyl, octyl, 2,2,4 trimethylpentyl, nonyl, decyl, the various branched chain isomers thereof, and the like. Each alkyl group may optionally be substituted with at least one substituent (e.g., about 1 to 4) which include, for example, alkyl, halo (such as F, Cl, Br, I), haloalkyl (e.g., CCl₃ or CF₃), alkoxy, alkylthio, hydroxy, methoxy, carboxyl, oxo, epoxy, alklyoxycarbonyl, alkylcarbonyloxy, amino, carbamoyl (e.g., NH₂C(=0)⁻ or NHRC(=0)⁻, wherein R is an alkyl), urea (⁻NHCONH₂),
alkylurea, aryl, ether, ester, thioester, nitrile, nitro, amide, carbonyl, carboxylate and thiol. The term "lower alkyl" refers to an alkyl which contains 1 to 3 carbons and/or heteroatoms in the hydrocarbon chain.

The term "aryl," as employed herein, refers to monocyclic and bicyclic aromatic groups containing 6 to 10 carbons in the ring portion. Examples of aryl groups include, without limitation, phenyl, naphthyl, such as 1-naphthyl and 2-naphthyl, indolyl, and pyridyl, such as 3-pyridyl and 4-pyridyl. Aryl groups may be optionally substituted through available carbon atoms with 1 to about 4 groups. Exemplary substituents include those provided above for alkyl. The aromatic groups may be heteroaryl. "Heteroaryl" refers to an optionally substituted aromatic ring system that includes at least one, particularly from 1 to about 4 sulfur, oxygen, or nitrogen heteroatom ring members.

The following example provides illustrative methods of practicing the instant invention, and is not intended to limit the scope of the invention in any way.

EXAMPLE

Materials and Methods

General procedures

Melting points were determined on a Yanagimoto micro-melting apparatus and were uncorrected. The $^1$H NMR spectra were measured on a Varian Gemini-2000™ spectrometer using DMSO as solvent unless otherwise specified. Chemical shifts for $^1$H NMR (300 MHz) were expressed in ppm units with TMS as an internal standard. Multiplicities were recorded as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), m...
(multiplet). Mass spectra were obtained on an LC-MS-2010A spectrometer with ESI. Elemental analyses were performed on an elemental analyser (Vario El). Thin-layer chromatography (TLC) was performed on Merck silica gel plates (DC-60 F254). Curcuminoids (1-3) were isolated from the extract of Curcuma longa Linn (Du et al. (2006) Eur. J. Med. Chem., 41:213). All reagents (highest grade) were used as received unless otherwise stated.

Synthesis of curcumin analogs

A total of 61 curcumin analogs were synthesized using a modified version of a synthesis method (Du et al. (2006) Eur. J. Med. Chem., 41:213). A mixture of the appropriate aldehyde (0.01 mol) and the ketone (0.005 mol) was dissolved in glacial acetic acid saturated with anhydrous hydrogen chloride and heated in a water bath at 25-30°C for 2 hours. After standing for 2 days, the mixture was treated with cold water and filtered. The solid obtained was then washed and dried. The crude product was recrystallized from appropriate solvents (methanol or ethanol).

1. (2E, 6E) -2,6-Bis (4-fluorobenzylidene) cyclohexanone (A1). Yield 85%. m.p. 155-157°C. $^1$H NMR (CDCl$_3$, 300 MHz) 5(ppm): 7.75 (s, 2H, -CH=), 7.45 (d, J = 8.1 Hz, 4H, arom), 7.10 (d, $J = 8.1$ Hz, 4H, arom), 2.91 (t, $J = 5.7$ Hz, 4H, -CH$_2$-C-CH$_2$-), 1.82 (q, $J = 5.7$ Hz, 2H, -C-CH$_2$-C-). ESI-MS (m/z): 311(M+1)$^+$. Anal. Calc. for C$_{20}$H$_{15}$F$_2$: 310, C 77.40, H 5.20. Found: C 77.38, H 5.23.

2. (2E, 6E) -2,6-Bis (4-chlorobenzylidene) cyclohexanone (A2). Yield 83%. m.p. 147-149°C. $^1$H NMR (CDCl$_3$, 300 MHz) 5(ppm): 7.72 (s, 2H, -CH=), 7.42-7.28 (m, 8H, arom), 2.65 (t, $J = 5.7$ Hz, 4H, -CH$_2$-C-CH$_2$-, 1.96-1.85 (m, 2H, -C-CH$_2$-C-). ESI-MS (m/z): 344 (M+1)$^+$. Anal. Calc. for C$_{20}$H$_{15}$Cl$_2$: 343; C 69.98, H 4.70. Found: C 69.90, H 4.76.
3. \((2E, 6E)\) -2,6-Bis (4-bromobenzylidene) cyclohexanone (A3). Yield 82%. m.p. 149-151°C. \(^1\)H NMR (CDCl\(_3\), 300 MHz) 5 (ppm): 7.70 (s, 2H, -CH=), 7.53 (d, \(J = 8.1\) Hz, 4H, arom), 7.31 (d, \(J = 8.1\) Hz, 4H, arom), 2.94 (t, \(J = 5.7\) Hz, 4H, -CH\(_2\)-), 1.88-1.79 (m, 2H, -C-CH\(_2\)-C). ESI-MS (m/z): 433 (M+1)\(^+\). Anal. Calc. for C\(_{20}\)H\(_{16}\)Br\(_2\): C 78.89, H 6.70. Found: C 78.89, H 6.70.

4. \((2E, 6E)\) -2,6-Bis (4-Hydroxybenzylidene) cyclohexanone (A4). This compound is characterized in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 278-280°C.

5. \((2E, 6E)\) -2,6-Bis (4-methoxybenzylidene) cyclohexanone (A6). Yield 90%, m.p. 148-149°C. \(^1\)H NMR (DMSO-d\(_6\), 300 MHz) \(\delta\) (ppm): 7.54 (s, 2H, -CH=), 7.48 (d, \(J = 8.1\) Hz, 4H, arom), 6.98 (d, \(J = 8.1\) Hz, 4H, arom), 3.78 (s, 6H, -OCH\(_3\) \(i\)); 2.85 (t, \(J = 5.7\) Hz, 4H, -CH\(_2\)-C-CH\(_2\)-C-). ESI-MS (m/z): 335 (M+1)\(^+\). Anal. Calc. for C\(_{22}\)H\(_{20}\)O\(_3\): 334; C 79.02, H 6.63. Found: C 78.89, H 6.70.

6. \((2E, 6E)\) -2,6-Bis (4-Hydroxy-3-methoxybenzylidene) cyclohexanone (A7). This compound is characterized as A3 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 178-179°C.

7. \((2E, 6E)\) -2,6-Bis (3,4-dimethoxybenzylidene) cyclohexanone (A8). This compound is characterized as A5 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 138-140°C.

8. \((2E, 6E)\) -2,6-Bis (4-Hydroxy-3,5-dimethoxybenzylidene) cyclohexanone (A9). This compound is characterized as A6 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 134-135°C.

9. \((2E, 6E)\) -2,6-Bis (3,4,5-trimethoxybenzylidene) cyclohexanone (A10). Yield 78%. m.p. 196-198°C. \(^1\)H NMR (CDCl\(_3\), 300 MHz) : 7.72 (s, 2H, -CH=), 6.70 (s, 4H, arom), 3.89 (s, 18H, -OCH\(_3\)), 2.96 (t, \(J = 5.7\) Hz, 2H, -CH\(_2\)-C-CH\(_2\)-C-), 1.84 (q, \(J = 5.7\) Hz, 2H, -C-CH\(_2\)-C-). ESI-MS (m/z): 455 (M+1)\(^+\). Anal. Calc. for C\(_{26}\)H\(_{27}\)O\(_7\): 454; C 68.71, H 6.65. Found: C 68.68, H 6.73.

10. \((2E, 6E)\) -2,6-Bis (2,3,4 -trimethoxybenzylidene) cyclohexanone (A11). Yield 75%. m.p. 201-204°C. \(^1\)H NMR (CDCl\(_3\), 300 MHz) : 7.93 (s, 2H, -CH=), 7.38 (s, 4H, 2H, arom), 7.27 (d, \(J = 8.1\) Hz, 2H, arom), 4.12 (s, 6H, -OCH\(_3\)), 4.06 (s, 6H, -OCH\(_3\)), 3.98 (s, 6H, -OCH\(_3\)). 2.98(t,
J = 5.7 Hz, 4H, -CH=CH) 1.85 (q, J = 5.7 Hz, 2H, -CH2-CH-) ESI-MS (m/z): 455 (M+1)+. Anal. Calc. for C24H30O7: 454; C 68.71, H 6.65. Found: C 68.68, H 6.69.

11. (2E, 5E)-2,5-Bis (4-fluorobenzylidene) cyclopentanone (B1). Yield 81%. m.p. 212-214°C. 1H NMR (CDCl3, 300 MHz) 5 (ppm): 7.57 (d, J = 8.1 Hz, 4H, arom), 7.56 (s, 2H, -CH=), 7.13 (t, J = 8.1 Hz, 4H, arom), 3.10 (s, 4H, -CH2-CH2−). ESI-MS (m/z): 297 (M+1)+. Anal. Calc. for C19H14F2O: 296; C 77.01, H 4.76. Found: C 76.93, H 4.81.

12. (2E, 5E)-2,5-Bis (4-chlorobenzylidene) cyclopentanone (B2). Yield 78%. m.p. 230-232°C. 1H NMR (CDCl3, 300 MHz) 5 (ppm): 7.52 (d, J = 8.1 Hz, 4H), 7.51 (s, -CH=, arom), 7.41 (d, J = 8.1 Hz, 4H, arom), 3.10 (s, 4H, -CH2-CH2−). ESI-MS (m/z): 329 (M+1)+. Anal. Calc. for C19H14Cl2O: 328; C 69.32, H 4.29. Found: C 69.30, H 4.35.

13. (2E, 5E)-2,5-Bis (4-bromobenzylidene) cyclopentanone (B3). Yield 82%. m.p. 211-213°C. 1H NMR (CDCl3, 300 MHz) 5 (ppm): 7.57 (d, J = 8.1 Hz, 4H, arom), 7.52 (s, 2H, -CH=), 7.44 (d, J = 8.1 Hz, 4H, arom), 3.09 (s, 4H, -CH2-CH2−). ESI-MS (m/z): 419 (M+1)+. Anal. Calc. for C19H14Br2O: 418; C 54.58, H 3.37. Found: C 54.55, H 3.42.

14. (2E, 5E)-2,5-Bis (4-Hydroxybenzylidene) cyclopentanone (B4). This compound is characterized as B3 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 204-207°C.

15. (2E, 5E)-2,5-Bis (4-methoxybenzylidene) cyclopentanone (B6). Yield: 92%; m.p. 158-161°C. 1H NMR (DMSO-de, 300 MHz) δ (ppm): 7.62 (d, J = 8.1 Hz, 4H, arom), 7.35 (s, 2H, -CH=), 7.03 (d, J = 8.1 Hz, 4H, arom), 3.79 (s, 6H, -OCH3), 3.03 (s, 4H, -CH2-CH2−). ESI-MS (m/z): 321 (M+1)+. Anal. Calc. for C21H20O3: 320; C 78.73, H 6.29. Found: C 78.52, H 6.33.

16. (2E, 5E)-2,5-Bis (4-Hydroxy-3-methoxybenzylidene) cyclopentanone (B7). This compound is characterized as B3 in ref 35. The m.p. was 212-214°C.

17. (2E, 5E)-2,5-Bis (3,4-dimethoxybenzylidene) cyclopentanone (B8). This compound was characterized earlier as B5 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 188-190°C.

18. (2E, 5E)-2,5-Bis (4-Hydroxy-3,5-dimethoxybenzylidene) cyclopentanone (B9). This compound...
is characterized as $B_6$ in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 226-228°C.

19. (2E, 5E) -2, 5-Bis (3,4,5-trimethoxybenzylidene) cyclopentanone (BIO). Yield 81%. m.p. 202-204°C. ¹H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.52 (s, 2H, -CH=), 6.85 (s, 4H, arom), 3.91 (s, 18H, -OCH₃), 3.16 (s, 4H, -CH₂-CH₂-). ESI-MS (m/z): 441 (M+1)⁺. Anal. Calc. for C₁₂H₁₈O₇: 440; C 68.17, H 6.41. Found: C 68.13, H 6.46.

20. (2E, 5E) -2, 5-Bis (2,3,4-trimethoxybenzylidene) cyclopentanone (BI1). Yield 66 %. m.p. 207-209°C. ¹H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.82 (s, 2H, -CH=), 7.26 (d, J = 8.1 Hz, 2H, arom), 7.15 (d, J = 8.1 Hz, 2H, arom), 4.08 (s, 6H, -OCH₃), 4.02 (s, 6H, -OCH₃). 3.18 (s, 4H, -CH₂-CH₂-). ESI-MS (m/z): 441 (M+1)⁺. Anal. Calc. for C₂₅H₂₈O₇: 440; C 68.17, H 6.41. Found: C 68.14, H 6.45.

21. (IE, 4E) -1, 5-Bis (4-fluorophenyl) -1,4-pentadiene-3-one (CI). Yield 63%. m.p. 146-148°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 7.71 (d, J = 15.9Hz, 2H, -CH=), 7.60 (d, J = 8.7Hz, 4H, arom), 7.11 (d, J = 8.7Hz, 4H, arom), 7.00 (d, J = 15.9Hz, 2H, -C=C-). ESI-MS (m/z): 271 (M+1)⁺. Anal. Calc. for C₁₇H₁₂F₂O: 270; C 75.55, H 4.48. Found: C 75.50, H 4.53.

22. (IE, 4E) -1, 5-Bis (4-chlorophenyl) -1,4-pentadiene-3-one (C2). Yield 70%. m.p. 192-193°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 7.68 (d, J = 15.9Hz, 2H, -CH=C-), 7.54 (d, J = 8.1Hz, 4H, arom), 7.38 (d, J' = 8.1Hz, 4H, arom), 7.03 (d, J = 15.9Hz, 2H, -C=CH-). ESI-MS (m/z): 303 (M+1)⁺. Anal. Calc. for C₁₇H₁₂Cl₂O: 302; C 67.35, H 3.99. Found: C 67.32, H 4.03.

23. (IE, 4E) -1, 5-Bis (4-bromophenyl) -1,4-pentadiene-3-one (C3). Yield 76%. m.p. 211-213°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 7.67 (d, J = 15.9Hz, 2H, -CH=C-), 7.55 (d, J = 8.1Hz, 4H, arom), 7.47 (d, J = 8.1Hz, 4H, arom), 7.04 (d, J = 11.9Hz, 2H, -CH=C-). ESI-MS (m/z): 393 (M+1)⁺. Anal. Calc. for C₁₉H₁₂Br₂O: 392; C 52.08, H 3.08. Found: C 52.06, H 3.10.

24. (IE, 4E) -1, 5-Bis (4-hydroxyphenyl) -1,4-pentadiene-3-one (C4). This compound is characterized as CI in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 243-245°C.
25. (IE, 4E)-1, 5-Bis (3,4-Dihydroxyphenyl) -1, 4-pentadiene-3-one (C5). This compound is characterized as C2 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 150-154°C.

26. (IE, 4E)-1, 5-Bis (4-methoxyphenyl) -1, 4-pentadiene-3-one (C6). Yield 79%. m.p. 121-122°C. 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 7.82-7.70 (m, 4H, arom), 7.67 (d, J = 15.9 Hz, 2H, -CH=), 7.15 (d, J = 15.9 Hz, 2H, -C=CH-), 7.10-6.98 (m, 4H, arom), 3.78 (s, 6H, -OCH3). ESI-MS (m/z): 295 (M+1) +. Anal. Calc. for C19H18O3: 294; C 77.53, H 6.16. Found: C 77.47, H 6.23.

27. (IE, 4E)-1, 5-Bis (4-hydroxy-3-methoxyphenyl) -1, 4-pentadiene-3-one (C7). This compound is characterized as C3 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 99-100°C.

28. (IE, 4E)-1, 5-Bis (3,4-dimethoxyphenyl) -1, 4-pentadiene-3-one (C8). This compound is characterized as C5 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 89-90°C.

29. (IE, 4E)-1, 5-Bis (4-Hydroxy-3,5-dimethoxyphenyl) -1, 4-pentadiene-3-one (C9). This compound is characterized as C6 in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 230-231°C.

30. (IE, 4E)-1, 5-Bis (3,4,5-trimethoxyphenyl) -1, 4-pentadiene-3-one (C10). Yield 66%, m.p. 118-120°C. 1H NMR (CDCl3, 300 MHz) δ 7.65 (d, J = 15.9 Hz, 2H, -CH=), 6.98 (d, J = 15.9 Hz, 2H, -C=CH-), 6.84 (s, 4H, arom), 3.89 (s, 18H, -OCH3). ESI-MS (m/z): 415(M+1) +. Anal. Calc. for C23H26O7: 414; C 66.65, H 6.32. Found: C 66.67, H 6.30.

31. (IE, 4E)-1, 5-Bis (2,3,4 -trimethoxyphenyl )-1,4-pentadiene-3-one (C11). Yield 56%. m.p. 169-171°C. 1H NMR (CDCl3, 300 MHz) δ ppm: 7.92 (d, J = 15.9 Hz, 2H, -CH=), 7.34 (d, J = 8.1 Hz, 2H, arom), 7.08 (d, J = 15.9 Hz, 2H, -C=CH-), 6.72 (d, J = 8.1 Hz, 2H, arom), 3.95 (s, 6H, -OCH3), 3.91 (s, 6H, -OCH3), 3.89 (s, 6H, -OCH3). ESI-MS (m/z): 415(M+1) +. Anal. Calc. for C23H25O7: 414; C66.65, H 6.32. Found: C 66.60, H 6.39.

32. (3E, 5E)-3,5-Bis (4-fluorobenzylidene) -4-piperidone (D1). Yield: 78%, m.p. 208-210°C. 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 9.61 (br, 2H, -N+=H), 7.85 (s, 2H, -CH=), 7.63-7.50 (m, 4H, arom), 7.40-7.25 (m, 4H, arom), 4.45 (s,
33. (3E, 5E) -3,5-Bis (4-chlorobenzylidene) -4-piperidone

Yield: 80%, m.p. 240-243°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 9.80 (br, 2H, -N⁺H), 7.84 (s, 2H, -CH=), 7.60-7.42 (m, 8H, arom), 4.45 (s, 4H, -CH₂-). ESI-MS (m/z): 345 (M+1)⁺. Anal. Calc. for C₁₉H₁₅F₂NO: C 73.30, H 4.86. Found: C 73.16, H 4.91.

34. (3E, 5E) -3,5-Bis (4-methoxybenzylidene) -4-piperidone (D3). Yield: 83%, m.p. 271-273°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 7.55 (s, 2H, -CH=), 7.28 (d, J = 8.1 Hz, 4H, arom), 6.85 (d, J = 8.1 Hz, 4H, arom), 4.85 (s, 4H, -CH₂-O-CH₂⁻). ESI-MS (m/z): 309 (M+1)⁺. Anal. Calc. for C₁₉H₁₅Br₂NO: 433; C 52.69, H 3.49. Found: C 52.58, H 3.53.

35. (3E, 5E) -3, 5-Bis (4-Hydroxybenzylidene) -4-piperidone (D4). This compound is characterized as Di in Du et al. (2006) Eur. J. Med. Chem., 41:213. The m.p. was 291-294°C.

36. (3E, 5E) -3,5-Bis (4-hydroxybenzylidene) -4-tetrahydropyran-4-one (E4). Yield 75%. m.p. 271-273°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 10.03 (brs, 2H, -OH), 7.40 (d, J = 8.7 Hz, 4H, arom), 7.02 (d, J = 8.7 Hz, 4H, arom), 4.88 (s, 4H, -CH₂-O-CH₂⁻), 3.81 (s, 6H, -OCH₃). ESI-MS (m/z): 337 (M+1)⁺. Anal. Calc. for C₁₉H₂₀O₄: 308; C 74.98, H 5.99. Found: C 74.92, H 6.01.

37. (3E, 5E) -3,5-Bis (4-methoxybenzylidene) -4-tetrahydropyran-4-one (E6). Yield: 78%; m.p. 177-180°C. ¹¹E NMR (DMSO-d₆, 300 MHz) δ (ppm): 7.60 (s, 2H, -CH=), 7.40 (d, J = 8.7 Hz, 4H, arom), 7.02 (d, J = 8.7 Hz, 4H, arom), 4.88 (s, 4H, -CH₂-O-CH₂⁻), 3.81 (s, 6H, -OCH₃). ESI-MS (m/z): 337 (M+1)⁺. Anal. Calc. for C₂₁H₂₀O₄: 336; C 74.98, H 5.99. Found: C 74.92, H 6.01.

38. (3E, 5E) -3,5-Bis (4-Hydroxy-3, 5-dimethoxybenzylidene) -4-tetrahydropyran-4-one (E9). Yield 63%. m.p. 226-228°C. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 9.03 (brs, 2H, -OH), 7.58 (s, 2H, -CH=), 6.70 (s, 4H, arom), 4.95 (s, 4H, -CH₂-O-CH₂⁻), 3.81 (s, 12H, -OCH₃). ESI-MS (m/z): 427 (M-1)⁺. Anal. Calc. for C₂₃H₄₀O₈: 428; C 64.48, H 5.65. Found: C 64.43, H 5.68.

39. (3E, 5E) -3,5-Bis (3,4,5-trimethoxybenzylidene) -4-tetrahydropyran-4-one (E10). Yield 61%. m.p. 248-250°C. ¹H
NMR (CDCl$_3$, 300 MHz) $\delta$ ppm: 7.76 (s, 2H, -CH=), 6.55 (s, 4H, arom), 4.97 (s, 4H, -CH$_2$-O-CH$_2$-) 3.90 (s, 18H, -OCH$_3$), 3.83 (s, 4H, -CH$_2$-S-CH$_2$-).

40. (3E, 5E) -3,5-Bis(2,3,4-trimethoxybenzylidene) tetrahydrothiopyran-4-one (Ell). Yield 53%. m.p. 162-164°C. $^1$H NMR (DMSO-de, 300 MHz) $\delta$ (ppm): 8.00 (s, 2H, -CH=), 7.51 (s, 2H, -CH=), 7.38 (d, $J = 8.1$ Hz, 4H, arom), 6.83 (d, $J = 8.1$ Hz, 4H, arom), 3.92 (s, 4H, -CH$_2$-S-CH$_2$-). ESI-MS (m/z): 457 (M+1$^+$. Anal. Calc. for C$_{25}$H$_{22}$O$_8$: 456; C 65.78, H 6.18. Found: C 65.73, H 6.23.

41. (3E, 5E) -3,5-Bis(4-hydroxybenzylidene) tetrahydrothiopyran-4-one (F4). Yield 65%. m.p. 242-245°C. $^1$H NMR (DMSO-de, 300 MHz) $\delta$ (ppm): 7.54 (s, 2H, -CH=), 7.49 (d, $J = 8.1$ Hz, 4H, arom), 7.00 (d, $J = 8.1$ Hz, 4H, arom), 3.96 (s, 4H, -CH$_2$-S-CH$_2$-), 3.80 (s, 6H, -OCH$_3$). ESI-MS (m/z): 323 (M-1$^+$. Anal. Calc. for C$_{19}$H$_{20}$O$_3$: 324; C 70.35, H 4.97. Found: C 70.31, H 4.99.

42. (3E, 5E) -3,5-Bis(4-methoxybenzylidene) tetrahydrothiopyran-4-one (F6). Yield: 73%; m.p. 179-181°C. $^1$H NMR (DMSO-de, 300 MHz) $\delta$ (ppm): 8.99 (brs, 2H, -OH), 7.53 (s, 2H, -CH=), 7.00 (d, $J = 8.1$ Hz, 4H, arom), 4.03 (s, 4H, -CH$_2$-S-CH$_2$-), 3.80 (s, 12H, -OCH$_3$). ESI-MS (m/z): 443 (M-1$^+$. Anal. Calc. for C$_{25}$H$_{22}$O$_3$: 444; C 62.15, H 5.44. Found: C 62.13, H 5.46.

43. (3E, 5E) -3,5-Bis(4-Hydroxy-3, 5-dimethoxybenzylidene) - tetrahydrothiopyran-4-one (F9). Yield 71%. m.p.149-152°C. $^1$H NMR (DMSO-d$_6$, 300 MHz) $\delta$ (ppm): 8.93 (brs, 2H, -OH), 7.53 (s, 2H, -CH=), 4.03 (s, 4H, -CH$_2$-S-CH$_2$-), 3.80 (s, 12H, -OCH$_3$). ESI-MS (m/z): 473 (M+1$^+$. Anal. Calc. for C$_{29}$H$_{20}$O$_3$: 474; C 63.54, H 5.97. Found: C 63.50, H 6.02.

44. (3E, 5E) -3,5-Bis(3,4,5-trimethoxybenzylidene) - tetrahydrothiopyran-4-one (F10). Yield 65%. m.p. 220-222°C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ (ppm): 7.71 (s, 2H, -CH=), 6.63 (s, 4H, arom), 3.96 (s, 4H, -CH$_2$-S-CH$_2$-), 3.89 (s, 18H, -OCH$_3$). ESI-MS (m/z): 473 (M+1$^+$. Anal. Calc. for C$_{29}$H$_{20}$O$_3$: 474; C 63.54, H 5.97. Found: C 63.50, H 6.02.

45. (3E, 5E) -3,5-Bis(2,3,4-trimethoxybenzylidene) - tetrahydrothiopyran-4-one (F11). Yield 61%. m.p. 173-175°C. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ (ppm): 7.84 (s, 2H, -CH=), 6.98 (d, $J = 8.7$ Hz, 2H, arom), 6.69 (d, $J = 8.7$ Hz, 2H, arom), 3.89 (s, 18H, -OCH$_3$), 3.83 (s, 4H, -CH$_2$-S-CH$_2$-).
ESI-MS (m/z) : 473(M+1) + . Anal. Calc. for C_{25}H_{28}O_{7}S : 472; C 63.54, H 5.97. Found: C 63.59, H 6.06.

46. (2E, 6E) -2,6-Bis (pyridin-2 -methylene) cyclohexanone

(AN1). Yield 85%. m.p. 124-127°C. ^1H NMR (CDCl_3, 300 MHz) 5 (ppm): 8.73-8.62 (m, 2H, arom), 7.75-7.60 (m, 4H, arom), 7.43(s, 2H, -CH=), 7.20-7.08 (m, 2H, arom), 3.30 (t, J = 6.3 Hz, 4H, -CH_2-C-CH_2-), 1.85 (q, J = 6.3 Hz, 2H, -C-CH_2-C-). ESI-MS (m/z): 263 (M+l) + . Anal. Calc. for C_{17}H_{14}N_2O : 262; C 77.84, H 5.38. Found: C 77.82, H 5.43.

47. (2E, 6E) -2,6-Bis (pyridin-3 -methylene) cyclohexanone

(AN2). Yield 69%. m.p. 140-143°C. ^1H NMR (DMSO-d_6, 300 MHz) 5 (ppm): 8.75-8.65 (m, 2H, arom), 8.60-8.47 (m, 2H, arom), 7.80-7.68 (m, 4H, arom), 7.34 (s, 2H, -CH=), 2.95 (t, J = 6.3 Hz, 4H, -CH_2-C-CH_2-), 1.85 (q, J = 6.3 Hz, 2H, -C-CH_2-C-). ESI-MS (m/z): 277 (M+l) + . Anal. Calc. for C_{18}H_9N_2O: 276; C 78.24, H 5.84. Found: C 78.08, H 5.88.

48. (2E, 6E)-2, 6-Bis (pyridin-4-methylene) cyclohexanone

(AN3). Yield 69%. m.p. 148-149°C. ^1H NMR (DMSO-d_6, 300 MHz) 5 (ppm): 8.72-8.60 (m, 4H, arom), 7.66 (s, 2H, -CH=), 7.32-7.18 (m, 4H, arom), 2.93 (t, J = 6.3 Hz, 4H, -CH_2-C-CH_2-), 1.84 (q, J = 6.3 Hz, 2H, -C-CH_2-C-). ESI-MS (m/z): 277 (M+l) + . Anal. Calc. for C_{18}H_{10}N_2O: 276; C 78.24, H 5.84. Found: C 78.23, H 5.86.

49. (2E, 5E)-2,5-Bis (pyridin-2-methylene) cyclopentanone

(BN1). Yield 83%. m.p. 195-196°C. ^1H NMR (CDCl_3, 300 MHz) 5 (ppm): 8.77-8.68 (m, 2H, arom), 7.75-7.65 (m, 2H, arom), 7.58-7.46 (m, 4H, arom), 7.20 (s, 2H, -CH=), 3.37 (s, 4H, -CH_2-CH_2-). ESI-MS (m/z): 263 (M+l) + . Anal. Calc. for C_{17}H_{14}N_2O: 262; C 77.84, H 5.38. Found: C 77.82, H 5.44.

50. (2E, 5E)-2,5-Bis (pyridin-3-methylene) cyclopentanone

(BN2). Yield 80%. m.p. 233-235°C. ^1H NMR (CDCl_3, 300 MHz) 5 (ppm): 8.90-8.80 (m, 2H, arom), 8.65-8.55 (m, 2H, arom), 7.92-8.82 (m, 2H, arom), 7.57 (s, 2H, -CH=), 7.42-7.32 (m, 2H, arom), 3.16 (s, 4H, -CH_2-CH_2-). ESI-MS (m/z): 263 (M+l) + . Anal. Calc. for C_{17}H_{14}N_2O: 262; C 77.84, H 5.38. Found: C 77.83, H 5.42.

51. (2E, 5E)-2,5-Bis (pyridin-4-methylene) cyclopentanone

(BN3). Yield 70%. m.p. 243-246°C. ^1H NMR (CDCl_3, 300 MHz) 5 (ppm): 8.70 (s, J = 6.3Hz, 4H, arom), 7.49 (s, 2H, -CH=), 7.41 (d, J = 6.3Hz, 4H, arom), 3.17 (s, 4H, -CH_2-CH_2-). ESI-MS (m/z): 263 (M+l) + . Anal. Calc. for C_{17}H_{14}N_2O: 262; C 77.84, H 5.38. Found: C 77.82, H 5.43.
52. (3E, 5E) -3, 5-Bis (pyridin-2 -methylene) tetrahydropyran-4-one (EN1). Yield 74%. m.p. 200-202°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 8.73-8.63 (m, 2H, arom), 7.75-7.65 (m, 2H, arom), 7.63 (s, 2H, -CH=), 7.49-7.41 (m, 2H, arom), 7.23-7.15 (m, 2H, arom), 5.31 (s, 4H, -H₂C=O-CH₂⁻). ESI-MS (m/z) : 279 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂O₂: 278; C 73.37, H 5.07. Found: C 73.33, H 5.09.

53. (3E, 5E) -3, 5-Bis (pyridin-3 -methylene) tetrahydropyran-4-one (EN2). Yield 71% .m.p. 169-171°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 8.65-8.55 (m, 2H, arom), 8.59 (s, 2H, arom), 7.80 (s, 2H, -CH=), 7.67-7.57 (m, 2H, arom), 7.40-7.30 (m, 2H, arom), 4.94 (s, 4H, -H₂C=O-CH₂⁻). ESI-MS (m/z) : 279 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂O₂: 278; C 73.37, H 5.07. Found: C 73.31, H 5.12.

54. (3E, 5E) -3, 5-Bis (pyridin-4-methylene) tetrahydropyran-4-one (EN3). Yield: 55%. m.p. 182-183°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 8.75-8.65 (m, 4H, arom), 7.72 (s, 2H, -CH=), 7.17 (s, 4H, arom), 4.91 (s, 4H, -H₂C=O-CH₂⁻). ESI-MS (m/z): 279 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂O₂: 278; C 73.37, H 5.07. Found: C 73.34, H 5.09.

55. (3E, 5E) -3, 5-Bis (pyridin-2-methylene) tetrahydrothiopyran-4-one (FN1). Yield 82%. m.p. 153-156°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 8.94-8.83 (m, 2H, arom), 8.70-8.58 (m, 2H, arom), 7.96-7.87 (m, 2H, arom), 7.62 (s, 2H, -CH=), 7.48-7.39 (m, 2H, arom), 3.80 (s, 4H, -H₂C=O-S-CH₂⁻). ESI-MS (m/z): 295 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂S: 294; C 69.36, H 4.79. Found: C 69.31, H 4.84.

56. (3E, 5E) -3, 5-Bis (pyridin-3-methylene) tetrahydrothiopyran-4-one (FN2). Yield 77%. m.p. 174-177°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 8.72-8.60 (m, 2H, arom), 8.63-8.52 (m, 2H, arom), 7.80 (s, 2H, -CH=), 7.68-7.57 (m, 2H, arom), 7.43-7.32 (m, 2H, arom), 3.91 (s, 4H, -H₂C=O-S-CH₂⁻). ESI-MS (m/z): 295 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂S: 294; C 69.36, H 4.79. Found: C 69.33, H 4.84.

57. (3E, 5E) -3, 5-Bis (pyridin-4-methylene) tetrahydrothiopyran-4-one (FN3). Yield 57%, m.p. 140-142°C. 
1H NMR (CDCl₃, 300 MHz) δ (ppm) : 9.02-8.90 (m, 4H, arom), 7.70 (s, 2H, -CH=), 7.50-7.38 (m, 4H, arom), 3.85 (s, 4H, -H₂C=S-CH₂⁻). ESI-MS (m/z): 295 (M+1)⁺. Anal. Calc. for C₁₅H₁₄N₂S: 294; C 69.36, H 4.79. Found: C 69.35, H 4.83.

58. (2E, 6E) -2, 6-Bis (thiophen-3-methylene) cyclohexanone (AS). Yield: 87%. m.p. 139-141°C. 
1H NMR (CDCl₃, 300 MHz)
δ (ppm) : 7.93-7.84 (m, 2H, arom), 7.72-7.58 (m, 4H, -CH=, arom), 7.42-7.32 (m, 4H, arom), 2.87(t, J = 6.7 Hz, 4H, -CH₂-C-CH₂-), 1.78(q, J = 6.7 Hz, 2H, -C-CH₂-C-). ESI-MS (m/z) : 287 (M+1)⁺. Anal. Calc. for C₁₅H₁₄O₂S₂ : 286; C 67.10, H 4.93. Found: C 67.13, H 4.96.

59. (2E, 5E) -2,5-Bis (thiophen-3'-methylene) cyclopentanone (BS). Yield: 73%. m.p. 210-212°C. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 7.65-7.55 (m, 4H, arom), 3.06(s, 4H, -CH₂-CH₂-). ESI-MS (m/z): 273 (M+1)⁺. Anal. Calc. for C₁₅H₁₀O₃S : 272; C 66.14, H 4.44. Found: C 66.18, H 4.46.

60. (3E, 5E) -3,5-Bis (thiophen-3'-methylene) -tetrahydropyran-4-one (ES). Yield: 76%. m.p. 189-192°C. ¹H NMR (CDCl₃, 300MHz) δ (ppm): 7.89-7.80 (m, 2H, arom), 7.72-7.58(m, 2H, arom), 7.63(s, 2H, -CH=), 7.31(s, 2H, arom), 4.89 (s, 4H, -CH₂-O-CH₂-). ESI-MS (m/z): 289 (M+1)⁺. Anal. Calc. for C₁₅H₁₀O₃S₂ : 288; C 62.47, H 4.19. Found: C 62.43, H 4.23.

61. (3E, 5E) -3,5-Bis (thiophen-3'-methylene) -tetrahydrothiopyran-4-one (FS). Yield: 78%. m.p. 161-163°C. ¹H NMR (CDCl₃, 300 MHz) δ (ppm): 7.98-7.88(m, 2H, arom), 7.70-7.60 (m, 2H, arom), 7.58(s, 2H, -CH=), 7.39 (s, 2H, arom), 3.99(s, 4H, -CH₂-S-CH₂-). ESI-MS (m/z): 305 (M+1)⁺. Anal. Calc. for C₁₅H₁₂O₃S₃ : 304; C 59.18, H 3.97. Found: C59.15, H 3.99.

30 Cell culture and reagents
PC-3, Panc-1 and HT-29 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). RPMI-1640 and DMEM tissue culture medium, penicillin-streptomycin, L-glutamine and fetal bovine serum (FBS) were from Gibco (Grand Island, NY). PC-3 cells were maintained in RPMI-1640 culture medium, and Panc-1 and HT-29 cells were maintained in DMEM medium. Both RPMI-1640 and DMEM media were supplemented with 10% FBS, 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% CO₂ and were passaged twice a week. Different curcumin analogs were
dissolved in DMSO and the final concentration of DMSO in all experiments was 0.1%.

**MTT and apoptosis assays**

PC-3, Panc-1 and HT-29 cells were seeded at a density of 0.2 x 10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 hours. The cells were then treated with various concentrations (0.05-30 μM) of the different curcumin analogs for 72 hours. After treatment, 200 μl 3-[4,5-dimethylthiazol-2-yl] - 2,5-diphenyl tetrazolium bromide (MTT; 5 mg/ml in PBS) was added to each well of the plate and incubated for 2 hours. Then the plate was centrifuged at 1,000 rpm for 5 minutes at 4°C. After careful removal of the medium, 0.1 ml DMSO was added to each well. The absorbance was recorded on a microplate reader at 540 nm. The effect of different curcumin analogs on growth was assessed as percent cell growth as compared to the DMSO-treated cells. The concentration of DMSO as a solvent for the different curcumin analogs was 0.1% in the culture medium used and was without any effect on cell growth. Apoptosis was determined by a morphological assay as described (Zheng et al. (2008) Int. J. Oncol., 32:257-264; Zheng et al. (2010) Cancer Prev. Res., 3:114-124; Motyl et al. (1998) Eur. J. Cell. Biol., 75:367-374).

**Results**

**Chemistry**

A series of heterocyclic analogs of curcumin were prepared. These compounds were synthesized by coupling the appropriate substituted benzaldehyde with cyclohexanone (group A), eylopentanone (group B), acetone (group C), 4-piperidinone (group D), tetrahydropyran-4-ones (group E) or tetrahydrothiopyran-
4-one (group F) (Figure 1). In groups AN, BN, EN and FN, the analogs were synthesized by coupling the appropriate pyridyl aldehyde with cyclohexanone (group AN), cyclopentanone (group BN), tetrahydropyran-4-one (group EN) and tetrahydrothiopyran-4-one (group FN) (Figure 2). In groups AS, BS, ES and FS, the analogs were synthesized by coupling thiophene aldehyde with cyclohexanone (AS), cyclopentanone (BS), tetrahydropyr an-4-one (ES) or tetrahydrothiopyran-4-one (FS) (Figure 3).

Inhibitory effects of curcumin analogs towards cultured human prostate, pancreas and colon cancer cells

The inhibitory effects of sixty-one curcumin analogs on the growth of cultured human prostate cancer cells (PC-3), pancreatic cancer cells (Panc-1) and colon cancer cells (HT-29) were determined by using the MTT assay. For each incubation, curcumin was evaluated as a positive control. The inhibitory effect of curcumin did not vary significantly between the different incubations. Data from all curcumin incubations in experiments with groups A to F compounds are averaged and presented in Table 1. Average data from curcumin incubations in experiments with groups AN to FN compounds are presented in Table 2, and average data from curcumin incubations in experiments with AS, BS, ES and FS are shown in Table 3. As shown in Table 1, all compounds in group A (reaction products of different substituted benzaldehydes and cyclohexanone) had stronger anticancer growth inhibitory effects on PC-3, Panc-1 and HT-29 cells than curcumin as determined by the MTT assay. The IC50 for this group of compounds ranged from 2.6 to 15.9 µM (Table 1). Group B compounds (reaction products of different substituted
benzaldehydes and cyclopentanone) had a weaker growth inhibitory effect on the cells when compared with group A compounds. Seven compounds in this group had a similar or weaker effect than curcumin and only 3 compounds had a better effect than curcumin (Table 1). Ten of the 11 compounds in Group C (reaction products of different substituted benzaldehydes and acetone) had a stronger anticancer effect than curcumin and one compound (C3) in this group was similar to curcumin when tested on PC-3 and Panc-1 cells (Table 1). As shown in Table 1, compounds in groups D, E and F (reaction products of different substituted benzaldehydes and piperidone, pyridine or thiophene furan ketone) all had stronger inhibitory effects on tumor cell growth than curcumin or group A compounds. This result indicates that substitution of the cyclohexanone with 4-piperidinone, tetrahydropyran-4-ones or tetrahydrothiopyran-4-one strongly increases the effectiveness of these compounds. Heteroatoms such as nitrogen, oxygen or sulfur in the 4-position of the central six-carbon ring (groups D, E and F) increased the inhibitory effect of these compounds on tumor cell growth.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>H</td>
<td>OCH3</td>
<td>O</td>
<td>H</td>
<td>PC-3</td>
</tr>
<tr>
<td>A1</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>19.37±2.5</td>
</tr>
<tr>
<td>A2</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>11.87±2.2</td>
</tr>
<tr>
<td>A3</td>
<td>H</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>11.31±1.5</td>
</tr>
<tr>
<td>A4 (A1)</td>
<td>H</td>
<td>H</td>
<td>O</td>
<td>H</td>
<td>13.35±2.0</td>
</tr>
<tr>
<td>A5</td>
<td>H</td>
<td>H</td>
<td>OCH3</td>
<td>H</td>
<td>13.25±0.5</td>
</tr>
<tr>
<td>A6</td>
<td>H</td>
<td>H</td>
<td>OCH3</td>
<td>H</td>
<td>12.35±1.4</td>
</tr>
<tr>
<td>A7 (A2)</td>
<td>H</td>
<td>OCH3</td>
<td>O</td>
<td>H</td>
<td>5.89±0.9</td>
</tr>
<tr>
<td>A8 (A3)</td>
<td>H</td>
<td>OCH3</td>
<td>OCH3</td>
<td>H</td>
<td>5.20±1.0</td>
</tr>
<tr>
<td>A9 (A4)</td>
<td>H</td>
<td>OCH3</td>
<td>O</td>
<td>OCH3</td>
<td>4.14±0.7</td>
</tr>
<tr>
<td>A10</td>
<td>H</td>
<td>OCH3</td>
<td>OCH3</td>
<td>OCH3</td>
<td>6.17±1.3</td>
</tr>
<tr>
<td>A11</td>
<td>OCH3</td>
<td>OCH3</td>
<td>OCH3</td>
<td>H</td>
<td>8.22±1.5</td>
</tr>
<tr>
<td>B1</td>
<td>H</td>
<td>H</td>
<td>F</td>
<td>H</td>
<td>18.99±3.0</td>
</tr>
<tr>
<td>B2</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>24.98±3.5</td>
</tr>
<tr>
<td>B3</td>
<td>H</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>&gt;30</td>
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</tbody>
</table>
The inhibitory effects of groups A to F compounds on the growth of human prostate cancer PC-3, pancreas cancer Panc-1 and colon cancer HT-29 cells. PC-3, Panc-1 and HT-29 cells were seeded at a density of 0.2 x 10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 hours. The cells were then treated with various concentrations (0.05-30 μM) of the different compounds for 72 hours. Effects of the different compounds on the growth of PC-3, Panc-1 and HT-29 cells were determined by the MTT assay.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>X1</th>
<th>X2</th>
<th>X3</th>
<th>IC_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PC-3</td>
</tr>
<tr>
<td>Curcumin</td>
<td></td>
<td></td>
<td></td>
<td>20.16±2.3</td>
</tr>
<tr>
<td>AN1</td>
<td>N</td>
<td>C</td>
<td>C</td>
<td>1.28±0.2</td>
</tr>
<tr>
<td>AN2</td>
<td>C</td>
<td>N</td>
<td>C</td>
<td>2.16±0.2</td>
</tr>
<tr>
<td>AN3</td>
<td>C</td>
<td>C</td>
<td>N</td>
<td>2.12±0.4</td>
</tr>
<tr>
<td>BN1</td>
<td>N</td>
<td>C</td>
<td>C</td>
<td>2.94±0.3</td>
</tr>
<tr>
<td>BN2</td>
<td>C</td>
<td>N</td>
<td>C</td>
<td>6.97±0.8</td>
</tr>
</tbody>
</table>
Table 2: Inhibitory effects of groups AN, BN, EN, FN compounds on the growth of human prostate cancer PC-3, pancreas cancer Panc-1 and colon cancer HT-29 cells. PC-3, Panc-1 and HT-29 cells were seeded at a density of 0.2 x 10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 hours. The cells were then treated with various concentrations (0.05-30 µM) of the different compounds for 72 hours. Effects of the different compounds on the growth of PC-3, Panc-1 and HT-29 cells were determined by the MTT assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC-3</td>
</tr>
<tr>
<td>Curcumin</td>
<td>19.94±2.2</td>
</tr>
<tr>
<td>AS</td>
<td>14.55±2.5</td>
</tr>
<tr>
<td>BS</td>
<td>23.0±6.0</td>
</tr>
<tr>
<td>ES</td>
<td>6.59±1.4</td>
</tr>
<tr>
<td>FS</td>
<td>4.13±1.0</td>
</tr>
</tbody>
</table>

Table 3: Inhibitory effects of AS, BS, ES and FS on the growth of human prostate cancer PC-3, pancreas cancer Panc-1 and colon cancer HT-29 cells. PC-3, Panc-1 and HT-29 cells were seeded at a density of 0.2 x 10^5 cells/ml of medium in 96-well plates (0.2 ml/well) and incubated for 24 hours. The cells were then treated with various concentrations (0.1-30 µM) of different compounds for 72 hours. Effects of the different compounds on the growth of PC-3, Panc-1 and HT-29 cells were determined by the MTT assay.

The results for the growth inhibitory effect of compounds in groups AN, BN, EN and FN on PC-3, Panc-1 and HT-29 cells are summarized in Table 2. All compounds in these four groups had a stronger inhibitory effect on tumor cell growth than curcumin, and the IC_{50} values ranged from 0.51 to 7.88 µM. Compounds in the
AN, EN and FN groups had a stronger inhibitory effect on tumor cell growth than compounds in the BN group (Table 2). The results for the growth inhibitory effect of compounds in the AS, BS, ES and FS groups on PC-3, Panc-1 and HT-29 cells are summarized in Table 3. AS and BS had a similar or higher IC₅₀ than curcumin. ES and FS had a stronger inhibitory effect on tumor cell growth than curcumin (Table 3).

Among the 61 curcumin analogs studied, IC₅₀ measurements indicated that compounds E1O, F1O, FN1 and FN2 exhibited the most potent inhibitory effects on the growth of cultured PC-3, Panc-1 and HT-29 cancer cells (21- to 117-fold more active than curcumin). The IC₅₀ values for these compounds were lower than 1 µM in all three cell lines. The higher activity of E1O, F1O, FN1 and FN2 were confirmed by using the trypan blue assay for evaluation of cell growth. E1O was 72-, 46- and 117-fold more active than curcumin (IC₅₀) for inhibiting the growth of PC-3, Panc-1 and HT-29 cells, respectively (Table 1), whereas F1O was 69-, 34- and 72-fold more potent than curcumin for inhibiting the growth of PC-3, Panc-1 and HT-29 cells, respectively (Table 1). FN1 and FN2 had about the same inhibitory effect as E1O and F1O towards Panc-1 cells but were less active than E1O and F1O towards PC-3 and HT-29 cells (Tables 1 and 2). The structures of E1O, F1O, FN1, FN2 and curcumin are shown in Figure 4.

Treatment of PC-3 cells with E1O, F1O, FN1 or FN2 for 96 hours had a dramatic stimulatory effect on apoptosis at concentrations <1µM whereas curcumin had little or no effect at these low concentrations (Fig. 5). E1O was the most potent stimulator of apoptosis followed by F1O and FN1. Although FN2 was less effective than the other three compounds, it was also a
strong stimulator of apoptosis. The stimulatory effect of E10, F10, FN1 and FN2 on apoptosis in PC-3 cells paralleled their activities for inhibiting the growth of PC-3 cells (Tables 1 and 2).

5

Structure–activity relationship analysis

Based on analysis of the relationship between the structures of curcumin analogs and their ability to inhibit the growth of cultured cancer cells, the presence of groups on the two aromatic rings and the linker between the aromatic rings both played key roles in determining the anticancer activity of the various analogs.

Among the different series of curcumin analogs, linear or cyclic linkers between the two aromatic rings of curcumin analogs showed different activity trends. In general, the activities of compounds with a tetrahydrothiopyran-4-one (group F) or a tetrahydropryan-4-one (group E) linker exhibited the strongest activities, and that of compounds with piperidin-4-one (group D) had moderate activity whereas compounds with cyclohexanone (group A), acetone (group C) or cyclopentanone (group B) linker were less active. The activities of compounds with a heteroatom linker (compounds in groups D, E and F) were better than those without a heteroatom linker (groups A, B and C), which indicates that a flexible cycle linker could exhibit stronger activity than that of a small and highly rigid linker. Some curcumin analogs with piperidin-4-one possess potent anticancer activity, such as compound 14 (Adams et al. (2004) Bioorg. Med. Chem., 12:3871-3883), compound B10 (Yadav et al. (2010) Bioorg. Med. Chem., 18:6701-6707), and compound EF24 (Subramaniam et al. (2008) Cancer Res., 68:1962-1969) (Figure 6). The
instant curcumin analogs with a tetrahydropyran-4-one linker (group F) or a tetrahydrothiopyran-4-one linker (group E) were not previously reported to have anticancer activity, but E9, E10, F9 and F10 from groups E and F were strongly active. Comparing compounds with different heterocyclic ketones as a linker, the inhibitory effect of certain analogs with a sulfur or oxygen heterocyclic ketone linker and distal benzene rings (e.g. compounds E10 and F10) on tumor cell growth were slightly more potent than that of analogs with a sulfur or oxygen heterocyclic ketone linker and distal nitrogen heterocyclic rings (e.g. compounds FN1 and FN2) described herein.

Comparing different substituted groups on curcumin analogs with the same linker, it was found that introduction of methoxy groups on the aromatic rings enhanced anticancer activity. In both the E and F series, compounds with methoxy groups at R2, R3 and R4 (compounds E10 and F10) had exceptionally high anticancer activity whereas methoxy groups at R1, R2 and R3 (compounds E11 and F11) resulted in markedly reduced activity when compared with E10 and F10. It was observed that methoxy groups at R2, R3 and R4 in the A, B or C group of compounds (A10, B10 and C10) had stronger anticancer activity than curcumin and moving the methoxy groups to R1, R2 and R3 (compounds A11, B11 and C11) decreased activity slightly when compared with A10, B10 and C10. Compounds with three methoxy groups on each aromatic ring were also demonstrated to have strong anticancer activity (compounds B10 and GO-Y016, Figure 6). The later compound is the same as C10 in Table 1 and Figure 1. GO-Y016 was reported to be 12- to 60-fold more active than curcumin for inhibiting the growth of several cancer cell lines (Ohori et al. (2006).
Herein, the same compound (CIO) was only 4- to 11-fold more active than curcumin for inhibiting the growth of PC-3, Panc-1 and HT-29 cells (Table 1).

Introduction of halogen at R3 in group A, B or C compounds did not result in strongly active compounds (compounds A1, A2, A3, B1, B2, B3, C1, C2 and C3) whereas introduction of halogen at R3 in the D series (compounds D1, D2 and D3) resulted in compounds that had substantially more activity than curcumin.

An interesting observation was that the growth inhibitory effects of curcumin became stronger when the normal benzene rings in curcumin were replaced by distal heterocyclic aromatic rings (compounds in the AN, BN, EN, FN, ES and FS groups; Table 2). These results indicate that distal heteroatoms enhance the growth inhibitory effects of curcumin on cultured prostate, pancreas and colon cancer cells. The compound was 9- to 10-fold more active than curcumin for inhibition of the growth of PC-3, Panc-1 and HT-29 cells (Table 2).

Comparing the analogs containing 1-, 2- and 3-pyridine, it was found that the growth inhibitory effects of these compounds were similar or considerably stronger than curcumin.

Taken together, compounds with three methoxy groups at R2, R3 and R4 on each distal aromatic ring and a heterocyclic ketone linker, such as E10 and F10 as well as compounds with distal heterocyclic aromatic rings and a heterocyclic ketone linker, such as FN1 and FN2 (Figure 4), exhibited more potent anticancer effects than the other compounds studied.

The inhibitory effect of E10 on the growth of prostate and pancreatic xenograft tumors in SCID mice was also determined. In these experiments, SCID mice
were injected subcutaneously with human prostate cancer PC-3 or human pancreatic cancer Panc-1 cells (2x10^6 cells/mouse). After 4 weeks, mice with PC-3 or Panc-1 tumors (0.6-1.0 cm long and 0.6-1.0 cm wide) received i.p. injections of vehicle, curcumin, or E10 once a day for 28 days. Tumor size (length x width; cm^2) and body weight (g) were measured three times per week (Monday, Wednesday and Friday). It was found that E10 inhibited the growth of PC-3 and Panc-1 tumors with activity similar to that of curcumin.

In additional experiments, the in vivo anti-inflammatory effect of E10 was tested in a mouse model (12-0-tetradecanoy lphorhol-13-acetate-induced ear inflammation). It was determined that the anti-inflammatory effect of E10 was similar to that of curcumin.

Herein, several groups of curcumin analogs were synthesized and evaluated for anticancer activity using cultured human prostate, pancreas and colon cancer cells. Curcumin analogs with a tetrahydropyran-4-one (groups E and EN) or tetrahydrothiopyran-4-one (groups F and FN) as the core structure had a more potent effect on cancer cells than other curcumin analogs. Compounds E10, F10, FN1 and FN2 exhibited particularly potent inhibitory effects on the growth of cultured prostate cancer PC-3 cells, pancreas cancer Panc-1 cells and colon cancer HT-29 cells. The IC50 for these compounds in all three cell lines was lower than 1 µM. E10 was 72-, 46- and 117-fold more active than curcumin (comparison of IC50 values) for inhibiting the growth of PC-3 prostate cancer cells, Panc-1 pancreas cancer cells and HT-29 colon cancer cells, respectively, whereas F10 was 69-, 34- and 72-fold more potent than curcumin for inhibiting the growth of cultured PC-3, Panc-1 and HT-29
cells, respectively. FN1 and FN2 were also highly active inhibitors of the growth of these cancer cell lines, but overall they were less active than E10 and F10. It was found that (1) tetrahydropyran-4-one or tetrahydrothiopyran-4-one as a core structure, (2) methoxy groups at R2, R3 and R4 in the aromatic rings and (3) nitrogen heterocycles in the distal rings yield potent anticancer activity.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.
What is claimed is:

1. A compound having the formula

   \[
   \begin{array}{c}
   Y \leftarrow \underbrace{\begin{array}{c}
   O \\
   \end{array}}_{n} \rightarrow Z
   \end{array}
   \]

   wherein \( n = 1, 2, 3, 4, 5, 6, 7, \text{ or } 8 \), wherein the carbon chain optionally comprises at least one heteroatom; and
   wherein \( Y \) and \( Z \) are each independently an aryl.

2. The compound of claim 1, wherein \( Y \) and \( Z \) are heteroaryls.

3. The compound of claim 1, wherein \( n \) is 2 or 3.

4. The compound of claim 1, wherein the heteroatom is oxygen, sulfur, or nitrogen.

5. The compound of claim 1, wherein \( Y \) and \( Z \) are the same.

6. The compound of claim 1, wherein \( Y \) and \( Z \) are selected from the group consisting of:

   \[
   \begin{array}{c}
   \begin{array}{c}
   N \\
   \end{array}
   \end{array}
   \]

   \[
   \begin{array}{c}
   \begin{array}{c}
   N \\
   \end{array}
   \end{array}
   \]

   \[
   \begin{array}{c}
   \begin{array}{c}
   N \\
   \end{array}
   \end{array}
   \]

   from the group consisting of:
7. The compound of claim 1, having the formula:

wherein $R_p$ is absent, $H$, $CH_3$, alkyl, or lower alkyl; wherein $X'$ is 0, $S$, $NH$, or $NR_nR_m$ - wherein $R_n$ and $R_m$ are each independently $H$, $CH_3$, alkyl, or lower alkyl; and wherein $R_i$, $R_2$, $R_3$, $R_4$, and $R_5$ are each independently $H$, $OH$, halo, $OCH_3$, $OCF_3$, $O(C=O)CH_3$, $OCH_2CH_3$, 0-alkyl or 0-lower alkyl.

8. The compound of claim 1, having the formula:

wherein $X$ is 0, $S$, $NH$, or $NR_nR_m$, wherein $R_n$ and $R_m$ are each independently $H$, $CH_3$, alkyl, or lower alkyl.
wherein $R_1'$, $R_2'$, $R_3'$, $R_4'$, $R_5'$, $R_i$, $R_2$, $R_3$, $R_4$, and $R_5$ are each independently $H$, $OH$, halo, $OCH_3$, $OCF_3$, $O(C=0)CH_3$, $OCH_2CH_3$, $O$-alkyl or $O$-lower alkyl.

9. The compound of claim 8, having the formula:

wherein $X$ is $0$, $s$, $NH$, or $NR_nR_m$, wherein $R_n$ and $R_m$ are each independently $H$, $CH_3$, alkyl, or lower alkyl.

10. The compound of claim 1, selected from the group consisting of:

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11. A method for inhibiting cancer in a subject in need thereof, said method comprising administering to said subject a composition comprising at least one compound of claim 1 and at least one pharmaceutically acceptable carrier.

12. The method of claim 11, wherein said cancer is prostate, pancreatic, or colon cancer.

13. The method of claim 11, further comprising administering at least one other chemotherapeutic agent.

14. A method for inhibiting an inflammation disorder in a patient in need thereof, said method comprising administering to said subject a composition comprising at least one compound of claim 1 and at least one pharmaceutically acceptable carrier.

15. The method of claim 14, wherein said inflammation disorder is a neurodegenerative disease.

16. A composition comprising the compound of claim 1 and at least one pharmaceutically acceptable carrier.
Figure 1
Figure 2

EtOH, NaOH, 96h
or HCl(g), AcOH, 96h

O

X₂ X₂' X₃

CHO +

O

X₂ X₂' X₃

CHO +

O

X₂ X₂' X₃

X₂ X₂' X₃

X₂ X₂' X₃

X₂ X₂' X₃
Figure 3

AS or BS (or HCl(g), AcOH, 96h)

EtOH, NaOH, 96h

CHO +

ES or FS (or HCl(g), AcOH, 96h)

CHO +
Figure 4
Figure 6

Compound B33

Compound GO-YO31

Compound B10

Compound B16

Compound GO-YO30

Compound 14

Compound EF24

Compound GO-YO16
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/47403

According to International Patent Classification (IPC) or to both national classification and IPC

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 43/16; A61K 31/35 (2011.01)
USPC - 514/457

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/457

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/277, 279, 430, 432, 449, 451, 453, 455 (see search terms below)

Electronic database consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPAB,USPT,EPAB,JPAB), Google Scholar
Search terms: curcumin, curcuminoid, analog, derivative, mimetic, mimic, cancer, tumor, neoplasS, treats, amelioratS, inflammatS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 6,664,272 B2 (SNYDER et al.) 16 December 2003 (16.12.2003) col 2, In 7-17; col 5, In 21 to col 6, In 17; col 6, In 40-60. Table; col 12, In 3-17; In 53-59</td>
<td>1-16</td>
</tr>
<tr>
<td>Public Chemical Database CID 2740872 19 July 2005, pg 1-2; pg 1</td>
<td>1, 3-10</td>
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</table>

Further documents are listed in the continuation of Box C.

Date of the actual completion of the international search 06 December 2011 (06.12.2011)

Date of mailing of the international search report 22 DEC 2011

Authorized officer: Lee W. Young

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