

Synthesis, characterization, crystal structure and cytotoxicities of 2-aryl-3-aryl-5*H*-furo[3,2-*g*]chromene derivatives

Shi-Hui Wang,^a Yan Wang,^a Yu-Ying Zhu,^a Jian Han,^a Yi-Fan Zhou,^a Diwa Koirala,^b
Da-Wei Li,^b and Chun Hu^{a*}

^aKey Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, China
Department of Organic Chemistry, School of Pharmaceutical Engineering,
Shenyang Pharmaceutical University, Shenyang 110016, China

^bSchool of Pharmacy, Shanghai Jiao Tong University, Shanghai, 300193, China
E-mail: chunhu@syphu.edu.cn

DOI: <http://dx.doi.org/10.3998/ark.5550190.0011.b17>

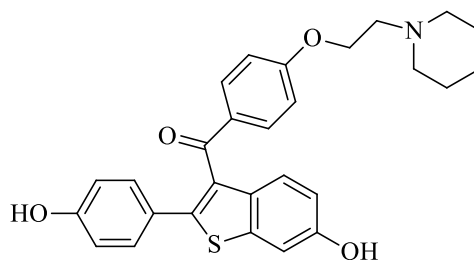
Abstract

Five 2-aryl-3-aryl-5*H*-furo[3,2-*g*]chromene derivatives have been synthesized, and their structures were characterized by IR, NMR, ESI-MS and elemental analysis. The crystal structure for 2-benzoyl-3-(4-methoxyphenyl)-6,7-dihydro-5*H*-furo[3,2-*g*]chromene has been determined by single-crystal X-ray diffraction. X-ray analysis reveals that the pyran ring adopts a half-chair conformation, while the fused furo[3,2-*g*]chromene ring is approximately coplanar with a slight distortion. The preliminary pharmacological test showed all target compounds exhibit cytotoxicities against the U2OS-EGFP-4F12G cell line.

Keywords: Synthesis; characterization; crystal structure; furo[3,2-*g*]chromene; cytotoxicity

Introduction

Selective estrogen receptor modulators (SERMs) are of great value in the treatment of various estrogen dependent diseases such as breast cancer, osteoporosis, Alzheimer's disease, and coronary heart disease. Tamoxifen is being used frequently as a drug for the prevention and treatment of breast cancer.¹ Raloxifene, another SERM, is commonly used in the prevention and treatment of osteoporosis.² However, the clinical usefulness of marketed SERMs is often accompanied by adverse effects such as increased incidence of endometrial cancer and hot flashes,^{3,4} therefore, it is necessary and urgent to find more effective SERMs.



Raloxifene

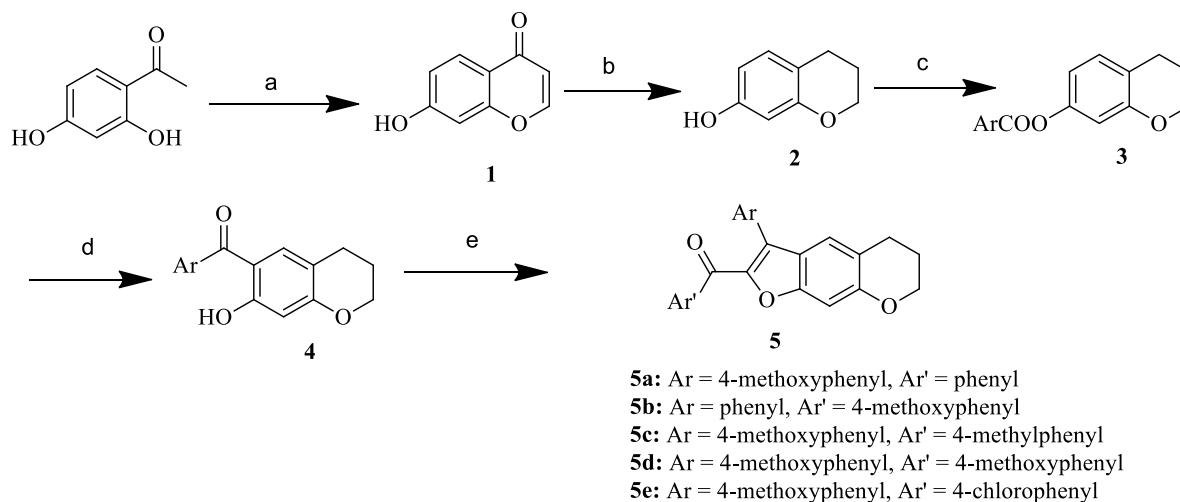
As known, the furochromene ring is an interesting structure for its various biological activities including anticancer,⁵ antitumor,⁶ and antimycobacterial activity.⁷ 2-Aroylbenzofuran derivatives exhibit selective cytotoxicity against a tumorigenic cell line.⁸ By use of a panel of 24 different human tumor cell lines, mean IC₅₀ values of the most potent 2-arylbenzofurans with N-hydroxyacrylamide is 0.75 μM.⁹ According to the structure-activity relationship of raloxifene¹⁰⁻¹² and the principle of bioisosterism and hybridization, based on replacing the central core of the benzothiophene in raloxifene structure with a furo[3,2-*g*]chromene moiety, a series of 2-aryl-3-aryl-5*H*-furo[3,2-*g*]chromene derivatives were designed and synthesized as the target compounds.

The target compounds were characterized by IR, NMR, ESI-MS and elemental analysis. Fortunately, a single crystal of **5a** was grown, and the structure was characterized by X-ray diffraction analysis. The preliminary pharmacological test showed all target compounds exhibit cytotoxicities against U2OS-EGFP-4F12G cell line.

Results and Discussion

Chemistry

The synthesis of the target is described in Scheme 1, 2',4'-dihydroxyacetophenone was easily converted into 7-hydroxychromone **1**, subsequently the catalytic hydrogenation of **1** resulted in 7-hydroxychroman **2**, then 6-aryl-7-hydroxychroman **4** was synthesized by esterification and Fries arrangement. Many reagents, for example, stannic chloride, titanium tetrachloride, and aluminum chloride were explored as catalyst to optimize this Fries arrangement reaction: stannic chloride was found to be the most efficient reagent for the preparation of the rearranged product **4**. The condensation of **4** with substituted phenacyl chlorides led to 2-aryl-3-aryl-6,7-dihydro-5*H*-furo[3,2-*g*]chromene derivatives **5**.



Scheme 1. Synthesis route of the target compounds. Reagents and conditions: a: $\text{CH}(\text{OEt})_3$, 70% HClO_4 , RT, 85%; b: H_2 , 10% Pd-C, reflux, 95%; c: ArCOCl , RT, 91-96%; d: SnCl_4 , reflux, 80-85%; e: $\text{Ar}'\text{COCH}_2\text{Cl}$, 30% K_2CO_3 , TBAB, CH_2Cl_2 , reflux, 72-83%.

Compounds **4** were synthesized by Fries rearrangement with stannic chloride as the solvent and catalyst. The two single peaks at $\delta = 6.43$ and 7.30 ppm in the proton NMR indicated the rearrangement may occur at the C6 position of the chroman ring, because the two protons do not couple. When the rearrangement occurred at the C8 position of the chroman ring, the two protons must couple. So we speculated that the product of the rearrangement reaction should be 6-aryl-7-hydroxychroman derivatives. In order to confirm the structures of the products, a single crystal of **5a** was grown for X-ray diffraction analysis. X-ray analysis reveals that our speculation was correct. The molecular structure of the target compound **5a** is shown in Figure 1, and the crystal packing of the target compound **5a** in Figure 2.

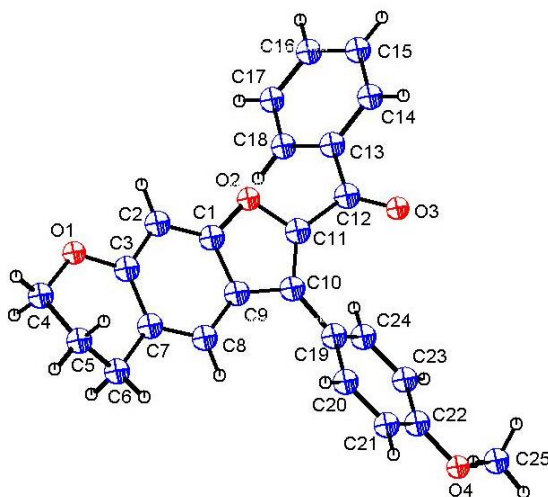


Figure 1. Structure of the target compound **5a**.

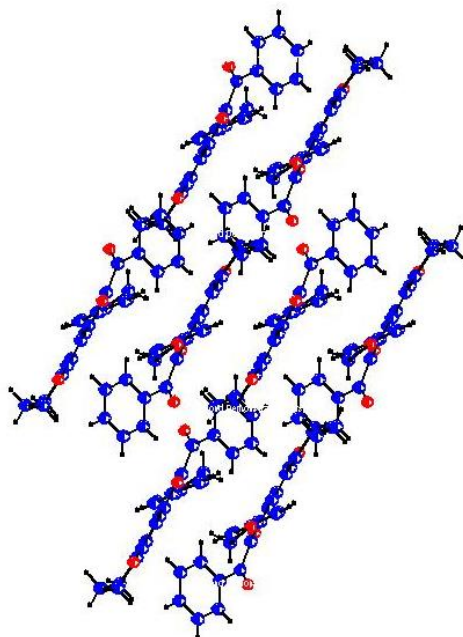


Figure 2. Crystal packing of the target compound **5a** viewed along the C axis.

Crystal structure

The aromatic C–C bond distances in the target compound ranging from 1.3691(19) to 1.3976(18) Å are almost within the normal ranges. The pyran ring (O(1)/C(3)~C(7)) adopts a half-chair conformation : atoms O(1), C(3), C(4), C(6), and C(7) are coplanar, while atom C(5) deviates from the plane by 0.6883 Å. The basal plane (O(1)–C(3)–C(4)–C(6)–C(7)) makes a dihedral angle of 3.626(49)° to the benzene ring (C(1)~C(3)/ C(7)~C(9)). The dihedral angle between the furan ring (O(2)/C(1)/C(9)~C(11)) and benzene ring (C(1)~C(3)/ C(7)~C(9)) is 1.065 (41)°. So the fused furo[3,2-*g*]chromene ring is approximately coplanar except C(5). The dihedral angles between the furan ring and two benzene rings are 60.070 (49)° for C(13)~C(18) and 38.226 (41)° for C(19)~C(24). The result confirms the screw structure (Figure 3) constructed by the furo[3,2-*g*]chromene ring and the different benzene rings. However, the crystal belongs to the triclinic crystal system and space group of P-1 (Figure 2).

Crystallographic data for structure **5a** has been deposited at the Cambridge Crystallographic Data Center, CCDC No. 775960 for compound **5a**. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk or www: <http://www.ccdc.cam.ac.uk>).

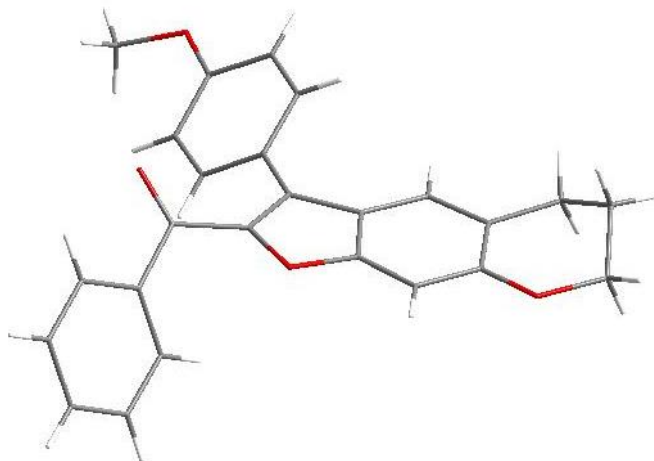


Figure 3. The screw structure (right) in the target compound **5a**.

Cytotoxicity

The target compounds were evaluated for cytotoxicity *in vitro* by MTT assay against the U2OS-EGFP-4F12G (human osteosarcoma) cell line. The screening result indicates that all target compounds exhibit cytotoxicities (Table 1), and the 5*H*-furo[3,2-*g*]chromene scaffold becomes a novel moiety with cytotoxicity.

Table 1. Cytotoxicity of the target compounds against U2OS-EGFP-4F12G cell line *in vitro*

Compound	IC ₅₀ (μ M)	Compound	IC ₅₀ (μ M)
5a	8.02	5d	20.24
5b	57.20	5e	21.71
5c	76.48	doxorubicin	0.46

Compound **5a** was superior to compound **5b**, which indicated that the methoxy group at the *para* position on the phenyl group at C3 position of furo[3,2-*g*]chromene scaffold was good for cytotoxicity.

Among all target compounds, the cytotoxicity of compound **5a** without substituents was more potent than those of the others, while the compound **5c** with methyl group, compound **5d** with methoxy group and compound **5e** with chloro group led to a huge decrease in biological activity, which indicated that the steric effect played an important role in cytotoxicity.

Conclusions

The target compounds were synthesized and the corresponding molecular structures were experimentally characterized by IR, NMR, ESI-MS and elemental analysis. The crystal structure for compound **5a** has been determined by single-crystal X-ray diffraction with a triclinic space

group P-1. X-ray analysis reveals that the pyran ring adopts a half-chair conformation. The fused furo[3,2-*g*]chromene ring is approximately coplanar except atom C(5), while the whole molecular forms the screw structure. All target compounds showed cytotoxicities against U2OS-EGFP-4A12G cells.

Experimental Section

General. The melting point was determined on open capillary tubes and the thermometer was uncorrected. IR spectra were recorded in the range of 4000-400 cm^{-1} using KBr pellets on a Bruker AFS55 spectrometer. Proton NMR spectra was measured on Bruker spectrometers operating at 300 MHz or 600 MHz with CDCl_3 as the solvent and TMS as the internal standard. ^{13}C -NMR spectra was measured on Bruker spectrometers operating at 100 MHz with $\text{DMSO-}d_6$ as the solvent. Mass spectra were recorded on a electrospray (ESI) ion source in a Waters spectrometer at 3.5 kV spray voltage. The single-crystal structure was determined on a Bruker SMART 1000 CCD diffractometer. Elemental analysis was carried out on a Perkin Elmer 2400 elemental analyzer. All the reagents were of analytical reagent grade.

Synthesis of 7-hydroxy-4-chromone (1). To a solution of 2',4'-dihydroxyacetophenone (15.2 g, 100 mmol) and ethyl orthoformate (83 mL, 500 mmol) was added 70% perchloric acid (10 mL, 120 mmol). The mixture was stirred at room temperature for 1 h and poured to 200 mL ethyl ether, subsequently, the solution was filtered and the solid was washed with cold water and recrystallized from ethanol to give **1** as a white solid, yield 85%, m.p.: 214-215 °C (lit.¹³: 215-216 °C); $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 6.21 (d, 1H, Ar-H, $J = 6.0$ Hz), 6.85 (d, 1H, Ar-H, $J = 2.0$ Hz), 6.90-6.93 (dd, 1H, Ar-H, $J_1 = 8.7$ Hz, $J_2 = 2.0$ Hz), 7.87 (d, 1H, Ar-H, $J = 8.7$ Hz), 8.15 (d, 1H, Ar-H, $J = 6.0$ Hz), 10.78 (s, 1H, OH).

Synthesis of 7-hydroxychroman (2). A round-bottomed flask (250 mL) was charged with **1** (7.60 g, 50 mmol), 10% palladium over charcoal (0.05 g, 5 mmol) and ethanol (100 mL) in hydrogen gas, and the mixture was refluxed for 4 h. Upon cooling, the mixture was filtered, then the filtrate was concentrated to dryness and the residue recrystallized from ethanol to give **2** as a white solid, yield 95%, m.p.: 87-89 °C (lit.¹⁴ 91 °C). $^1\text{H-NMR}$ (300 MHz, CDCl_3): δ 1.97 (m, 2H, CH_2), 2.70 (t, 2H, CH_2), 4.15 (t, 2H, CH_2), 6.29 (d, 1H, Ar-H, $J = 2.5$ Hz), 6.34 (dd, 1H, Ar-H, $J_1 = 8.3$ Hz, $J_2 = 2.5$ Hz), 6.88 (d, 1H, Ar-H, $J = 8.3$ Hz).

General procedure for preparing of 7-aryloxchromans (3)

To a solution of **2** (40 mmol) and triethylamine (44 mmol) in 80 mL dichloromethane, was added aroyl chloride (44 mmol). The mixture was stirred at room temperature for 3h and monitored by TLC. The mixture was washed with diluted hydrochloric acid (1 mol/L) and water (20 mL \times 3), and the combined organic layer was dried over MgSO_4 . After distillation under

reduced pressure, the residue was recrystallized from ethanol.

7-(4-Methoxybenzoyloxy)chroman (3a). A white solid, yield 91%, m.p.: 92-94 °C; ESI-MS: m/z 285.1[M+H]⁺; ¹H-NMR (300 MHz, CDCl₃): δ 1.97-2.05 (m, 2H, CH₂), 2.79 (t, 2H, CH₂), 3.89 (s, 3H, OCH₃), 4.18 (t, 2H, CH₂-O), 6.64 (d, 1H, Ar-H, $J = 2.1$ Hz), 6.66-6.70 (dd, 1H, Ar-H, $J_1 = 8.2$ Hz, $J_2 = 2.1$ Hz), 6.97 (d, 2H, Ar-H, $J = 8.8$ Hz), 7.05 (d, 1H, Ar-H, $J = 8.2$ Hz), 8.14 (d, 2H, Ar-H, $J = 8.8$ Hz).

7-Benzoyloxychroman (3b). A white solid, yield 96%, m.p.: 77-79 °C; ESI-MS: m/z 255.1[M+H]⁺; ¹H-NMR (300 MHz, CDCl₃): δ 1.97-2.05 (m, 2H, CH₂), 2.79 (t, 2H, CH₂), 4.19 (t, 2H, CH₂-O), 6.66 (d, 1H, Ar-H, $J = 2.2$ Hz), 6.66-6.70 (dd, 1H, Ar-H, $J_1 = 8.1$ Hz, $J_2 = 2.2$ Hz), 7.06 (d, 1H, Ar-H, $J = 8.1$ Hz), 7.47-7.52 (m, 2H, Ar-H), 7.60-7.65 (m, 1H, Ar-H), 8.18 (d, 2H, Ar-H, $J = 8.5$ Hz).

General procedure for preparing of 6-aroil-7-hydroxychromans (4)

A mixture of **3** (30 mmol), stannic chloride (150 mmol) was refluxed for 8 h. The solution was poured to ice water and appeared the precipitation, the solid was washed with cold water and recrystallized from ethanol.

7-Hydroxy-6-(4-methoxybenzoyl)chroman (4a). A white solid, yield 80%, m.p.: 148-150 °C; ¹H-NMR (600 MHz, CDCl₃): δ 1.99-2.01 (m, 2H, CH₂), 2.68 (t, 2H, CH₂), 3.90 (s, 3H, OCH₃), 4.24 (t, 2H, CH₂-O), 6.43 (s, 1H, Ar-H), 6.99 (d, 2H, Ar-H, $J = 9.0$ Hz), 7.30 (s, 1H, Ar-H), 7.65-7.66 (dd, 2H, Ar-H, $J_1 = 9.0$ Hz, $J_2 = 2.4$ Hz), 12.24 (s, 1H, OH); ESI-MS: m/z 285.1[M+H]⁺.

6-Benzoyl-7-hydroxychroman (4b). A white solid, yield 85%, m.p.: 113-114 °C; ¹H-NMR (300 MHz, CDCl₃): δ 1.99-2.00 (m, 2H, CH₂), 2.66 (t, 2H, CH₂), 4.23 (t, 2H, CH₂-O), 6.44 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.49-7.64 (m, 5H, Ar-H), 12.27 (s, 1H, OH); ESI-MS: m/z 255.1[M+H]⁺.

General procedure for preparing of 2-aroil-3-aryl-6,7-dihydro-5H-furo[3,2-g]chromenes (5)

A mixture of **4** (20 mmol), substituted phenacyl chloride (20 mmol), tetrabutyl ammonium bromide (10 mmol), 30 mL 30% aqueous potassium carbonate and 70 mL dichloromethane was refluxed for 12 h. The mixture was washed with diluted hydrochloric acid (1 mol/L) and water (20 mL × 3), and the combined organic layer was dried over MgSO₄. After distillation under reduced pressure, the residue was recrystallized from ethanol.

2-Benzoyl-3-(4-methoxyphenyl)-6,7-dihydro-5H-furo[3,2-g]chromene (5a). A yellow crystal, yield 74% m.p.: 107-109 °C; IR: (KBr, cm⁻¹) 2933(m), 1628(s), 1602(s), 1551(s), 1506(s), 1334(s), 1311(s), 1290(s), 1251(s), 1152(s), 1127(m), 1002(s), 829(s), 719(s), 695(s); ¹H-NMR (600 MHz, CDCl₃): δ 2.04-2.06 (m, 2H, CH₂), 2.91 (t, 2H, CH₂), 3.83 (s, 3H, OCH₃), 4.27 (t, 2H, CH₂-O), 6.89-6.91 (dd, 2H, Ar-H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz), 7.01 (s, 1H, Ar-H), 7.31-7.35 (m, 3H, Ar-H), 7.41-7.47 (m, 3H, Ar-H), 7.83-7.85 (dd, 2H, Ar-H, $J_1 = 8.4$ Hz, $J_2 = 1.2$ Hz). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 184.4, 159.2, 156.1, 153.7, 145.7, 137.4, 132.3, 131.1 (2C), 129.2, 128.9 (2C), 128.1 (2C), 122.6, 122.1, 120.9, 120.8, 113.7 (2C), 98.6, 66.5, 55.1, 24.7, 21.6.

ESI-MS: m/z 385.2 $[M+H]^+$, 407.2 $[M+Na]^+$, 423.1 $[M+K]^+$; Anal. Calcd. for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24; Found: C, 78.19; H, 5.18.

2-(4-Methoxybenzoyl)-3-phenyl-6,7-dihydro-5H-furo[3,2-g]chromene (5b). A yellow solid, yield 76%, m.p.: 126-128 °C; IR: (KBr, cm^{-1}) 2928(m), 1626(s), 1599(s), 1551(s), 1508(s), 1334(m), 1309(s), 1260(s), 1241(s), 1176(s), 1159(s), 1127(m), 997(s), 842(s), 762(s); 1H -NMR (300 MHz, $CDCl_3$): δ 2.03-2.06 (m, 2H, CH_2), 2.91 (t, 2H, CH_2), 3.83 (s, 3H, OCH_3), 4.27 (t, 2H, CH_2-O), 6.81-6.84 (dd, 2H, Ar-H, $J_1 = 7.0$ Hz, $J_2 = 1.9$ Hz), 7.03 (s, 1H, Ar-H), 7.30 (s, 1H, Ar-H), 7.35-7.38 (m, 3H, Ar-H), 7.46-7.50 (m, 2H, Ar-H), 7.89-7.92 (dd, 2H, Ar-H, $J_1 = 6.9$ Hz, $J_2 = 2.0$ Hz); ^{13}C -NMR (100 MHz, $DMSO-d_6$): δ 182.8, 162.9, 155.8, 153.5, 146.2, 131.8 (2C), 130.8, 129.7, 129.6 (2C), 128.9, 128.3 (2C), 128.1, 127.9, 121.8, 120.9, 120.6, 113.6 (2C), 98.6, 66.5, 55.5, 24.7, 21.6. ESI-MS: m/z 385.2 $[M+H]^+$; Anal. Calcd. for $C_{25}H_{20}O_4$: C, 78.11; H, 5.24; Found: C, 78.21; H, 5.30.

3-(4-Methoxyphenyl)-2-(4-methylbenzoyl)-6,7-dihydro-5H-furo[3,2-g]chromene (5c). A yellow solid, yield 72%, m.p.: 113-115 °C; IR: (KBr, cm^{-1}) 2933(m), 1640(s), 1606(s), 1555(s), 1506(s), 1334(s), 1291(s), 1250(s), 1176(m), 1152(s), 1126(m), 1003(m), 831(s), 773(s), 761(s); 1H -NMR (600 MHz, $CDCl_3$): δ 2.04-2.06 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 2.91 (t, 2H, CH_2), 3.83 (s, 3H, OCH_3), 4.26 (t, 2H, CH_2-O), 6.91-6.92 (dd, 2H, Ar-H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz), 7.00 (s, 1H, Ar-H), 7.14 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.31 (s, 1H, Ar-H), 7.44-7.45 (dd, 2H, Ar-H, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz), 7.79 (d, 2H, Ar-H, $J = 8.4$ Hz). ^{13}C -NMR (100 MHz, $DMSO-d_6$): δ 184.0, 159.2, 155.9, 153.6, 145.8, 142.8, 134.7, 131.3, 131.1 (2C), 129.4 (2C), 128.7 (2C), 128.4, 122.7, 122.0, 120.8 (2C), 113.7 (2C), 98.6, 66.5, 55.1, 24.7, 21.6, 21.1. ESI-MS: m/z 399.2 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{22}O_4$: C, 78.37; H, 5.57; Found: C, 78.46; H, 5.51.

2-(4-Methoxybenzoyl)-3-(4-methoxyphenyl)-6,7-dihydro-5H-furo[3,2-g]chromene (5d). A yellow solid, yield 83%, m.p.: 130-132 °C; IR: (KBr, cm^{-1}) 2936(m), 1628(s), 1598(s), 1551(m), 1505(s), 1335(s), 1312(s), 1251(s), 1173(s), 1155(s), 1127(m), 999(s), 845(s), 831(s), 767(s); 1H -NMR (600 MHz, $CDCl_3$): δ 2.03-2.07 (m, 2H, CH_2), 2.91 (t, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.27 (t, 2H, CH_2-O), 6.85 (d, 2H, Ar-H, $J = 9.0$ Hz), 6.93 (d, 2H, Ar-H, $J = 9.0$ Hz), 7.01 (s, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.45 (d, 2H, Ar-H, $J = 9.0$ Hz), 7.92 (d, 2H, Ar-H, $J = 9.0$ Hz). ^{13}C -NMR (100 MHz, $DMSO-d_6$): δ 182.8, 162.8, 159.2, 155.8, 153.5, 146.0, 131.7 (2C), 131.0 (2C), 129.8, 127.9, 122.8, 121.9, 120.8, 120.7, 113.7 (2C), 113.5 (2C), 98.6, 66.5, 55.4, 55.1, 24.7, 21.6. ESI-MS: m/z 415.2 $[M+H]^+$; Anal. Calcd. for $C_{26}H_{22}O_5$: C, 75.35; H, 5.35; Found: C, 75.29; H, 5.44.

2-(4-Chlorobenzoyl)-3-(4-methoxyphenyl)-6,7-dihydro-5H-furo[3,2-g]chromene (5e). A yellow solid, yield 80%, m.p.: 136-138 °C; IR: (KBr, cm^{-1}) 2957(m), 1638(s), 1589(s), 1563(s), 1507(m), 1490(s), 1348(s), 1284(s), 1255(s), 1162(s), 1124(m), 1026(m), 843(s), 790(s), 622(s); 1H -NMR (600 MHz, $CDCl_3$): δ 2.04-2.06 (m, 2H, CH_2), 2.91 (t, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.27 (t, 2H, CH_2-O), 6.91-6.92 (dd, 2H, Ar-H, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz), 7.00 (s, 1H, Ar-H), 7.31-7.32 (m, 3H, Ar-H), 7.40-7.42 (m, 2H, Ar-H), 7.79-7.81 (dd, 2H, Ar-H, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz). ESI-MS: m/z 419.2 $[M+H]^+$, 441.2 $[M+Na]^+$, 457.1 $[M+K]^+$; Anal. Calcd. for $C_{25}H_{19}ClO_4$: C, 71.69; H, 4.57; Found: C, 71.75; H, 4.49.

Crystal data and structure determination

The yellow needle crystal of compound **5a** with dimensions of 0.18 mm × 0.16 mm × 0.10 mm was selected for X-ray diffraction analysis. The data were collected by a Bruker Smart 1000 CCD diffractometer equipped with a graphite-monochromatic Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) using a φ - ω scan mode at 113(2) K. In the range of $2.22 \leq \theta \leq 25.02^\circ$, a total of 6627 reflections were collected with 3235 unique ones ($R_{\text{int}} = 0.0245$), of which 2622 were observed with $I > 2\sigma(I)$ and used in the succeeding refinements. Intensity data were corrected for L_p factors and empirical absorption. The structure was solved by direct methods and expanded by difference Fourier techniques with SHELXS-97 program.^{15,16} All of the non-hydrogen atoms were located with successive difference Fourier syntheses. The structure was refined by full-matrix least-squares techniques on F^2 using anisotropic thermal parameters for all non-hydrogen atoms. The hydrogen atoms were added according to theoretical models. The crystallographic data and refinement information for **5a** are summarized in Table 2.

Table 2. Crystal data and details of the structure determination of **5a**

Compound 5a			
<i>Empirical formula</i>	C ₂₅ H ₂₀ O ₄	<i>Volume</i>	936.6 (3) Å ³
<i>Formula weight</i>	384.41	<i>Z, Calculated density</i>	2, 1.363 g/cm ³
<i>Temperature</i>	113(2) K	<i>Absorption coefficient</i>	0.092 mm ⁻¹
<i>Wavelength</i>	0.71073 Å	<i>F(000)</i>	404
<i>Crystal system, space group</i>	Triclinic, P-1	<i>Crystal size</i>	0.18 × 0.16 × 0.10 mm
<i>Unit cell dimensions</i>		a = 9.3968 (19) Å	α = 115.00 (3) °
		b = 10.406 (2) Å	β = 99.23 (3) °
		c = 11.571 (2) Å	γ = 105.74 (3) °
<i>θ range for data collection</i>		2.22 to 25.02 °	
<i>Collection h/k/l</i>	-11/-12/-13 deg	<i>Goodness-of-fit on F²</i>	1.028
<i>Reflections collected</i>	6627	<i>Final R indices</i>	R ¹ = 0.0349,
		<i>[I > 2σ(I)]</i>	wR ² = 0.0995
<i>Independent reflections</i>	3235[R(int) = 0.0245]	<i>R indices (all data)</i>	R ¹ = 0.0416,
			wR ² = 0.1043
<i>Data/restraints/parameters</i>	3235/0/263	<i>Largest diff. peak and hole</i>	0.199 and -0.24 e.Å ⁻³

Screening method

The method of MTT assay was followed.¹⁷ Human osteosarcoma U2OS-EGFP-4A12G cells were plated at a density of 1.5×10^4 per well in a 96-well plate, then incubated at 37 °C, 95% O₂/5% CO₂ atmosphere for 24 h. Cells were treated with the compound of choice at 100 μM concentration, then incubated for a further 72 h. Following incubation 50 μL aliquots of medium were removed to a fresh 96-well plate. Cytotoxicity was determined using and LDH assay kit

obtained from Sigma, following the manufacturer's instructions for use. Briefly, 50 μL per well LDH substrate mixture was added to each well and the plate left in darkness at room temperature for equilibration. The reaction was stopped by 50 μL of 1 N HCl solution added to all wells before reading the absorbance at 490 nm. A control of 100% lysis was determined for a set of untreated cells which were lysed through the addition of 20 μL lysis solution to the media 45 min prior to harvesting. Calculate the IC_{50} values.

Acknowledgements

This work was partially supported by the National Natural Science Foundation of China (NSFC) for the grant No. 20474053. The authors would like to thank Professor Hai-Bing Song from Nankai University for measurement and refinement of single-crystal structure, and Ms He Zhu from Shandong University for measuring the NMR spectra.

References

1. Yuzuru, A.; Christian, F. H.; Hiroyuki, K.; Erlio, G. *Cancer Res.* **1989**, *49*, 2362.
2. Bruce, E.; Dennis, M. B.; Bruce, H. M.; Ronald, K. K.; Thomas, N.; Harry, K. G.; Claus, C.; Pierre, D. D.; Jose, R. Z.; Jacob, S.; Claus, C. G.; Kathryn, K.; Fredric, J. C.; Stephen, E.; Kristine, E. E.; Louis, V. A.; Paul, L.; Steven, R. C. *J. Am. Med. Assoc.* **1999**, *282*, 637.
3. Fisher, B.; Costantino, J. P.; Wickerham, D. L.; Redmond, C. K.; Kavanah, M.; Cronin, W. M.; Vogel, V.; Robidoux, A.; Dimitrov, N.; Atkins, J.; Daly, M.; Wieand, S.; Tan-Chiu, E.; Ford, L.; Wolmark, N. *J.Nati.Cancer Inst.* **1998**, *90*, 1371.
4. Walsh, B. W.; Kuller, L. H.; Wild, R. A.; Paul, S.; Farmer, M.; Lawrence, J. B.; Shah, A. S.; Anderson, P. W. *J. Am. Med. Assoc.* **1998**, *279*, 1445.
5. Niho, N.; Mutoh, M.; Sakano, K.; Takahashi, M.; Hirano, S.; Nukaya, H.; Sugimura, T.; Wakabayashi, K. *Cancer Sci.* **2006**, *97*, 248.
6. Abdel-Hafez, O. M.; Amin, K. M.; Abdel-Latif, N. A.; Mohamed, T. K.; Ahmed, E. Y.; Maher, T. *Eur. J. Med.Chem.* **2009**, *44*, 2967.
7. Alvey, L.; Prado, S.; Huteau, V.; Saint-Joanis, B.; Michel, S.; Koch, M.; Cole, S. T.; Tillequin, F.; Janin, Y. L. *Bioorg. Med. Chem.* **2008**, *16*, 8264.
8. Hayakawa, I.; Shioya, R.; Agatsuma, T.; Furukawa, H.; Narutoc, S.; Suganoa, Y. *Bioorg Med Chem.Lett.* **2004**, *14*, 455.
9. Mahboobi, S.; Sellmer, A.; Höcher, H.; Garhammer, C.; Pongratz, H.; Maier, T.; Ciossek, T.; Beckers, T. *J. Med. Chem.* **2007**, *50*, 4405.
10. Grese, T. A.; Pennington, L. D.; Sluka, J. P.; Adrian, M. D.; Cole, H. W.; Fuson, T. R.;

- Magee, D. E.; Phillips, D. L.; Rowley, E. R.; Shetler, P. K.; Short, L. L.; Venugopalan, M.; Yang, N. N.; Sato, M.; Glasebrook, A. L.; Bryant, H. U. *J. Med. Chem.* **1998**, *41*, 1272.
11. Renaud, J.; Bischoff, S. F.; Buhl, T.; Floersheim, P.; Fournier, B.; Halleux, C.; Kallen, J.; Keller, H.; Schlaeppli, J. M.; Stark, W. *J. Med. Chem.* **2003**, *46*, 2945.
12. Renaud, J.; Bischoff, S. F.; Buhl, T.; Floersheim, P.; Fournier, B.; Geiser, M.; Halleux, C.; Kallen, J.; Keller, H.; Ramage, P. *J. Med. Chem.* **2005**, *48*, 364.
13. Pfeifer, P.; Oberlin, H.; Konermann, E. *Ber. Dtsch. Chem. Ges.* **1925**, *58B*, 1947.
14. Naylor, P.; Ramage, G. R.; Schofield, F. *J. Chem. Soc.* **1958**, 1190.
15. Sheldrick, G. M. *SHELXS97 and SHELXL97*, University of Göttingen, Germany 1997.
16. Bruker. *SAINTE and SHELXTL*, Bruker AXS Inc., Madison, Wisconsin, USA 1998.
17. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.