



2-20-2018

Halogenated Diarylacetylenes and Methods of Treating Cancer

Vitaliy M. Sviripa

University of Kentucky, vitaliy.sviripa@uky.edu

Wen Zhang

University of Kentucky, wen.zhang@uky.edu

Chunming Liu

University of Kentucky, chunming.liu@uky.edu

David S. Watt

University of Kentucky, [dwatt@uky.edu](mailto:d watt@uky.edu)

[Click here to let us know how access to this document benefits you.](#)

Follow this and additional works at: https://uknowledge.uky.edu/ps_patents

 Part of the [Pharmacy and Pharmaceutical Sciences Commons](#)

Recommended Citation

Sviripa, Vitaliy M.; Zhang, Wen; Liu, Chunming; and Watt, David S., "Halogenated Diarylacetylenes and Methods of Treating Cancer" (2018). *Pharmaceutical Sciences Faculty Patents*. 173.
https://uknowledge.uky.edu/ps_patents/173

This Patent is brought to you for free and open access by the Pharmaceutical Sciences at UKnowledge. It has been accepted for inclusion in Pharmaceutical Sciences Faculty Patents by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.



US009895324B2

(12) **United States Patent**
Sviripa et al.

(10) **Patent No.:** **US 9,895,324 B2**

(45) **Date of Patent:** **Feb. 20, 2018**

(54) **HALOGENATED DIARYLACETYLENES AND METHODS OF TREATING CANCER**

(71) Applicant: **UNIVERSITY OF KENTUCKY RESEARCH FOUNDATION**,
Lexington, KY (US)

(72) Inventors: **Vitaliy M. Sviripa**, Lexington, KY (US); **Wen Zhang**, Lexington, KY (US); **Chunming Liu**, Lexington, KY (US); **David Watt**, Lexington, KY (US)

(73) Assignee: **University of Kentucky Research Foundation**, Lexington, KY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/668,865**

(22) Filed: **Mar. 25, 2015**

(65) **Prior Publication Data**

US 2015/0272908 A1 Oct. 1, 2015

Related U.S. Application Data

(60) Provisional application No. 61/970,657, filed on Mar. 26, 2014.

(51) **Int. Cl.**
A61K 31/136 (2006.01)

(52) **U.S. Cl.**
CPC **A61K 31/136** (2013.01)

(58) **Field of Classification Search**
CPC **A61K 31/136**
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,951,889 B2 * 10/2005 Hansen et al. 514/564
8,664,276 B2 3/2014 Watt et al.
8,716,355 B2 5/2014 Tsai

FOREIGN PATENT DOCUMENTS

JP 08-184867 * 7/1996 C09K 9/02
W 2001029011 A2 4/2001

W 2003073350 A2 6/2003
W 2009038759 A2 3/2009
W 2010092043 A1 8/2010
W 2012149048 A1 11/2012
W 2012149049 A1 11/2012

OTHER PUBLICATIONS

Yu et al 'Synthesis or 2-arylindole derivatives and evaluation as nitric oxide syntase and NFKB inhibitors' **Organic and Biomolecular Chemistry**, vol. 10, p. 8835-8847, 2012.*
English machine translation of JP 08-184867.*
Peckham et al 'Oxford Textbook of Oncology' Oxford University Press, vol. 1, p. 451, 1995.*
Hadfield, J.A. et al., "Preparation and Evaluation of Diarylalkynes as Antitumour Agents", **Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry**, vol. 28:8, pp. 1421-1431, 1998.
Idham, J.W. et al., "Genetic Toxicity of a Pharmacologically Active Group of ortho-(Arylalkynyl) phenoxypropanolamines." **Fundamental and Applied Toxicology**, vol. 14, pp. 376-385, 1990.
Sviripa, V.M. et al., "Halogenated Diarylacetylenes Repress c-myc Expression in Cancer Cells." **Bioorganic Medicinal Chemistry Letters**, vol. 24, pp. 3638-3640, 2014.
Zhang, W. et al. "Fluorinated N,N-Dialkylaminostilbenes for Wnt Pathway Inhibition and Colon Cancer Repression." **Journal of Medicinal Chemistry**, vol. 54, pp. 1288-1297, 2011.
Zhang, W. et al., "Fluorinated N,N-Dialkylaminostilbenes Repress Colon Cancer by Targeting Methionine S-Adenosyltransferase 2A." **ACS Chemical Biology**, vol. 8, pp. 796-803, 2013.

* cited by examiner

Primary Examiner — Craig D Ricci
Assistant Examiner — Christopher R Stone
(74) *Attorney, Agent, or Firm* — McDermott Will & Emery LLP

(57) **ABSTRACT**

Halogenated diarylacetylenes, e.g., diarylacetylenes having at least one halo substituent in one aryl ring and an amine in the opposing aryl ring, can inhibit the proliferation of LS174T colon cancer cells through the inhibition of c-myc and induction of the cyclin-dependent kinase inhibitor-1 (i.e., p21(Wif1/Cip1)). Such compounds are useful as anti-neoplastic agents.

12 Claims, 2 Drawing Sheets

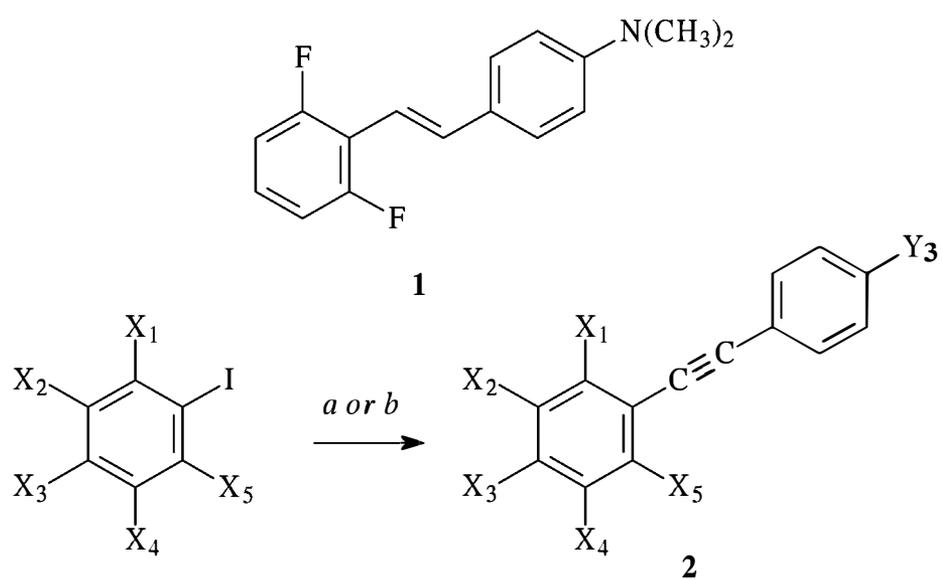


FIG. 1

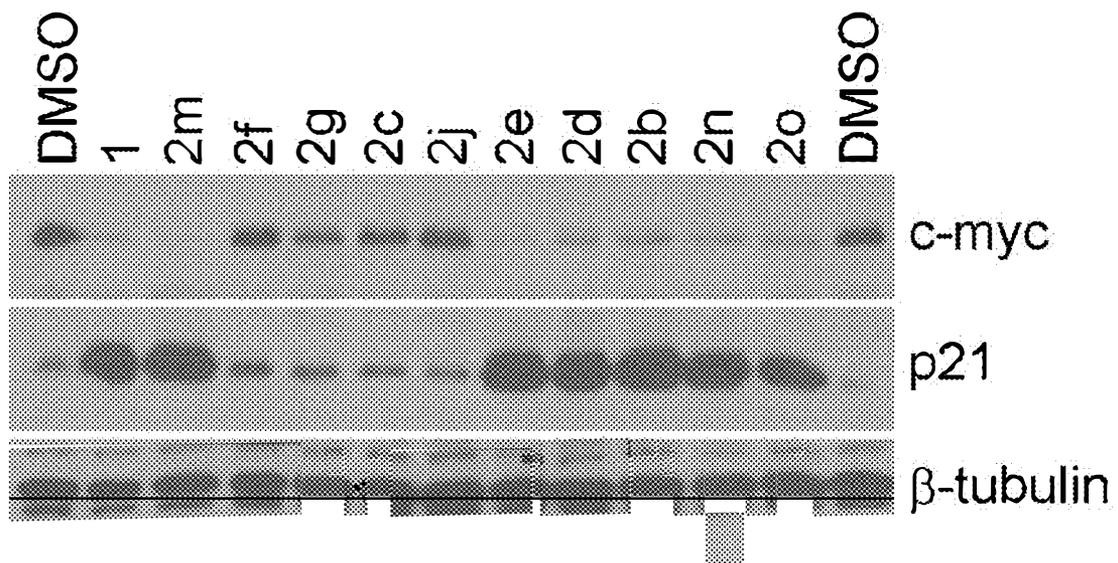


FIG. 2

1

HALOGENATED DIARYLACETYLENES AND METHODS OF TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 61/970,657 filed Mar. 26, 2014 the entire disclosure of which is hereby incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under R21 CA139359 and R01 CA172379. The Government has certain rights in the invention.

TECHNICAL FIELD

The present disclosure relates to halogenated diarylacetylenes for use as antineoplastic agents.

BACKGROUND

A family of fluorinated N,N-dialkylaminostilbene analogs (FIDAS agents) that inhibit the expression of Wnt target genes, such as c-myc, and repress colon cancer cell growth in vitro and in vivo was recently described. See, e.g., *J Med Chem* 2011: 54:1288-1297; *ACS Chem Biol* 2013: 8(4):796-803; U.S. Pat. No. 8,664,276.

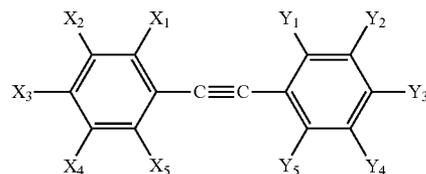
In addition, certain diarylacetylenes are known for certain medicinal uses. See, e.g., WO2012149049; WO2012149048; WO2010092043; WO2009038759; WO2008073350; and WO2001029011. Further, U.S. Pat. No. 8,716,355 to Tsai discloses hydroxylated tolans and related compounds in the treatment of cancer and Hadfield et al disclose preparation and evaluation of diarylalkynes as antitumor agents. Hadfield et al, *Synthetic Communications* 1998: 28(8):1421-1431. However, there is an ongoing need for additional compounds that can be used to treat cancer and other ailments.

SUMMARY OF THE DISCLOSURE

Advantages of the present disclosure include halogenated diarylacetylenes and compositions having antineoplastic activity and methods of inhibiting cancer cell growth and/or treating cancer in a patient by administering one or more of the halogenated diarylacetylenes or pharmaceutical compositions thereof.

One aspect of the present disclosure is directed to halogenated diarylacetylenes that are useful for killing hyperproliferating cells such as cancer cells for the treatment of human malignant and benign cancers, including without limitation, colorectal cancer (CRC), breast cancer, lung cancer, prostate cancer and liver cancer. In this aspect of the disclosure, there are provided certain halogenated diarylacetylenes having anti-neoplastic activity against cancerous cells. The halogenated diarylacetylenes of the present disclosure include compounds according to formula (I):

2



(I)

or a pharmaceutically acceptable salt thereof, wherein each of X_1 through X_5 independently represents H, a lower alkyl, or halo, provided that at least one of X_1 through X_5 is a halo; and each of Y_1 through Y_5 independently represents H, a lower alkyl, or NR_1R_2 , provided that at least one of Y_1 through Y_5 is NR_1R_2 , wherein each of R_1 and R_2 independently represents H, or a lower alkyl. In one aspect, of the present disclosure the compound of formula (I) does not include groups such as ester, hydroxyl, sulfonamide, amide, urethane, and carboxyl groups.

The halogenated diarylacetylenes of formula (I) or pharmaceutically acceptable salts thereof can be included in a pharmaceutical composition with a pharmaceutically acceptable carrier.

Another aspect of the present disclosure is directed to methods of treating cancer, e.g., inhibiting cancer cell growth and/or inhibiting tumor growth in a mammal, such as a human, or treating diseases associated with hyperproliferating cells. In one embodiment of this aspect of the disclosure, an effective amount of one or more halogenated diarylacetylenes, pharmaceutical salts and/or pharmaceutical compositions thereof is administered to a patient in need of treatment of cancer sufficient to treat/inhibit cancer cell growth in the patient.

In an embodiment of this aspect of the disclosure, a therapeutically effective amount of one or more halogenated diarylacetylenes, pharmaceutical salts and/or pharmaceutical compositions thereof is administered to a patient suffering from colorectal cancer. In another embodiment, a therapeutically effective amount of one or more halogenated diarylacetylenes, pharmaceutical salts and/or pharmaceutical compositions thereof is administered to a patient suffering from liver cancer or prostate cancer.

Additional advantages of the present invention will become readily apparent to those skilled in this art from the following detailed description, wherein only the preferred embodiment of the invention is shown and described, simply by way of illustration of the best mode contemplated of carrying out the invention. As will be realized, the invention is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the invention. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

Reference is made to the attached drawings, wherein elements having the same reference numeral designations represent similar elements throughout and wherein:

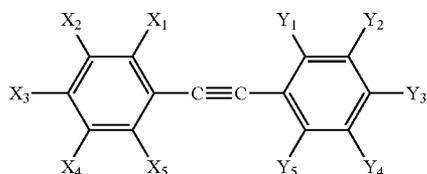
FIG. 1 is a schematic illustration of the synthesis of halogenated diarylacetylenes 2. Reagents: a, $HC\equiv CHC_6H_4Y$; 0.5% $Pd(PPh_3)_4$, 1% CuI, H_2O , $75^\circ C.$, 1-2 h; b, a, $HC\equiv CHC_6H_4NH_2$; 0.5% $Pd(PPh_3)_4$, 1% CuI, H_2O , $75^\circ C.$, 1-2 h followed by CH_3I , K_2CO_3 , acetone, 5 h, $56^\circ C.$

FIG. 2 is a blot showing inhibition of c-myc and induction of p21(Wif1/Cip1) by diarylacetylenes 2 in colon cancer cells. LS174T cells were treated with 1 μ M of each diarylacetylenes 2 for 36 h. DMSO and 1 were used as control. Cell lysates were analyzed by western blotting with β -tubulin as a loading control.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure relates to halogenated diarylacetylenes, their salts and their pharmaceutical compositions and methods of inhibiting cancer cell growth and/or treating cancer in a patient by administering one or more of the halogenated diarylacetylenes, a pharmaceutical salt thereof, or a pharmaceutical composition thereof. It was found that halogenated diarylacetylenes having at least one, preferably two, halo substituents in one aryl ring and an amine in the opposing aryl ring, e.g., N-methylamino or N,N-dimethylamino, inhibit the proliferation of LS174T colon cancer cells through the inhibition of c-myc and induction of the cyclin-dependent kinase inhibitor-1 (i.e., p21(Wif1/Cip1)). Such compounds and compositions are useful as antineoplastic agents.

The halogenated diarylacetylenes of the present disclosure include at least one amine group, e.g., a primary, secondary or tertiary amine, on the aryl ring. Such compounds are useful as antineoplastic agents and can be represented by the following formula:



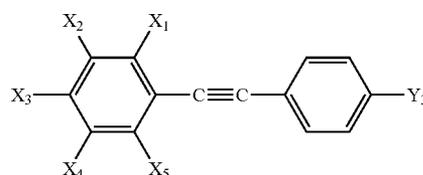
or a pharmaceutically acceptable salt thereof. The substituents of X_1 through X_5 each independently represent H, a lower alkyl, or halo, provided that at least one of X_1 through X_5 is a halo. A halo group means an F, Cl, Br, I, or At group. Preferably substituents X_1 through X_5 include at least two halo groups, e.g., an F and/or Cl. Each of Y_1 through Y_5 independently represents H, a lower alkyl, or NR_1R_2 , provided that at least one of Y_1 through Y_5 is NR_1R_2 , wherein each of R_1 and R_2 independently represents H, or a lower alkyl. In one aspect, of the present disclosure the compound of formula (I) does not include groups such as ester, hydroxyl, sulfonamide, amide, urethane, and carboxyl groups.

The term "lower alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups having from one to about ten carbons (C_1 - C_{10}), e.g., from one to about six carbon atoms (C_1 - C_6) in its backbone structure. In one aspect of the present disclosure, the lower alkyl groups specifically include methyl, ethyl, propyl, isopropyl, n-butyl, etc.

Embodiments of the halogenated diarylacetylenes of the present disclosure include wherein at least two of X_1 through X_5 is are halo groups, e.g., wherein X_1 through X_5 is either (i) a fluoro and chloro, (ii) both fluoro, (iii) both chloro groups, and wherein both R_1 and R_2 are lower alkyl groups,

e.g., methyl, ethyl, or butyl groups. Preferably X_1 and/or X_5 are halo groups, e.g., the diarylacetylenes have one or two halogen substituents at ortho-positions relative to the acetylenic linkage, and X_2 through X_4 are H or a lower alkyl. In some embodiments, the halogenated diarylacetylenes include compounds, or a pharmaceutically acceptable salt thereof, where Y_3 is NR_1R_2 , and Y_1 , Y_2 , Y_4 , and Y_5 independently represent H, a lower alkyl, or NR_1R_2 . In other embodiments, Y_3 is NR_1R_2 , and Y_1 , Y_2 , Y_4 , and Y_5 independently represent H or a lower alkyl, e.g. Y_1 , Y_2 , Y_4 , and Y_5 represent H. In still further embodiments, at least one of R_1 , or R_2 , is a lower alkyl.

In another embodiment of the present disclosure, the halogenated diarylacetylenes include compounds according to formula (II):



or a pharmaceutically acceptable salt thereof, wherein Y_3 is NR_1R_2 , and R_1 , R_2 , X_1 through X_5 and are as defined for the compounds of formula (I) including the various embodiments for formula (I). Preferably, at least one of R_1 , or R_2 , is a lower alkyl and at least two of X_1 through X_5 are either (i) a fluoro and chloro, (ii) both fluoro, (iii) both chloro groups. In another embodiment, both R_1 , and R_2 , are lower alkyl, e.g., methyl, and at least two of X_1 through X_5 are either: (i) a fluoro and chloro, (ii) both fluoro, (iii) both chloro groups. In some embodiments, X_1 and/or X_5 are halo groups, e.g., either a fluoro or chloro group.

Pharmaceutical compositions of the present disclosure include one or more of compounds according to formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. Preferable, pharmaceutical compositions of the present disclosure include one or more of compounds according to formula (II) and a pharmaceutically acceptable carrier.

In one aspect of the present disclosure, the compounds of formula (I) or (II), a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable composition thereof is used in the treatment of cancer. The method comprises administering to a patient in need of such treatment an effective amount of one or more of the halogenated diarylacetylenes, a pharmaceutical salt thereof, or a pharmaceutical composition thereof. Embodiments of the method include wherein the cancer treated is selected from the group consisting of colorectal cancer, breast cancer, lung cancer, prostate cancer and liver cancer.

In the course of developing new agents for the treatment of cancers, we identified a family of fluorinated N,N-dialkylaminostilbene analogs (FIDAS agents) that inhibit the expression of Wnt target genes, such as c-myc, and repress colon cancer cell growth in vitro and in vivo. Recently, we found that (E)-4-(2',6'-difluorostyryl)-N,N-dimethylaniline (1) (FIG. 1) targeted exclusively the catalytic subunit of methionine S-adenosyltransferase-2 (MAT-2). See Zhang et al. *ACS Chem Biol* 2013; 8(4):796-803. MAT-2 serves as a source of S-adenosylmethionine (SAM) in colorectal and liver cancers where MAT-2 is upregulated. See

Cai et al. *Hepatology* 1996: 24:1090-1097; Chen et al. *Gastroenterology* 2007: 133:207-218; Ito et al. *Surg Today* 2000: 30:706-710; and Liu et al. *J Biol Chem* 2011: 286: 17168-17180.

It is believed that neoplastic tissues make effective use of SAM from this isoform of MAT to manage crucial epigenetic modifications of histone proteins and thereby regulate gene expression. Interference with this process would represent a new approach for developing potential antineoplastic agents. It is believed that the compounds of the present disclosure inhibit c-myc. In addition, the present compounds would avoid the facile E/Z-isomerizations that afflict the stilbenes and complicate pharmacodynamic and pharmacokinetic studies.

The diarylacetylenes 2 (FIG. 1), e.g., compounds of formula (II), are a preferred group of halogenated diarylacetylenes. Prior reports of acetylenic compounds as antineoplastic agents include monoalkylacetylenes from aquatic organisms and diarylacetylenic analogs of combrestatin. See Dembitsky et al. *Nat. Prod. Commun.* 2006: 1:773-812 and Hadfield et al. *Synth. Commun.* 1998: 28:1421-1431. The latter compounds showed cytotoxic activity against a murine leukemia cell line and one showed activity as an inhibitor of tubulin polymerization. The Sonogashira coupling of 4-(N,N-dimethylamino)phenylacetylene with various aryl iodides provided access to the desired diarylacetylenes 2 (Table 1). (Additional information about Sonogashira coupling can be found in Bhattacharya et al. Sengupta, S. *Tetrahedron Lett.* 2004: 47:8733-8736 and Okuro et al. *J. Org. Chem.* 1993: 58:4716-4721). Prior work from our laboratories established that stilbenes with N-methylamino and N,N-dimethylamino groups in a para-orientation relative to the central double bond as well as 2,6-difluoro, 2-chloro-6-fluoro or 2,6-dichloro halogenation patterns in the other aromatic ring were the most potent analogs in the inhibition of LS174T cell proliferation. See Zhang et al. *J Med Chem* 2011: 54:1288-1297.

Table 1 below provides IC₅₀ values for the inhibition of LS174T cell proliferation for certain halogenated diarylacetylenes of the present disclosure and stilbene compound 1 as shown in FIG. 1.

TABLE 1

Compound	X ₁	X ₂	X ₃	X ₄	X ₅	Y ₃	Inhibition of LS174T Cell Proliferation IC ₅₀ (nM)
1	F				F	N(CH ₃) ₂	59 ± 7.5
2a	F				F	NH ₂	55 ± 7.8
2b	F				F	NHCH ₃	23 ± 10.3
2c						N(CH ₃) ₂	>3000
2d	F					N(CH ₃) ₂	39 ± 6.0
2e	Cl					N(CH ₃) ₂	31 ± 3.1
2f		F				N(CH ₃) ₂	>3000
2g			F			N(CH ₃) ₂	>3000
2h	F	F				N(CH ₃) ₂	119 ± 4.6
2i	F		F			N(CH ₃) ₂	56 ± 8.1
2j		F	F			N(CH ₃) ₂	>3000
2k		F		F		N(CH ₃) ₂	>3000
2l	F			F		N(CH ₃) ₂	55 ± 6.0
2m	F			F		N(CH ₃) ₂	23 ± 6.0
2n	F			Cl		N(CH ₃) ₂	19 ± 5.0
2o	Cl			Cl		N(CH ₃) ₂	52 ± 7.1

SAR studies were undertaken for those diarylacetylenes 2 that possessed fluorine or chlorine substituents in one aryl ring and N-methylamino or N,N-dimethylamino in the other aryl ring. We reported previously that stilbenes repressed colon cancer cell proliferation by inhibiting c-myc expres-

sion and inducing the cell cycle inhibitor, p21(Wif1/Cip1). See Zhang et al. *J Med Chem* 2011: 54:1288-1297. The similarity of the diarylacetylenes to the stilbenes also prompted an in silico modeling study of the binding of (E)-4-(2',6'-difluorostyryl)-N,N-dimethylaniline (1) and 4((2,6-difluorophenyl)ethynyl)-N,N-dimethylaniline (2m). It was believed that para-oriented amino-substituents, such as the N-methylamino and N,N-dimethylamino groups, were associated with potent MAT2A inhibition. Using an artificially constructed homodimer of MAT2A, we observed that 1 and 2m bound to the same active site and that diarylacetylene 2m inhibited MAT2A at concentrations comparable to that of the stilbene 1 (data not shown). Variability in the MAT2A inhibition assay made the measurement of c-myc inhibition a preferred analytical tool for assessing the potency of diarylacetylenes.

We tested the effect of these diarylacetylenes 2 on the proliferation of LS174T colon cancer cells. The expression of c-myc and p21(Wif1/Cip1) were analyzed by western blotting (FIG. 2). The most active diarylacetylenes 2 inhibited c-myc expression at 1 μM concentrations and as expected for a c-myc inhibitor, induced p21(wif1/Cip1) at the same time. Consistent with prior results in the stilbene family, the diarylacetylenes 2 lacking halogen substituents (e.g., 2c) or possessing only one fluorine substituent at a meta- or para-position relative to the acetylenic linkage (e.g., 2g) had very low potency (Table 1). Diarylacetylenes with one or two halogen substituents at ortho-positions relative to the acetylenic linkage (e.g., 2b, 2d, 2e, 2m and 2n) possessed potencies as inhibitors of LS174T cell proliferation that exceeded that of the related stilbene 1 with IC₅₀ values less than 50 nM (Table 1). Isomers of these diarylacetylenes (e.g., 2f, 2g, 2j and 2k) with halogens in meta- or para-positions were significantly less active than the diarylacetylenes with ortho-halogens. Once again, these results are in consistent with the SAR findings in the stilbene family of c-myc inhibitors. Zhang et al. *J Med Chem* 2011: 54:1288-1297. Finally, the N-methylation pattern in the diarylacetylenes suggested that N-methyl and N,N-dimethylaniline subunits led to equipotent inhibitors of c-myc (i.e., IC₅₀ of 2b≈IC₅₀ of 2m) but the desmethyl analog was considerably less active (IC₅₀ of 2a=55±7.8 nm).

It was found that diarylacetylenes 2 have a dramatic effect on the proliferation of LS174T colon cancer cells by altering the expression of c-myc and thereby inducing p21(Wif1/Cip1). These results are consistent with similar findings using halogenated stilbenes and suggest that diarylacetylenes and stilbenes repress colon cancer proliferation through similar mechanisms.

In an aspect of the present disclosure, the following particular halogenated diarylacetylenes and their pharmaceutical salts and pharmaceutical compositions can be used to treat cancer, CRC: 4-((2,6-Difluorophenyl)ethynyl)aniline (2a); 4-((2,6-Difluorophenyl)ethynyl)-N-methylaniline (2b); N,N-Dimethyl-4-(phenylethynyl)aniline (2c); 4-((2-Fluorophenyl)ethynyl)-N,N-dimethylaniline (2d); 4-((2-Chlorophenyl)ethynyl)-N,N-dimethylaniline (2e); 4-((3-Fluorophenyl)ethynyl)-N,N-dimethylaniline (2f); 4-((4-Fluorophenyl)ethynyl)-N,N-dimethylaniline (2g); 4-((2,3-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2 h); 4-((2,4-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2i); 4-((3,4-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2j); 4-((3,5-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2k); 4-((2,5-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2l); 4-((2,6-Difluorophenyl)ethynyl)-N,N-dimethylaniline (2m),

7

4-((2-Chloro-6-fluorophenyl)ethynyl)-N,N-dimethylaniline (2n), and/or 4-((2,6-Dichlorophenyl)ethynyl)-N,N-dimethylaniline (2o).

EXAMPLES

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

Cell Proliferation Assay.

LS174T cells were grown in RPMI medium (Mediatech) supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin. For cell proliferation assays, 3×10^4 cells/well growing in 12-well plates were treated with DMSO or inhibitors. The cell numbers and viability were analyzed by Vi-Cell Cell Viability Analyzer after 4 days. The IC_{50} values were calculated with GraphPad Prim 5.

Western Blotting.

Western blot was performed as described previously¹. The following antibodies were used: anti-c-myc (Epitomics, 1472-1), anti-p21(Wif1/Cip1) (Cell Signaling, 2947), anti- β -tubulin (DSHB, E7).

Materials.

Chemicals were purchased from Sigma Aldrich or Fisher Scientific or were synthesized according to literature procedures. Solvents were used from commercial vendors without further purification unless otherwise noted. Nuclear magnetic resonance spectra were determined on a Varian instrument (¹H, 400 MHz; ¹³C, 100 Mz). High resolution electrospray ionization (ESI) mass spectra were recorded on a LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, Mass., USA). The FT resolution was set at 100,000 (at 400 m/z). Samples were introduced through direct infusion using a syringe pump with a flow rate of 5 μ L/min. Purity of compounds was established by combustion analyses by Atlantic Microlabs, Inc., Norcross, Ga. Compounds were chromatographed on preparative layer Merck silica gel F254 unless otherwise indicated.

Synthesis of Diarylacetylenes.

A general procedure for the synthesis of diarylacetylenes 2 involved the addition of 2.0 mmol of arylacetylene to a mixture of 2.1 mmol of an aryl iodide, 3.0 mmol of diisopropylethylamine, 0.02 mmol of Pd(PPh₃)₄, and 0.02 mmol of CuI in water (7 mL). The mixture was stirred for 1-2 h at 75° C. After cooling, the product was collected by filtration or extracted using dichloromethane and purified by recrystallization and/or chromatography.

Characterization and Analytical Data For diarylacetylenes 2.

4-((2,6-Difluorophenyl)ethynyl)aniline (2a)

Purified by chromatography on silica gel using 1:2ethyl acetate-hexane ($R_f=0.43$). Yield 65%, mp 104-105° C. ¹H NMR (DMSO-d₆): δ 7.46-7.39 (m, 1H), 7.22 (d, 2H, J=8.8 Hz), 7.20-7.16 (m, 2H), 6.58 (d, 2H, J=8.8 Hz), 5.69 (s, 2H, NH₂). ¹³C NMR (DMSO-d₆): δ 161.72 (dd, J₁=248.9 Hz, J₂=5.3 Hz, two C), 150.21, 132.77 (two C), 129.89 (t, J=9.9 Hz), 113.60 (two C), 111.68 (dd, J₁=18.2 Hz, J₂=6.1 Hz, two C), 106.96, 101.96 (t, J=19.8 Hz), 102.28 (d, J=2.7 Hz), 73.11. HRMS (ESI) calcd for C₁₄H₉F₂N [MH⁺]: 230.07758. Found: 230.07660. Anal. Calcd for C₁₄H₉F₂N: C, 73.36; H, 3.96. Found: C, 73.10; H, 4.03.

8

4-((2,6-Difluorophenyl)ethynyl)-N-methylaniline (2b)

To a solution of 200 mg (0.87 mmol) of 2a in acetone (4 mL) was added successively 145 mg (1.04 mmol, 1.2 equiv) of potassium carbonate and 161 mg (1.13 mmol, 1.3 equiv) of iodomethane. The mixture was refluxed for 5 h. After cooling, the product was diluted with water, extracted with dichloromethane, dried over MgSO₄ and concentrated. The product was purified by chromatography on silica gel using 1:5ethyl acetate-hexane ($R_f=0.48$) to afford 68 mg (32%) of 2b. Mp 69-70° C. ¹H NMR (CDCl₃): δ 7.40 (d, 2H, J=8.8 Hz), 7.24-7.15 (m, 1H), 6.92-6.87 (m, 2H), 6.53 (d, 2H, J=8.8 Hz), 3.95 (br s, 1H), 2.84 (s, 3H). ¹³C NMR (CDCl₃): δ 162.92 (dd, J₁=251.2 Hz, J₂=5.3 Hz, two C), 149.92, 133.35 (two C), 128.77 (t, J=9.8 Hz), 112.08 (two C), 111.25 (dd, J₁=19.0 Hz, J₂=6.0 Hz, two C), 110.34, 103.39 (t, J=19.7 Hz), 100.89 (t, J=3.1 Hz), 74.16, 30.48. HRMS (ESI) calcd for C₁₅H₁₁F₂N [MH⁺]: 244.09323. Found: 244.09241. Anal. Calcd for C₁₅H₁₁F₂N: C, 74.06; H, 4.56. Found: C, 73.89; H, 4.71.

N,N-Dimethyl-4-(phenylethynyl)aniline (2c)

Purified by recrystallization from hexane. Yield 88%, mp 104-106° C. ¹H NMR (DMSO-d₆): δ 7.49-7.47 (m, 2H), 7.41-7.33 (m, 5H), 6.71 (d, 2H, J=9.2 Hz), 2.94 (s, 6H). ¹³C NMR (DMSO-d₆): δ 150.10, 132.38 (two C), 130.86 (two C), 128.61 (two C), 127.83, 123.29, 111.84 (two C), 108.41, 90.90, 87.16, 39.66 (two C). HRMS (ESI) calcd for C₁₆H₁₅N [MH⁺]: 222.12773. Found: 222.12713.

4-((2-Fluorophenyl)ethynyl)-N,N-dimethylaniline (2d)

Purified by recrystallization from hexane. Yield 62%, mp 94-96° C. ¹H NMR (DMSO-d₆): δ 7.57-7.53 (m, 1H), 7.43-7.34 (m, 3H), 7.31-7.22 (m, 1H), 7.24-7.20 (m, 1H), 6.71 (d, 2H, J=9.2 Hz), 2.95 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.47 (d, J=246.7 Hz), 150.32, 132.96, 132.46 (two C), 129.91 (d, J=7.6 Hz), 124.64 (d, J=3.8 Hz), 115.55 (d, J=20.5 Hz), 111.81 (two C), 111.63 (d, J=15.2 Hz), 107.83, 96.02 (d, J=3.0 Hz), 80.38, 39.62 (two C). HRMS (ESI) calcd for C₁₆H₁₄FN [MH⁺]: 240.11830. Found: 240.11726. Anal. Calcd for C₁₆H₁₄FN: C, 80.31; H, 5.90. Found: C, 80.04; H, 6.03.

4-((2-Chlorophenyl)ethynyl)-N,N-dimethylaniline (2e)

Purified by recrystallization from ethanol. Yield 56%, mp 108-110° C. ¹H NMR (DMSO-d₆): δ 7.60-7.54 (m, 2H), 7.38-7.34 (m, 4H), 6.73 (d, 2H, J=8.8 Hz), 2.96 (s, 6H). ¹³C NMR (DMSO-d₆): δ 150.40, 134.00, 133.33, 132.76, 132.55 (two C), 129.28, 127.27, 122.96, 111.85 (two C), 107.80, 96.27, 84.08, 39.67 (two C). HRMS (ESI) calcd for C₁₆H₁₄ClN [MH⁺]: 256.08875. Found: 256.08805.

4-((3-Fluorophenyl)ethynyl)-N,N-dimethylaniline (2f)

Purified by recrystallization from hexane. Yield 87%, mp 99-100° C. ¹H NMR (DMSO-d₆): δ 7.45-7.31 (m, 5H), 7.22-7.17 (m, 1H), 6.71 (d, 2H, J=9.2 Hz), 2.95 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.90 (d, J=242.8 Hz), 150.30, 132.55 (two C), 130.66 (d, J=8.4 Hz), 127.19 (d, J=2.2 Hz), 125.32 (d, J=9.9 Hz), 117.31 (d, J=22.7 Hz), 115.02 (d, J=21.3 Hz),

9

111.81 (two C), 107.79, 92.09, 86.07 (d, J=3.0 Hz), 39.64 (two C). HRMS (ESI) calcd for C₁₆H₁₄FN [MH⁺]: 240.11830. Found: 240.11750. Anal. Calcd for C₁₆H₁₄FN: C, 80.31; H, 5.90. Found: C, 80.03; H, 6.10.

4-((4-Fluorophenyl)ethynyl)-N,N-dimethylaniline
(2g)

Purified by recrystallization from methanol. Yield 92%, mp 132-134° C. ¹H NMR (DMSO-d₆): δ 7.54-7.51 (m, 2H), 7.35 (d, 2H, J=8.8 Hz), 7.24-7.20 (m, 2H), 6.70 (d, 2H, J=9.2 Hz), 2.94 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.47 (d, J=245.1 Hz), 150.12, 133.07 (d, J=8.4 Hz, two C), 132.35 (two C), 119.74 (d, J=3.8 Hz), 115.80 (d, J=22.1 Hz, two C), 111.83 (two C), 108.26, 90.56, 86.07, 39.65 (two C). HRMS (ESI) calcd for C₁₆H₁₄FN [MH⁺]: 240.11830. Found: 240.11752.

4-((2,3-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2 h)

Purified by recrystallization from methanol. Yield 65%, mp 104-106° C. ¹H NMR (DMSO-d₆): δ 7.46-7.37 (m, 4H), 7.24-7.19 (m, 1H), 6.72 (d, 2H, J=8.0 Hz), 2.91 (s, 6H). ¹³C NMR (DMSO-d₆): δ 149.78 (dd, J₁=244.4 Hz, J₂=11.4 Hz), 149.37 (dd, J₁=247.1 Hz, J₂=13.8 Hz), 132.62 (two C), 128.15 (d, J=4.0 Hz), 125.14 (d, J=4.6 Hz), 125.06 (d, J=4.6 Hz), 117.21 (d, J=16.7 Hz), 113.87 (d, J=11.4 Hz), 111.80 (two C), 107.20, 97.54 (d, J=3.8 Hz), 79.23 (d, J=4.6 Hz), 39.60 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10818. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.43; H, 5.22.

4-((2,4-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2i)

Purified by recrystallization from methanol. Yield 82%, mp 116-118° C. ¹H NMR (DMSO-d₆): δ 7.65-7.59 (m, 1H), 7.40-7.34 (m, 3H), 7.15-7.11 (m, 1H), 6.71 (d, 2H, J=9.2 Hz), 2.95 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.79 (dd, J₁=249.3 Hz, J₂=12.5 Hz), 150.35, 161.63 (dd, J₁=247.8 Hz, J₂=11.8 Hz), 134.11 (dd, J₁=9.8 Hz, J₂=2.3 Hz), 132.44 (two C), 112.15 (dd, J₁=22.0 Hz, J₂=3.8 Hz), 111.81 (two C), 108.31 (dd, J₁=15.6 Hz, J₂=4.2 Hz), 107.68, 104.50 (t, J=25.8 Hz), 95.75, 79.35, 39.63 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10806. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.61; H, 5.23.

4-((3,4-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2j)

Purified by recrystallization from methanol. Yield 93%, mp 100-101° C. ¹H NMR (DMSO-d₆): δ 7.60-7.55 (m, 1H), 7.48-7.41 (m, 1H), 7.37-7.32 (m, 3H), 6.71 (d, 2H, J=9.2 Hz), 2.95 (s, 6H). ¹³C NMR (DMSO-d₆): δ 150.29, 149.21 (dd, J₁=249.0 Hz, J₂=14.5 Hz, two C), 132.49 (two C), 128.26 (dd, J₁=6.4 Hz, J₂=3.4 Hz), 128.68 (dd, J₁=8.0 Hz, J₂=4.2 Hz), 119.76 (d, J=18.2 Hz), 118.00 (d, J=16.7 Hz), 111.80 (two C), 107.69, 91.58, 85.21, 39.62 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10787. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.80; H, 5.11.

4-((3,5-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2k)

Purified by recrystallization from methanol. Yield 84%, mp 72-74° C. ¹H NMR (DMSO-d₆): δ 7.37 (d, 2H, J=8.8

10

Hz), 7.28-7.19 (m, 3H), 6.71 (d, 2H, J=9.2 Hz), 2.96 (s, 6H). ¹³C NMR (DMSO-d₆): δ 162.27 (dd, J₁=245.1 Hz, J₂=14.4 Hz, two C), 150.47, 132.70 (two C), 126.29 (t, J=12.1 Hz), 113.95 (dd, J₁=19.0 Hz, J₂=7.6 Hz, two C), 111.78 (two C), 107.22, 103.94 (t, J=25.8 Hz), 93.42, 85.33 (t, J=3.8 Hz), 39.60 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10787. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.43; H, 5.20.

4-((2,5-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2l)

Purified by recrystallization from methanol. Yield 79%, mp 106-107° C. ¹H NMR (DMSO-d₆): δ 7.45-7.41 (m, 1H), 7.39-7.32 (m, 3H), 7.28-7.22 (m, 1H), 6.72 (d, 2H, J=9.2 Hz), 2.96 (s, 6H). ¹³C NMR (DMSO-d₆): δ 157.89 (dd, J₁=243.6 Hz, J₂=2.3 Hz), 157.80 (dd, J₁=239.9 Hz, J₂=2.3 Hz), 150.52, 132.62 (two C), 118.82 (d, J=25.8 Hz), 117.04 (dd, J₁=24.3 Hz, J₂=9.1 Hz), 116.53 (dd, J₁=24.3 Hz, J₂=8.3 Hz), 113.01 (dd, J₁=18.2 Hz, J₂=10.7 Hz), 111.80 (two C), 107.23, 97.22 (d, J=3.8 Hz), 79.61, 39.61 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10812. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.56; H, 5.21.

4-((2,6-Difluorophenyl)ethynyl)-N,N-dimethylaniline
(2m)

Purified by recrystallization from hexane. Yield 59%, mp 98-100° C. ¹H NMR (DMSO-d₆): δ 7.49-7.41 (m, 1H), 7.37 (d, 2H, J=8.8 Hz), 7.23-7.18 (m, 2H), 6.72 (d, 2H, J=8.8 Hz), 2.97 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.75 (dd, J₁=248.9 Hz, J₂=5.3 Hz, two C), 150.58, 132.59 (two C), 130.12 (t, J=10.3 Hz), 111.76 (dd, J₁=17.9 Hz, J₂=4.3 Hz, two C), 111.82 (two C), 107.21, 101.83 (t, J=19.8 Hz), 100.88 (t, J=3.1 Hz), 73.83, 39.64 (two C). HRMS (ESI) calcd for C₁₆H₁₃F₂N [MH⁺]: 258.10888. Found: 258.10801. Anal. Calcd for C₁₆H₁₃F₂N: C, 74.69; H, 5.09. Found: C, 74.47; H, 5.24.

4-((2-Chloro-6-fluorophenyl)ethynyl)-N,N-dimethylaniline
(2n)

Purified by chromatography on silica gel using 1:5 ethyl acetate-hexane (R_f=0.74). Yield 53%, mp 86-88° C. ¹H NMR (DMSO-d₆): δ 7.45-7.30 (m, 5H), 6.73 (d, 2H, J=8.8 Hz), 2.97 (s, 6H). ¹³C NMR (DMSO-d₆): δ 161.89 (d, J=249.7 Hz), 150.62, 135.11 (d, J=3.0 Hz), 132.64 (two C), 129.90 (d, J=9.1 Hz), 125.37 (d, J=3.0 Hz), 114.37 (d, J=21.2 Hz), 112.37 (d, J=18.2 Hz), 111.84 (two C), 107.24, 101.55 (d, J=3.8 Hz), 77.59, 39.64 (two C). HRMS (ESI) calcd for C₁₆H₁₃ClFN [MH⁺]: 274.07933. Found: 274.07858. Anal. Calcd for C₁₆H₁₃ClFN: C, 70.20; H, 4.79. Found: C, 70.43; H, 4.76.

4-((2,6-Dichlorophenyl)ethynyl)-N,N-dimethylaniline
(2o)

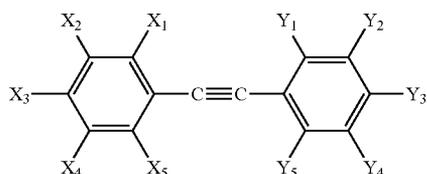
Purified by recrystallization from ethanol. Yield 54%, mp 96-98° C. ¹H NMR (DMSO-d₆): δ 7.52 (d, 2H, J=8.0 Hz), 7.37-7.29 (m, 3H), 6.70 (d, 2H, J=9.2 Hz), 2.93 (s, 6H). ¹³C NMR (DMSO-d₆): δ 150.66, 135.26, 133.31, 132.67 (two C), 129.40, 127.97 (two C), 122.79, 111.82 (two C), 107.22, 101.91, 81.62, 39.61 (two C). HRMS (ESI) calcd for C₁₆H₁₃Cl₂N [MH⁺]: 290.04978. Found: 290.04888. Anal. Calcd for C₁₆H₁₃Cl₂N: C, 66.22; H, 4.52. Found: C, 66.06; H, 4.70.

11

Only the preferred embodiment of the present invention and examples of its versatility are shown and described in the present disclosure. It is to be understood that the present invention is capable of use in various other combinations and environments and is capable of changes or modifications within the scope of the inventive concept as expressed herein. Thus, for example, those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances, procedures and arrangements described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

What is claimed is:

1. A method of treating colorectal cancer, the method comprising administering to a patient in need of such treatment an effective amount of a compound according to the following formula:



or a pharmaceutically acceptable salt thereof, wherein each of X_1 through X_5 independently represents H, a lower alkyl, or halo, provided that at least one of X_1 through X_5 is a halo; and each of Y_1 through Y_5 independently represents H, a lower alkyl, or NR_1R_2 , provided that at least one of Y_1 through Y_5 is NR_1R_2 , wherein each of R_1 and R_2 independently represents H, or a lower alkyl, provided that at least one of R_1 or R_2 is a lower alkyl.

2. The method of claim 1, wherein at least two of X_1 through X_5 are halo groups.

3. The method of claim 2, wherein X_1 through X_5 are either (i) a fluoro and chloro, (ii) both fluoro, or (iii) both chloro groups.

4. The method of claim 1, wherein both R_1 and R_2 are lower alkyl groups.

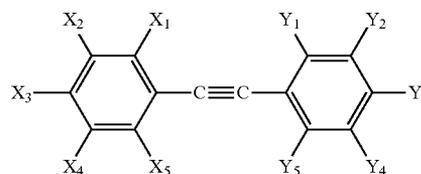
12

5. The method of claim 4, wherein either X_1 or X_5 or both X_1 and X_5 are halo groups and X_2 through X_4 are H or a lower alkyl.

6. The method of claim 1, wherein X_1 and/or X_5 are halo groups, X_2 through X_4 are H or a lower alkyl, Y_3 is NR_1R_2 , and Y_1 , Y_2 , Y_4 , and Y_5 independently represent H or a lower alkyl.

7. The method of claim 1, wherein X_1 and/or X_5 are fluoro and/or chloro and at least one of R_1 or R_2 is a lower alkyl.

8. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to the following formula:



or a pharmaceutically acceptable salt thereof, wherein each of X_1 through X_5 independently represents H, a lower alkyl, a fluoro or a chloro, provided that at least one of X_1 through X_5 is a fluoro or chloro; and each of Y_1 through Y_5 independently represents H, a lower alkyl, or NR_1R_2 , provided that at least one of Y_1 through Y_5 is NR_1R_2 , wherein each of R_1 and R_2 independently represents H, or a lower alkyl, provided that at least one of R_1 or R_2 is a lower alkyl; and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is acceptable to be administered to a mammal.

9. The pharmaceutical composition of claim 8, wherein at least two of X_1 through X_5 are fluoro, or chloro groups and the remaining X_1 through X_5 are H.

10. The pharmaceutical composition of claim 8, wherein X_1 and X_5 are either (i) a fluoro and chloro, (ii) both fluoro, (iii) both chloro groups.

11. The pharmaceutical composition of claim 8, wherein at least one of R_1 , or R_2 , is a lower alkyl.

12. The pharmaceutical composition of claim 11, wherein the lower alkyl is methyl or ethyl.

* * * * *