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ORIGINAL RESEARCH

Synthesis and evaluation of linear furanocoumarins as potential anti-breast and anti-prostate cancer agents

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Abstract A series of 22 furanocoumarin derivatives were synthesized and evaluated for cytotoxicity against breast cancer (MCF-7 and MDA-MB-231) and prostate cancer (PC-3) cell lines along with normal cell line. Several analogs were synthesized by replacing prenyl moiety with alkyl, aromatic, and heteroaromatic functionality to study the structure–activity relationship. Compounds **20** and **22** with adamantoylamino, diprenylamino and substituted benzene sulfonamide substituents showed potent antiproliferative activity in MCF-7 cell line with IC_{50} values of 0.48 and 0.53 μM, respectively. Both the compounds showed higher IC_{50} value in MCF-10A cell lines indicating nontoxicity in normal cell lines.

Keywords Anticancer · Breast cancer · Cytotoxic · Furanocoumarin · Imperatorin · Prostate cancer

Abbreviations

TBAB	Tetrabutylammonium bromide
FBS	Fetal bovine serum
MTT	Methylthiazolyl tetrazolium assay
SAR	Structure–activity relationship
DMEM	Dulbecco's modified Eagle medium
EDTA	Ethylenediaminetetraacetic acid

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Introduction

There were an estimated 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide; of these, 57 % (8 million) of new cancer cases, 65 % (5.3 million) of the cancer deaths, and 48 % (15.6 million) of the 5-year prevalent cancer cases occurred in the less-developed regions. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 million within the next two decades (http://www.who.int/mediacentre/factsheets/fs297/en/). Breast cancer is the top cancer among women, while prostate cancer is the most frequently diagnosed cancer in men and is a second leading cause of cancer morbidity. However, most of the currently used anticancer drugs cause undesirable side effects due to lack of tumor specificity and multidrug resistance.

Therefore, the search for potent, safe, and selective anticancer compounds is crucial for new drug development in cancer research. Over 60 % of the current anticancer drugs have their origin in one way or another from natural sources (Newman and Cragg, 2007). Nature continues to be the most prolific source of biologically active and diverse chemotypes. Natural products, due to their structural diversity, provide excellent templates for the construction of novel compounds (Newman, 2008).

Over the past few years, there has been a growing interest in furanocoumarin derivatives; they are widespread, occurring not only in lemon and lime oils, but also in the medicinal plants such as *Aegle marmelos*, *Angelica dahuricae*, and *Angelica*

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archangelica (Sancho *et al.*, 2004). These compounds have shown potent anti-inflammatory, anti-HIV, and antitumoral activities (Pae *et al.*, 2002; Luo *et al.*, 2011). Linear furanocoumarin derivatives such as imperatorin (**1**), isoimperatorin (**2**) xanthotoxin (**3**), bergapten (**4**), and psoralen (**5**) have been studied for their antiproliferative activities in various human cancer cell lines (Fig. 1) (Sancho *et al.*, 2004; Pae *et al.*, 2002; Luo *et al.*, 2011; Kostova, 2007). Prenylated furanocoumarin derivative imperatorin and isoimperatorin have been thoroughly exploited for their cytotoxic and apoptotic potential (Sancho *et al.*, 2004). Imperatorin (**1**) was found active in breast cancer, epithelial sarcoma, and leukemia cancer cell lines. Recently, Pae *et al.* showed that imperatorin induced cytochrome C-dependent apoptosis in human promyelocytic leukemia, HL-60 cells. Possible mechanisms for anticarcinogenicity of imperatorin (**1**) could be suppression of cell proliferation and induction of apoptosis together with decrease of metabolic activation, increase of detoxification, antioxidative properties, inhibition of DNA adduct formation, and oncogene activation (Pae *et al.*, 2002). Isoimperatorin (**2**) showed potent antiproliferation activity in breast cancer ($IC_{50} = 6.56$ μM, MCF-7) and induced apoptosis in prostate cancer cell line (DU-145) (Pae *et al.*, 2002). Other furanocoumarins including xanthotoxin (**3**), bergapten (**4**), and psoralen (**5**) also showed potent cytotoxic potential against breast cancer and prostate cancer cell lines with IC_{50} value in lower micromolar range (Diwan and Malpathak, 2009). As a part of our continuing efforts to synthesize naturally occurring compounds and their analogs to explore their biological potential and based on these literature reports of potent anticancer activity of furanocoumarin derivatives, 22 linear furanocoumarin derivatives were synthesized and tested in breast cancer and prostate cancer cell lines.

Materials and methods

General

Melting points were recorded on capillary melting point apparatus and used as uncorrected. ^{1}H NMR spectra were recorded on 400 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard, and the chemical shifts are reported in δ units. Mass spectra were recorded on LCMS (APCI/ESI). All chromatographic purifications were performed with silica gel (60–120 mesh), whereas all TLC (silica gel) development was performed on silica gel-coated (Merck Kiesel $60F_{254}$, 0.2-mm thickness) sheets. All chemicals were purchased from Sigma-Aldrich, SD fine chemicals, Lancaster, and CDH. Solvents used for the chemical synthesis purchased from commercial sources were of analytic grade and were used without further purification unless otherwise stated.

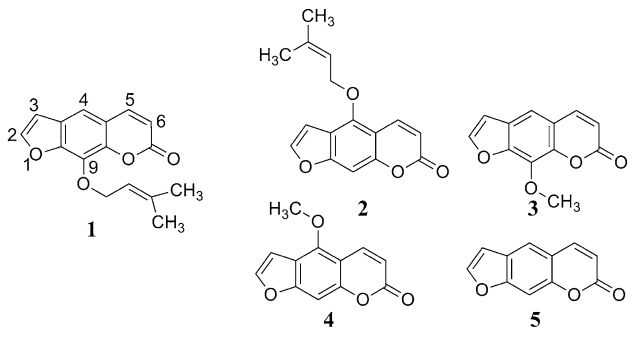


Fig. 1 Anti-HIV and anticancer furanocoumarins from plants

Cell culture

The MCF-7, MDA-MB-231, MCF-10A, and PC-3 cell lines were obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in DMEM containing 10 % FBS and 1 % penicillin–streptomycin. DMEM and FBS were obtained from Sigma chemicals. Antibiotic was purchased from Invitrogen. Cell cultures were maintained in flasks under standard conditions: incubation at 37 °C and 5 % CO_2. All subcultures were used prior to passage 15. Cells were routinely passaged using 0.25 % trypsin/0.1 % EDTA.

Cell viability assay

Cell *via*bility was determined by MTT assay. Cells were plated at 90 % confluence and incubated in the presence or absence of different compounds at various concentrations. After 24-h incubation, cells were treated with MTT solution for 4 h in a CO_2 incubator at 37 °C and 5 % CO_2. MTT which is a tetrazolium salt is converted into insoluble formazan by mitochondrial dehydrogenases in live cells. Formazan is dissolved in DMSO, and absorbance was measured at dual wavelengths of 550 and 630 nm on an ELISA plate spectrophotometer (Biotrek instruments). The total number of viable cells relative to cells in untreated control was calculated.

Chemistry

General method for synthesis of O-alkylated analogs (7–11)

A mixture of xanthotaxol **6** (202 mg, 1 mmol), anhydrous potassium carbonate (207 mg, 1.5 mmol), methyl iodide (124 μL, 2 mmol), and dry acetone (5 mL) was heated to reflux for a period from 4 to 5 h under nitrogen atmosphere, and the reaction was monitored by TLC until the starting material fully reacted. After cooling to room temperature, water was added to the reaction mixture and

extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed, and the residue was purified by flash chromatography to yield **7**. The reactions using allyl chloride (163 µL, 2 mmol), 1-chloro-2-methylpropane (222 µL, 2 mmol), benzyl alcohol (230 µL, 2 mmol), and 1-naphthyl chloride (325 mg, 2 mmol) as alkyl halide yielded compounds **8**, **9**, **10**, and **11**, respectively.

*9-Methoxy-7H-furo[3,2-g][1]benzopyran-7-one (**7**)* Yield: 85 %; cream colored crystalline solid, mp 140–141 °C (Lit. (Kilic *et al.*, 2006) 140–142 °C); UV ($CHCl_3$): λ_{max} (log ε) 252 (3.82), 298 (3.55); IR (neat): ν_{max} 1717, 1587, 1401, 1260, 1275, cm^1; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.77 (d, $J = 7.2$ Hz, 1H), 7.69 (d, $J = 2.1$ Hz, 1H), 7.35 (s, 1H), 6.82 (d, $J = 2.1$ Hz, 1H), 6.36 (d, $J = 7.2$ Hz, 1H), 4.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.5, 147.6, 146.6, 144.4, 142.9, 132.7, 126.1, 116.5, 114.7, 112.9, 106.7, 61.3. CIMS *m/z* 217 $[M+1]^+$

*9-(2-Propen-1-yloxy)-7H-furo[3,2-g][1]benzopyran-7-one (**8**)* Yield: 86 %; mp 82–83 °C [lit. (Li *et al.*, 2011) 80–81 °C]; UV ($CHCl_3$): λ_{max} (log ε) 254 (3.89), 302 (3.90); IR (neat):$\nu\nu_{max}$ 1725, 1623, 1586, 1465, 1400, 1292; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, $J = 9.6$ Hz, 1H), 7.69 (d, $J = 2.4$ Hz, 1H), 7.37 (s, 1H), 6.82 (d, $J = 2.4$ Hz, 1H), 6.37 (d, $J = 9.6$ Hz, 1H), 6.15 (m, 1H), 5.43 (dd, $J = 1.5$ Hz, $J = 17$ Hz, 1H), 5.20 (dd, $J = 1.2$ Hz, $J = 10.3$ Hz, 1H), 5.02 (d, $J = 3.6$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.5, 148.3, 146.6, 144.4, 143.5, 133.3, 131.3, 125.9, 118.9, 116.5, 114.7, 113.4, 106.8, 74.3; CIMS *m/z* 243.2 $[M+1]^+$

*9-(3-Methylpropoxy)-7H-furo[3,2-g][1]benzopyran-7-one (**9**) (IP N.Y.-Y. et al., 2011)* Yield: 87 %; mp 155–156 °C; UV ($CHCl_3$): λ_{max} (log ε) 252 (4.41), 301 (4.24); IR (neat): ν_{max} 2960, 1728, 1626, 1588, 1466, 1440, 1293; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, $J = 9.6$ Hz, 1H), 7.68 (d, $J = 2.4$ Hz, 1H), 7.34 (s, 1H), 6.81 (d, $J = 2.4$ Hz, 1H), 6.36 (d, $J = 9.6$ Hz, 1H), 4.25 (d, $J = 6.8$ Hz, 2H) 2.17 (m, 1H), 1.10 (d, $J = 6.8$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 148.1, 146.6, 144.3, 143.4, 132.3, 125.9, 116.5, 114.7, 112.8, 106.7, 80.4, 29.1, 19.0 CIMS *m/z* 259.1 $[M+1]^+$.

*9-(Phenylmethoxy)-7H-furo[3,2-g][1]benzopyran-7-one (**10**)* Yield: 71 %; mp 117–118 °C [lit. (Deng *et al.*, 2010) 120–122 °C]; UV ($CHCl_3$): λ_{max} (log ε) 255 (4.44), 299 (4.12); IR (neat): ν_{max} 2961, 1722, 1625, 1582, 1460, 1431, 1288; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, $J = 9.6$ Hz, 1H), 7.67 (d, $J = 2.4$ Hz, 1H), 7.56 (s, 1H), 7.55 (s, 1H), 7.27–7.37 (m, 4H), 6.79 (d, $J = 2.4$ Hz, 1H), 6.33 (d, $J = 9.6$ Hz, 1H), 5.53 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.5, 148.2, 146.7, 144.4, 143.5, 136.7, 131.3, 128.8, 128.4, 128.3, 128.1, 126.6, 116.4, 114.6, 113.9, 113.5, 106.7, 75.2; CIMS *m/z* 293 $[M+1]^+$.

*9-Naphthyloxy-7H-furo[3,2-g][1]benzopyran-7-one (**11**)* Yield: 73 %; mp 147–148 °C; UV ($CHCl_3$): λ_{max} (log ε) 256 (4.33), 301 (3.88); IR (neat): ν_{max} 2961, 1722, 1625, 1582, 1460, 1431, 1288; ^{1}H NMR (400 MHz, $CDCl_3$): δ, 8.77 (d, $J = 7.2$ Hz, 1H), 8.88 (d, $J = 8.6$ Hz, 1H), 8.11 (d, $J = 8.0$ Hz, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.88 (d, $J = 9.6$ Hz, 1H), 7.72 (d, $J = 2.0$ Hz, 1H), 7.45–7.65 (m, 4H), 6.83 (d, $J = 2.0$ Hz, 1H), 6.42 (d, $J = 9.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): 164.0, 148.7, 147.3, 144.1, 144.0, 135.5, 132.1, 131.9, 128.8, 128.4, 126.7, 125.8, 125.6, 124.8, 124.6, 124.4, 123.2, 117.0, 116.4, 115.0, 106.9; CIMS *m/z* 329 $[M+1]^+$.

*General method for synthesis of O-acylated analogs (**12** and **13**)*

A mixture of xanthotaxol (**6**) (202 mg, 1 mmol), pyridine (40 µL, 0.5 mmol), 1-naphthoyl chloride (180 µL, 1.2 mmol) for compound **12**, and anhydrous dichloromethane (5 mL) was stirred under nitrogen atmosphere and refluxed for 3 h. Reaction was monitored by TLC until the starting material fully reacted. After cooling to room temperature, the reaction mixture was filtered and washed with acetone. The solvent was removed on a rotary evaporator, and the crude product was purified by flash chromatography to give **12**. Similar reaction using 2-mercaptobenzoyl chloride (206 mg, 1.2 mmol) gave compound **13**.

*9-Naphthoyloxy-7H-furo[3,2-g][1]benzopyran-7-one (**12**)* Yield: 91 %; mp 162–163 °C; UV ($CHCl_3$): λ_{max} (log ε) 245 (4.31), 303 (4.08); IR (neat): ν_{max}1730, 1674, 1592, 1512, 1259, 1147, 1061 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.07 (d, $J = 8.4$ Hz, 1H), 8.70 (d, $J = 7.2$ Hz, 1H), 8.16 (d, $J = 8.0$ Hz, 1H), 7.96 (d, $J = 8.0$ Hz, 1H), 7.82 (d, $J = 9.6$ Hz, 1H), 7.71 (d, $J = 2.0$ Hz, 1H), 7.55–7.70 (m, 4H), 6.89 (d, $J = 2.0$ Hz, 1H), 6.40 (d, $J = 9.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): 164.0, 159.8, 148.5, 147.4, 144.2, 144.0, 135.5, 132.3, 131.9, 128.8, 128.5, 126.3, 125.4, 125.8, 124.5, 124.5, 124.5, 123.3, 117.1, 116.1, 114.0, 106.8; CIMS *m/z* 357 $[M+1]^+$; Anal. Calcd for $C_{22}H_{12}O_5$: C, 74.16; H, 3.39; Found: C, 74.09; H, 3.29.

*9-(2-Thienoyloxy)-7H-furo[3,2-g][1]benzopyran-7-one (**13**)* Yield: 83 %; mp 188–189 °C; UV ($CHCl_3$): λ_{max} (log ε) 250 (4.55), 282 (4.39); IR (neat): ν_{max} 1735, 1638, 1412, 1260, 1275, 1142, 1082 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, $J = 2$ Hz, 1H), 7.81 (d, $J = 9.6$ Hz, 1H), 7.75 (d, $J = 4.8$ Hz, 1H), 7.69 (s, 1H), 7.60 (s, 1H), 7.

23 (t, $J = 6$ Hz, 1H), 6.87 (s, 1H), 6.38 (d, $J = 9.6$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.8, 158.9, 148.6, 147.3, 144.0, 135.9, 135.1, 134.5, 131.0, 128.2, 125.8, 122.8, 117.2, 116.3, 114.9, 106.9; CIMS *m/z* 313.3 $[M+1]^+$; Anal. Calcd for $C_{16}H_8O_5S$: C, 61.53; H, 2.58; S, 10.27; Found C, 61.48; H, 2.46; S, 10.13.

*General procedure for synthesis of N-alkyl analogs (**18–19**)*

A mixture of **17** (231 mg, 1 mmol), potassium carbonate (207 mg, 1.5 mmol), allyl chloride (97 µL, 1.2 mmol), and anhydrous acetone (5 mL) was stirred under a nitrogen atmosphere at reflux for 3 h. After cooling to room temperature, the reaction mixture was filtered and washed with acetone. The solvent was removed on a rotary evaporator, and the crude product was purified by flash chromatography to give compound **18** (Zhang *et al.*, 2010). Similar reaction using benzyl chloride (138 µL, 1.2 mmol) yielded compound **19**.

*9-Methoxy-4-(2-propenylamino)-7H-furo[3,2-g][1]benzopyran-7-one (**18**) (Abdel-Hafez and Farrag, 1994)* Yield: 75 %; mp 239–240 °C; UV ($CHCl_3$): λ_{max} (log ε) 300 (3.77); IR (neat): ν_{max} 3426, 2958, 1732, 1591, 1470, 1275, 1147 cm^{-1}; ^{1}H NMR (400 MHz, CDCl3): δ 7.90 (d, $J =$ 10 Hz, 1H), 7.58 (d, $J = 2.4$ Hz, 1H), 6.89 (d, $J = 2.4$ Hz, 1H), 6.23 (d, $J = 10$ Hz, 1H), 6.01–6.06 (m, 1H), 5.34 (dd, $J = 14$ Hz, $J = 2.8$ Hz, 1H), 5.24 (dd, $J = 12$ Hz, $J = 1.3$ Hz, 1H), 4.12 (s, 3H), 4.08 (d, $J = 5.6$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 150.2, 144.6, 144.4, 138.9, 135.0, 134.3, 126.9, 117.3, 114.4, 111.2, 105.8, 104.7, 61.7, 50.7; CIMS *m/z* 272.3 $[M+1]^+$.

*9-Methoxy-4-(benzylamino)-7H-furo[3,2-g][1]benzopyran-7-one (**19**) (Abdel-Hafez and Farrag, 1994)* Yield: 71 %; mp 201–203 °C; UV ($CHCl_3$): λ_{max} (log ε) 297 (4.05); IR (neat): ν_{max} 3432, 1725, 1580, 1389, 1138, 1021 cm^{-1}; ^{1}H NMR (400 MHz, CDCl3): δ 7.83 (d, $J = 10.0$ Hz, 1H), 7.54 (d, $J = 2.4$ Hz, 1H), 7.32–7.73 (m, 5H), 6.82 (d, $J = 2.4$ Hz, 1H), 6.19 (d, $J = 10.0$ Hz, 1H), 4.64 (s, 2H), 4.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 150.3, 144.7, 144.4, 138.8, 134.4, 129.0, 127.9, 127.5, 126.5, 126.2, 114.4, 111.2, 105.9, 104.6, 61.7, 52.5; CIMS *m/z* 322.3 $[M+1]^+$.

*General procedure for synthesis of N-acyl analogs (**20** and **21**)*

A mixture of **17** (231 mg, 1 mmol), potassium carbonate (207 mg, 1.5 mmol), 1-adamantoyl chloride (238 mg, 1.2 mmol), and anhydrous dichloromethane (5 mL) was stirred under a nitrogen atmosphere at reflux for 3 h. After cooling to room temperature, the reaction mixture was filtered and washed with acetone. The solvent was removed on a rotary evaporator, and the crude product was purified by flash chromatography to give **20**. Reaction using 1-naphthoyl chloride (180 µL, 1.2 mmol) gave compound **21**.

*N-(9-Methoxy-7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl)-adamantanamide (**20**)* Yield: 77 %; mp 193–194 °C; UV ($CHCl_3$): λ_{max} (log ε) 264 (3.68); IR (neat): ν_{max} 3428, 2908, 2851, 1735, 1634, 1480, 1260, 1137, 1041 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, $J = 9.6$ Hz, 1H), 7.63 (s, 1H), 7.47 (s, 1H), 6.62 (s, 1H), 6.31 (d, $J = 9.6$ Hz, 1H), 4.25 (s, 3H), 2.16 (s, 3H), 2.06 (s, 6H), 1.82 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 177.3, 160.0, 147.4, 146.3, 143.1, 139.6, 131.8, 123.7, 119.3, 114.5, 113.04, 105.0, 61.4, 41.5, 39.2, 36.3, 28.1; CIMS *m/z* 394.2 $[M+1]^+$. Anal. Calcd for $C_{23}H_{23}NO_5$: C, 70.21; H, 5.89; N, 3.56; Found: C, 69.91; H, 5.81; N, 3.46.

*N-(9-Methoxy-7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl)-naphthamide (**21**)* Yield: 89 %; mp 256–258 °C; UV ($CHCl_3$): λ_{max} (log ε) 292 (4.22), 306 (3.77); IR (neat): ν_{max} 3401, 1770, 1707, 1593, 1509, 1275, 1260, 1171, 1060 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.14 (d, $J = 8.4$ Hz, 2H), 8.44 (d, $J = 6.8$ Hz, 2H), 8.15 (d, $J = 8.4$ Hz, 2H), 7.95 (d, $J = 8.0$ Hz, 2H), 7.71 (t, $J = 7.2$ Hz, 2H), 7.54–7.63 (m, 2H), 4.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.9, 135.5, 133.9, 132.1, 131.9, 131.5, 128.8, 128.8, 126.7, 125.6, 124.9, 124.4, 61.2; CIMS *m/z* 385.1 $[M+1]^+$; Anal. Calcd for $C_{23}H_{15}NO_5$: C, 71.68; H, 3.92; N, 3.63; Found: C, 71.56; H, 3.88; N, 3.55.

*General procedure for synthesis of N,N-dialkyl analogs (**22** and **23**)*

A mixture of **17** (231 mg, 1 mmol), potassium carbonate (207 mg, 1.5 mmol), 1-chloro-3-methyl-2-butene (281 µL, 2.5 mmol), and anhydrous acetone (5 mL) was stirred under a nitrogen atmosphere at reflux for 3 h. After cooling to room temperature, the reaction mixture was filtered and washed with acetone. The solvent was removed on a rotary evaporator, and the crude product was purified by flash chromatography to give **22**. Reaction using benzyl chloride (287 µL, 2.5 mmol) gave compound **23**.

*9-Methoxy-4-[di-(3-methyl-2-butenyl)amino]-7H-furo[3,2-g][1]benzopyran-7-one (**22**)* Yield: 86 %; IR (neat): ν_{max} 3426, 2958, 2959, 2928, 1732, 1591, 1470, 1376, 1421, 1275, 1139, 1073 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, $J = 9.6$ Hz, 1H), 7.63 (d, $J = 2.4$ Hz, 1H), 6.96 (d, $J = 2.4$ Hz, 1H), 6.28 (d, $J = 9.6$ Hz, 1H), 5.14 (m, 2H),

4.25 (s, 3H), 3.74 (d, $J = 7.2$ Hz, 4H), 1.62 (s, 6H), 1.49 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.9, 148.7, 145.0, 143.6, 142.3, 139.1, 136.7, 135.5, 129.9, 122.9, 122.2, 121.1, 114.6, 112.7, 107.5, 106.3, 61.5, 52.0, 29.4, 25.7, 17.8; CIMS *m/z* 368.4 $[M+1]^+$; Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81; Found: C, 71.83; H, 6.75; N, 3.73.

*9-Methoxy-4-[di-(benzyl)amino]-7H-furo[3,2-g][1]benzopyran-7-one (**23**)* Yield: 71 %; UV ($CHCl_3$): λ_{max} (log ε) 261 (4.46); IR (neat): ν_{max} 3429, 1729, 1634 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, $J = 9.6$ Hz, 1H), 7.59 (s, 1H), 7.27 (m, 6H), 7.14 (s, 4H), 6.67 (s, 1H), 6.25 (d, $J = 9.6$ Hz, 1H), 4.29 (s, 4H), 4.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.6, 145.2, 143.5, 141.4, 137.6, 135.6, 130.3, 129.0 (5C), 128.4 (4C), 127.5 (3C), 123.3, 114.2, 113.2, 106.1, 61.5, 58.3 (2C); CIMS *m/z* 412.3 $[M+1]^+$; Anal. Calcd for $C_{26}H_{21}NO_4$: C, 75.90; H, 5.14; N, 3.40; Found, C, 75.95; H, 5.10; N, 3.34.

*General procedure for synthesis of N-sulfonyl analogs (**25–28**)*

A mixture of **24** (1 mmol), 4-fluoroaniline (94 µL, 1 mmol), and pyridine (0.05 mL), and anhydrous toluene (3 mL) was heated to reflux and stirred for 40 min under a nitrogen atmosphere. After completion of reaction, water (10 mL) was added to the mixture, followed by extraction with ethyl acetate (3 × 10 mL). The combined organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4. The organic phase was evaporated in vacuo, and the residue was purified by silica gel flash chromatography to yield **25**. Reaction using 3,4-difluoroaniline (99 µL, 1 mmol), *p*-anisidine (123 mg, 1 mmol), and cyclohexylamine (114 µL, 1 mmol) yielded compounds **26**, **27** and **28**, respectively.

*9-Methoxy-7-oxo-N-(4-fluorophenyl)-7H-furo[3,2-g][1]benzopyran-4-sulfonamide (**25**)* Yield: 87 %; mp 166–167 °C; UV ($CHCl_3$): λ_{max} (log ε) 260 (3.86); IR (neat): ν_{max} 1739, 1370, 1150, 1240, 1021 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ 8.78 (d, $J = 9.6$ Hz, 1H), 7.75 (d, $J = 2.4$ Hz, 1H), 7.38 (d, $J = 2.4$ Hz, 1H), 6.94 (m, 2H), 6.84 (m, 2H), 6.45 (d, $J = 9.6$ Hz, 1H), 4.40 (s, 3H); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz): δ 163.4, 152.0, 149.1, 146.5, 144.6, 140.5, 135.9, 131.8, 128.8, 128.7, 122.7, 120.0, 119.9, 119.7, 119.6, 118.6, 111.7, 65.1; CIMS *m/z* 390.1 $[M+1]^+$. Anal. Calcd for $C_{18}H_{12}FNO_6S$: C, 55.53; H, 3.11; N, 3.60; S, 8.24; Found: C, 55.43; H, 3.14; N, 3.63; S, 8.20.

*9-Methoxy-7-oxo-N-(3,4-difluorophenyl)-7H-furo[3,2-g][1]benzopyran-4-sulfonamide (**26**)* Yield: 85 %; mp 188–189 °C; UV ($CHCl_3$): λ_{max} (log ε) 260 (3.93), 309 (3.63); IR (neat): ν_{max} 3432, 1735, 1587, 1372, 1147, 1097 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (d, $J = 9.6$ Hz, 1H), 8.26 (s, 1H), 7.35 (s, 1H), 7.23 (q, 1H), 6.98 (t, $J = 10$, 1H) 6.73 (d, $J = 8.4$ Hz, 1H), 6.68 (d, $J = 9.6$ Hz, 1H), 4.31 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.1, 149.8, 144.7, 142.5, 139.7, 135.7, 127.1, 118.3, 118.1, 117.9, 117.8, 116.7, 114.3, 110.4, 110.2, 107.0, 61.1; CIMS *m/z* 408.2 $[M+1]^+$. Anal. Calcd for $C_{18}H_{11}F_2NO_6S$: C, 53.07; H, 2.72; N, 3.44; S, 7.87; Found: C, 52.95; H, 2.75; N, 3.41; S, 7.82.

*9-Methoxy-7-oxo-N-(4-methoxybenzyl)-7H-furo[3,2-g][1]benzopyran-4-sulfonamide (**27**)* Yield: 89 %; mp 187–189 °C; UV ($CHCl_3$): λ_{max} (log ε) 260 (4.13), 310 (3.83); IR (neat): ν_{max} 3399, 2951, 1739, 1275, 1033 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (d, $J = 10.0$ Hz, 1H), 8.63 (broad singlet, 1H), 8.23 (s, 1H), 7.40 (s, 1H), 6.78 (d, $J = 7.6$ Hz, 2H), 6.61 (d, $J = 9.0$ Hz, 1H), 6.51 (d, $J = 7.6$ Hz, 2H), 4.25 (s, 3H), 3.95 (d, $J = 4.72$, 2H), 3.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.3, 158.1, 149.1, 145.1, 142.5, 140.6, 134.8, 128.5 (2C), 128.3, 126.3, 121.2, 115.9, 113.8, 112.9 (2C), 107.4, 61.0, 54.9, 45.2; CIMS *m/z* 416.3 $[M+1]^+$; Calcd, C, 57.82; H, 4.12; N, 3.37; Found C, 57.78; H, 4.02; N, 3.43.

*9-Methoxy-7-oxo-N-(cyclohexyl)-7H-furo[3,2-g][1]benzopyran-4-sulfonamide (**28**)* Yield: 73 %; mp 212–214 °C; UV ($CHCl_3$): λ_{max} (log ε) 308 (4.23), 260 (4.49); IR (neat): ν_{max} 3418, 2923, 1737, 1370, 1152, 1241, 1021 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (d, $J = 10.4$ Hz, 1H), 7.80 (s, 1H), 7.54 (s, 1H), 6.55 (d, $J = 10.4$ Hz, 1H), 4.44 (s, 3H), 3.11 (broad singlet, 1H), 1.56–1.65 (m, 4H), 1.48 (d, 1H), 1.05–1.20 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.8, 148.0, 145.5, 143.1, 140.3, 136.2, 127.3, 120.6, 116.7, 114.5, 107.8, 61.3, 52.7, 33.8, 31.5, 24.9, 24.5, 22.6; CIMS *m/z* 378.7 $[M+1]^+$; Anal. Calcd for $C_{18}H_{19}NO_6S$: C, 57.28; H, 5.07; N, 3.71; Found: C, 57.01; H, 4.92; N, 3.88.

Results and discussion

Chemistry

Several linear furanocoumarins including ten new compounds were synthesized by substituting the parent nucleus at C-4 and C-9 positions with alkyl, aromatic, heteroaromatic, and substituted benzene sulfonamide functionalities (Schemes 1, 2, 3).

Synthesis of O-alkylated and O-acylated analogs

In this series, prenyl moiety of the parent compound **1** was replaced with linear alkyl chains and heteroaromatic groups. In order to synthesize *O*-alkylated and *O*-acylated

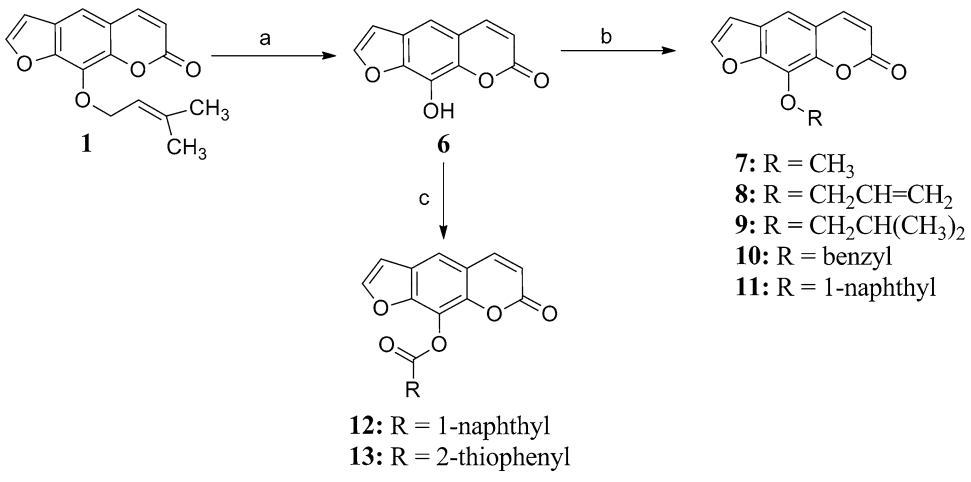
analogs of imperatorin (**1**), deprenylation of **1** was attempted with 20 mol% of 1:1 mixture of zirconium chloride and sodium iodide in acetonitrile at reflux temperature to yield xanthotaxol (**6**) (Sharma *et al.*, 2003). This deprenylation methodology was further simplified using 3 M HCl in methanol–water system to yield the desired product xanthotaxol (**6**) in quantitative yield (Scheme 1) (Waight *et al.*, 1987). The free hydroxyl of **6** was substituted with various alkyl and acyl moieties to study the effects of chain length and ring size on activity. Reaction of **6** with various alkyl and aryl halides in the presence of K_2CO_3 in acetonitrile or acetone gave the desired compounds (**7–11**) in 80–85 % yield (Scheme 1). Similarly, reaction of **6** with aromatic/heteroaromatic acyl chlorides in the presence of catalytic amount of pyridine in dichloromethane yielded the desired compounds (**12** and **13**).

Synthesis of N-alkylated and N-acylated analogs

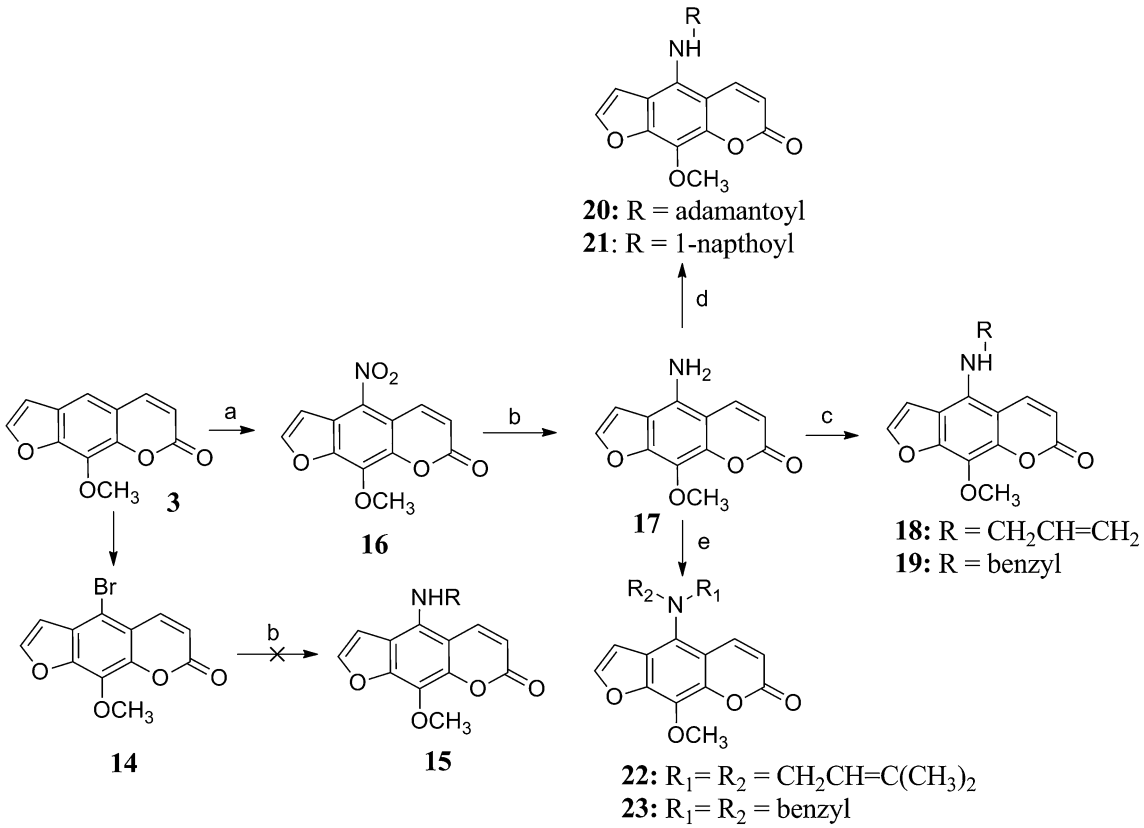
A few linear furanocoumarins were synthesized by substituting the C-4 position of basic psoralen nucleus with different aromatic or alkyl amine moieties. Synthetic strategy for this series of compounds involves introduction of bromine at the C-4 position of the nucleus and nucleophilic replacement of bromine moiety with the help of various alkyl or aromatic amines. Xanthotoxin (**3**) was treated with bromine water in acetic acid to yield the bromo derivative **14** (Zhang *et al.*, 2010). Next, the nucleophilic replacement of the bromine moiety of **14** with amines was investigated using C–N coupling agents such as CuI or palladium (II) acetate/DPPE. All the attempts to couple aromatic amines to **14** were unsuccessful. Hence, we adopted an alternative synthetic strategy by introducing amine functionality at the C-4 position via reduction of nitro group in the furanocoumarin nucleus. Various aliphatic and aryl acyl substitutions were carried out in the presence of suitable base to get the desired *N*-alkylated compounds (**18–21**) (Zhang *et al.*, 2010). Nitration of xanthotoxin (**3**) using combination of acetic acid and nitric acid resulted into formation of 9-methoxy-4-nitro-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**16**) which on further reaction with tin metal in concentrated HCl gave 4-amino-9-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**17**) (Zhang *et al.*, 2010). Different aliphatic and aryl substitutions on **17** were carried out using K_2CO_3 in acetonitrile or acetone to yield the desired analogs (**18–21**) (Scheme 3). The intermediate 4-amino-9-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one (**17**) was treated with excess of alkyl and aryl chlorides in the presence of K_2CO_3 in acetonitrile or acetone to provide *N, N*-dialkyl derivatives (**22** and **23**) (Scheme 2).

Synthesis of C-4-substituted sulfonamide analogs

Sulfonamides are reported to possess potent anticancer activity and representatives of this class of pharmacological agents are widely used in clinic as antibacterial (Chohan *et al.*, 2005), hypoglycemic, diuretic (Aumuller *et al.* 1966), antihypertensive (Takenaka *et al.*, 1982) and antiviral drugs (Scozzafava *et al.*, 2003). All these derivatives incorporate in their molecules a common chemical motif of aromatic/heterocyclic sulfonamide. There are a variety of mechanisms of their antitumor action, such as inhibition of tubulin polymerization, cell cycle perturbation in the G1 phase, carbonic anhydrase (CA) inhibition, functional suppression of the transcriptional activator NF-Y, angiogenesis (matrix metalloproteinase or integrin a2) inhibition, and so on (Scozzafava *et al.*, 2003). Hence, based on these literature reports, in this series, we have incorporated sulfonamide moiety at the C-4 position of psoralen nucleus. These sulfonamide analogs were synthesized in two steps.

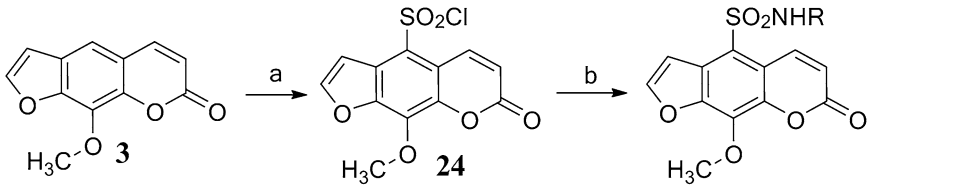


Scheme 1 *a* MeOH/H_2O, 3 M HCl, quantitative yield *b* R-X, K_2CO_3, acetone, 80–85 % yield *c* pyridine, CH_2Cl_2, RCOCl, 85–92 % yield



Scheme 2 *a* CH_3COOH, HNO_3, 95 % yield *b* Sn, conc HCl, 70 % yield *c* R-Cl, K_2CO_3, acetone, 80–85 % yield *d* R-COCl, pyridine, CH_2Cl_2, 85–92 % yield. *e* R-Cl, K_2CO_3, acetone, 71–86 % yield

Scheme 3 *a* Chlorosulfonic acid, $CHCl_3$, 0 °C, 95 % yield *b* R-NH_2, pyridine, toluene, 73–89 % yield



In the first step, chlorosulfonation was carried using excess of chlorosulfonic acid in chloroform to yield the intermediate sulfonyl chloride derivative (**24**) (Zhang *et al.*, 2010). This intermediate was further reacted with various aromatic and cycloaliphatic amines in the presence of pyridine to yield the desired C-4-substituted sulfonamide analogs (**25**–**28**) (Scheme 3) (Zhang *et al.*, 2010).

Anticancer activity of imperatorin analogs

All the synthesized compounds were evaluated for anticancer activity in breast cancer cell lines (MCF-7 and MDA-MB-231) and prostate cancer (PC-3) cell line. The IC_{50} values of the compounds are given in Table 1.

In order to establish structure–activity relationships in linear furanocoumarins for anticancer activity, several new and known furanocoumarins were synthesized varying the substitution on free hydroxyl of xanthotoxin with different alkyl and acyl substituents. The idea was to see the effects of the length and the size of alkyl and acyl chains on anticancer activity. Simple C-9 alkyl-substituted analogs such as compounds **7**–**9** showed increased activity than the parent nucleus of imperatorin. Similarly, compound **10** with benzyloxy substitution at the C-9 position showed

enhanced activity. Neither of the two *O*-acylated derivatives was active. The anticancer potential of *N*-alkyl derivatives of linear furanocoumarins is not explored in the literature.

Table 1 Cytotoxicity (IC_{50} in μM) of linear furanocoumarin derivatives against breast cancer and prostate cancer cell lines and normal cell lines

Comp. No.	MCF-7[a]	MDA-MB-231[b]	PC-3[c]	MCF^{-}10A[d]
1	100	100	100	ND
6	>100	100	100	ND
7	20	>30	25	ND
8	12	10	15	100
9	8.15	10	7	>100
10	7.07	8.5	10	>100
11	>100	100	100	ND
12	100	100	100	ND
13	>100	100	100	ND
14	5.69	6	8	>100
16	>100	100	90	ND
17	>100	100	75	ND
18	3.87	5	10	>100
19	1.09	3	5	>100
20	0.48	2	5	96
21	>100	100	100	ND
22	0.53	1	1	92
23	5	>15	19	>100
24	>100	100	100	ND
25	>100	100	100	ND
26	100	100	100	ND
27	100	100	100	ND
28	>100	100	100	ND
Tamoxifen citrate	4.6	1.6	1.6	>100

[a] Estrogenic breast cancer cell lines

[b] Non-estrogenic breast cancer cell lines

[c] Prostate cancer cell lines

[d] Normal cell lines

We synthesized *N*-alkyl and *N, N*-dialkyl derivatives (substitution at C-4) with similar functionalities as in compounds **7**–**11**. *N*-alkylated (C-4 substituted) derivatives of linear furanocoumarins showed better antiproliferative effect compared to *O*-alkyl derivatives (C-9 substituted). Compounds **20** and **22** with *N*-adamantoylamino and *N,N*-diprenylamino substituents showed the most potent activity in MCF-7 cell line with IC_{50} values of 0.48 and 0.53 μM, respectively. Similarly, other *N*-alkyl derivatives such as **18** and **19** also showed potent activity. The compound **22** with diprenylamino substituent showed better activity compared to parent compound imperatorin. The presence of two prenyl moieties in compound **22** may be responsible for the enhanced potency. The compounds with sulfonamide group were inactive. In general, the compounds showing activity in estrogenic cell line (MCF-7) were also active in non-estrogenic cell line (MDA-MB-231) and prostate cancer cell line (PC-3). Seven out of 22 compounds showed enhanced or similar potency compared to the standard tamoxifen citrate. Compounds **20** and **22** were found almost nine times more potent than the standard. All the active compounds were nontoxic in normal cell lines, which indicates their specificity for cancer cells. This structure–activity relationship study suggested the basic pharmacophore responsible for activity would be a 4-amino-9-methoxyfuranocoumarin with aliphatic substituents on amino group preferably with adamantoyl or diprenyl groups (Fig. 2).

Conclusion

In all, 22 linear furanocoumarin analogs of imperatorin have been synthesized and evaluated for their anticancer activity in breast cancer (MCF-7 and MDA-MB-231) and prostate cancer (PC-3) cell lines. Structure–activity relationship was established by synthesizing *O*-alkylated and

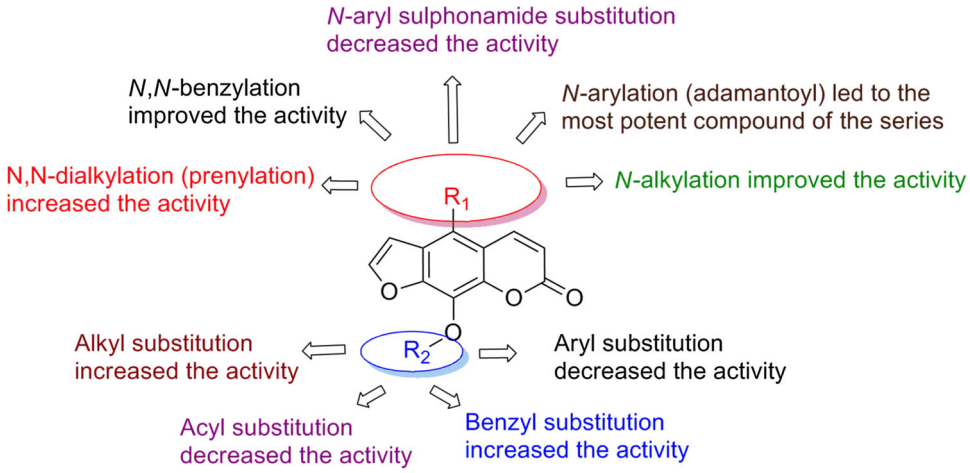


Fig. 2 Structure–activity relationship of linear furanocoumarins

acylated analogs, *N*-alkylated, and acylated and C-4-substituted benzene sulfonamide analogs of basic psoralen nucleus. Compounds **20** and **22** with adamantoylamino and diprenylamino substituents showed the most potent activity in breast cancer (MCF-7) cell lines with IC_{50} values of 0.48 and 0.53 μM, respectively. These two compounds could be a potential lead for further development of newer anticancer agents.

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Conflict of interest Authors declared no conflict of interest.

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