Study on Design, Synthesis and Anticancer Activity of 4*H*-Pyran Derivatives Bearing Arylurea Moiety

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ABSTRACT: OBJECTIVE To design and synthesize a series of 4*H*-pyran-arylurea derivatives and to evaluate their anticancer activities *in vitro*. **METHODS** Nitro-containing pyran intermediates was synthesized by "one-pot" condensation of 3-nitrobenzaldehyde, malononitrile, dimethyl 3-oxopentanedioate. Reduction of the nitro group of above compound with iron powder, and then condensed with isocyanates to afford target compounds. Human large cell lung cancer cells H460, human lung cancer cells A549 and human colon cancer cells HT-29 cell lines were employed to evaluate anticancer activities of these compounds using MTT-based assay. **RESULTS** Eleven 4*H*-pyran derivatives bearing arylurea moieties were synthesized. The results of *in vitro* antiproliferative activity showed 11 compounds had good inhibitory activities against 3 tumor cell lines. Among them, compound **7c** exhibited remarkable inhibitory activity against H460 and A549 cell lines with IC_{50} value of 0.82 and 0.98 μmol·L^{-1}, respectively, which were more potent than that of the positive control sorafenib(IC_{50}=3.20 and 2.83 μmol·L^{-1}). **CONCLUSION** 4*H*-pyran derivatives bearing arylurea moieties show excellent anticancer activities, and they can be used as the structural skeleton of anticancer compounds for futher study.

KEYWORDS: pyran; arylurea; synthesis; anticancer activity

含有芳基脲结构的 4*H*-吡喃类化合物的设计、合成及抗肿瘤活性研究

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摘要：目的 设计并合成一系列含有芳基脲结构的 4*H*-吡喃类化合物，评价该类化合物的体外抗肿瘤活性。**方法** 以间硝基苯甲醛、丙二腈和丙酮二羧酸二甲酯为原料，通过“一锅法”合成含有硝基的吡喃中间体，该中间体的硝基经铁粉还原为氨基，再与取代异氰酸苯酯反应得到一系列目标化合物。以人大细胞肺癌细胞 H460、人肺癌细胞 A549 和人结肠癌细胞 HT-29 3 种肿瘤细胞为测试细胞株，采用 MTT 法评价了目标化合物的抗肿瘤活性。**结果** 合成了 11 个含有芳基脲结构的 4*H*-吡喃类化合物。体外抗肿瘤活性试验表明，11 个化合物对 3 种肿瘤细胞株均具有很好的抑制活性。其中化合物 **7c** 活性突出，对 H460 和 A549 细胞的 IC_{50} 值分别为 0.82，0.98 μmol·L^{-1}，优于阳性对照药索拉非尼(IC_{50}=3.20，2.83 μmol·L^{-1})。**结论** 含有芳基脲结构的 4*H*-吡喃类化合物具有很好的抗肿瘤活性，可作为抗肿瘤化合物的结构骨架进一步研究。

关键词：吡喃；芳基脲；合成；抗肿瘤活性

中图分类号：R914.5　　**文献标志码：**A　　**文章编号：**1007-7693(2022)17-2192-06

DOI: 10.13748/j.cnki.issn1007-7693.2022.17.004

引用本文：黄婷，卢久富，苏明明，等. 含有芳基脲结构的 4*H*-吡喃类化合物的设计、合成及抗肿瘤活性研究[J]. 中国现代应用药学, 2022, 39(17): 2192-2197.

In recent years, pyran derivatives have received significant attention due to their biological importance[1-5], especially the 4*H*-pyran ring. The 4*H*-pyran derivatives often exhibit diverse biological activities, including anti-coagulant, spasmolytic, anticancer, antibacterial and antifungal, diuretic, specific IKCa channel blockers[6-9]. On the other hand, aryl urea system also belongs to the privileged structure in modern medicinal chemistry, particularly in discovery of new antitumor agents[10]. Recently, researchers embarked on a program for the optimization of aryl-urea scaffold. A variety of small-molecule inhibitors containing aryl urea moiety have emerged, such as sorafenib, linifanib,

基金项目：陕西省教育厅重点实验室科研计划项目(18JS023)；安康市科技局项目(AK2020-GY02-3)；国家级大学生创新创业训练计划项目(202111397015)；安康学院科研项目(2021AYPT14)

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tivozanib, BIRB796[11-13]. Previous research has not been fully considered to the compounds contain both 4*H*-pyran and arylurea units, which may possess novel bio-activities for screening. In the light of the above mentioned findings, a novel series compounds as hybrids of pyran and arylurea using combination principle was designed. Thus the synthesis and antiproliferative activity against H460, A549 and HT-29 cancer cell lines of a series of 4*H*-pyran derivatives containing arylurea moieties as new potential anticancer agents.

1 Materials and methods

1.1 Materials

All melting points were taken on a X-4 microscopy melting point apparatus(Beijing Taike) and were uncorrected; IR spectra were recorded as KBr pellets on a spcetrum one FT-IR spectrometer (Perkin-Elmer); ^{1}H-NMR spectra were recorded on a 300 MHz or 600 MHz nuclear magnetic resonance spectrometer(Bruker Biospin) using TMS; Mass spectra were recorded on a micromass quattro micro API mass spectrometer(Waters, ESI, direct injection); Elemental analysis of the newly synthesized compounds was carried out on a 1108 elemental analyzer(Carlo Erba) and were found within the range of theoretical value.

3-Nitrobenzaldehyde(batch number: ANX279), Dimethyl acetone-1,3-dicarboxylate(batch number: AQA299), Malononitrile(batch number: DPQ864), iron powder(batch number: BRW954), sorafenib (batch number: DZD727), DMSO-d_6(batch number: BQL437), $CDCl_3$ (batch number: BQL581) were obtained from Shanghai Bidepharm Co., Ltd with purity ⩾98.0%(HPLC). Other materials and reagents were all analytical pure, and obtained from commercial supplies without further purification.

1.2 Synthesis of target compounds

1.2.1 Synthesis of methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-(3-nitrophenyl)-4*H*-pyran-3-carboxylate(**4**) A mixture of 3-nitrobenzaldehyde (6.00 g, 39.70 mmol), malononitrile(2.62 g, 39.70 mmol), dimethyl 3-oxopentanedioate(6.91 g, 39.70 mmol)and triethylamine(4.02 g, 39.70 mmol) in EtOH(100 mL) was stirred at room temperature for 5 h. After completion of the reaction, the solid which formed was collected by filtration and recrystallization from EtOH to give compound **4**(9.40 g) as a light yellow solid in a 63.4% yield. mp 153–155 ℃. ^{1}H–NMR spectrum (600 MHz, $CDCl_3$), δ: 8.12 br(2H), 7.65 d(1H, J=7.8 Hz), 7.52 t(1H, J=7.8 Hz), 4.77 s(2 H), 4.61 s(1 H), 4.07 d(1H, J=16.8 Hz), 3.79 s(3 H), 3.71 d(1 H, J=16.8 Hz), 3.60 s(3 H). IR spectrum, v, cm^{-1}: 3 390.1(NH_2), 3 197.4(NH_2), 2 950.6, 2 923.2, 2 197.5(–CN), 1 754.9(C=O), 1 680.8(C=O), 1 637.1 (C=C_{arom}), 1 535.4(NO_2), 1 384.5, 1 354.2, 1 273.6, 1 070.2. MS(ESI) m/z(%): 395.9[M+Na]$^+$.

1.2.2 Synthesis of methyl 6-amino-4-(3-aminophenyl)-5-cyano-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(5)** A mixture of compound **4** (7.00 g, 18.75 mmol), iron powder(5.24 g, 93.75 mmol), acetic acid(11.26 g, 187.51 mmol), water(15 mL) and ethyl acetate(150 mL) was heated to reflux for 2 h. After completion of the reaction as indicated by TLC, the mixture was filtered immediately. The organic layer of the filtrate was separated, washed with water, dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure until precipitate appeared, which was filtered to obtain 3.78 g(yield 58.72%) of **5** as a light yellow solid, mp 145–148 ℃. ^{1}H-NMR spectrum(600 MHz, $CDCl_3$), δ: 7.09 t(1H, J=7.7 Hz), 6.65 d(1H, J=7.7 Hz), 6.59 t(1H, J=1.8 Hz), 6.57–6.52 m(1H), 4.49 s(2H), 4.37 s(1H), 3.92 d(1H, J=16.8 Hz), 3.81–3.57 m(9H). MS(ESI) m/z(%): 344.1[M+H]$^+$, 366.2[M+Na]$^+$.

1.2.3 General procedure for preparation of target compounds(**7a**–**7k**) Compound **5**(0.10 mmol) was dissolved in ethyl acetate(8 mL) and then treated with a stoichiometric amount of isocyanates **6a**–**6k**. The mixture was stirred at room temperature for 20 h, until completion(TLC monitoring). The heavy precipitate formed was filtered off, washed with ethyl acetate (20 mL), and dried in vacuo to afford the target compound **7a**–**7k** as light yellow solid.

1.3 *In vitro* anticancer activity test

The anticancer activities of compounds **7a–7k** were evaluated against HT-29 and A549 cell lines using the standard MTT assay *in vitro*, with sorafenib as the positive control. Individual wells of a 96-well tissue culture micro titer plate were inoculated with 100 μL of complete medium containing approximate 1×10^4 cells. The plates were incubated at 37 ℃ in a humidifi ed 5% CO_2 incubator for 24 h prior to the experiment. After medium removal, 100 μL of fresh medium containing the test compounds and sorafenib at different concentrations were added to each well and incubated at 37 ℃ for 72 h. Then the medium was discarded and replaced with 10 μL MTT dye. Plates were incubated at 37 ℃ for 4 h. The resulting formazan crystals solubilized in 100 μL extraction buffer. The optical density(OD) was measured at 570 nm with micro plate reader(Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded

0.25%. All compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner(Bliss) software.

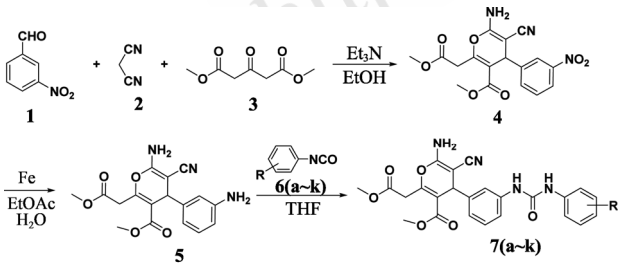
1.4 Acridine orange/ethidium bromide staining

H460 and A549 cells were seeded in 24-well plates(1×10^6 cells per well), and then the cells were incubated for 24 h. Cells were treated with **7c** at concentrations 0, 0.10, 1.00 and 10.00 μmol·L^{-1} for 48 h, cells were collected, washed with phosphate buffer saline(PBS) that stored at 4 ℃. Acridine orange/ethidium bromide(AO/EB) mixed solution 1.0 μL(100 μg·mL^{-1} AO and 100 μg·mL^{-1} EB) was added to each suspension, and then stained for 10 min, covered with a coverslip. The morphology of apoptotic cells was observed by CKX53 fluorescent microscope(Olympus).

2 Results and discussion

2.1 Chemistry

The synthetic methods for compounds **7a**–**7k** were outlined in Fig.1. First, methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-(3-nitrophenyl)-4*H*-pyran-3-carboxylate(**4**) was synthesized by one-pot three-component condensation of 3-nitrobenzaldehyde (**1**), malononitrile(**2**), dimethyl 3-oxopentanedioate(**3**) using triethylamine as catalyst in ethanol at room temperature. Reduction of the nitro group of **4** with iron powder and acetic acid in ethyl acetate/water(10∶1) provided aniline compounds **5**. Finally, intermediate **5** was condensed with stoichiometric amount of aromatic isocyanates to afford target compounds **7a**–**7k** with good yields and its structures were established by IR, ^{1}H-NMR, MS and elemental analyses. All data of the target compounds confirmed its structural integrity.



7a: R=H
7b: R=4-methoxy
7c: R=3-chloro
7d: R=2-(trifluoromethoxy)
7e: R=3-(trifluoromethyl)
7f: R=3,4-dimethoxy
7g: R=4-chloro-3-(trifluoromethyl)
7h: R=4-fluoro-3-(trifluoromethyl)
7i: R=2,4-dichloro
7j: R=2,4-difluoro
7k: R=5-fluoro-2-methyl

Fig. 1 Synthetic route of target compounds
图 1 目标化合物的合成路线

Methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-[3-(3-phenylureido)phenyl]-4*H*-pyran-3-carboxylate(**7a**): Yield 79.8%, mp 194–196 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 8.71 s(1H), 8.64 s(1H), 7.45 d(2H, J=7.8 Hz), 7.39 d(1H, J=7.8 Hz), 7.32–7.19 m(4H), 7.02–6.92 m(3H), 6.81 d(1H, J=7.4 Hz), 4.29 s(1H), 3.82 m(2H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 379.3(NH_2), 2 189.2(CN), 1 722.4(C=O), 1 668.4(C=O), 1 600.9(C=C_{arom}), 1 550.8(C=C_{arom}), 1 431.2, 1 311.6, 1 224.8, 1 068.6. MS(ESI) m/z(%): 463.2[M+H]$^+$, 485.1[M+Na]$^+$. Anal. calcd for $C_{24}H_{22}N_4O_6$: C, 62.33%; H, 4.80%; N, 12.12%; found: C, 62.45%; H, 4.85%; N, 12.22%.

Methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-{3-[3-(4-methoxyphenyl)ureido]phenyl}-4*H*-pyran-3-carboxylate**(7b)**: Yield 74.4%, mp 199–201 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO- d_6), δ: 8.60 s(1H), 8.41 s(1H), 7.44–7.29 m(3H), 7.27–7.15 m(2H), 6.97 s(2H), 6.87 d(2H, J=8.6 Hz), 6.79 d(1H, J=7.5 Hz), 4.28 s(1H), 3.89–3.75 m(2H), 3.71 s(3H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 375.4(NH_2), 2 185.4(CN), 1 726.3(C=O), 1 666.5(C=O), 1 604.8(C=C_{arom}), 1 546.9(C=C_{arom}), 1 317.4, 1 222.9, 1 066.6. MS(ESI) m/z(%): 493.1[M+H]$^+$, 515.1[M+Na]$^+$. Anal. calcd for $C_{25}H_{24}N_4O_7$: C, 60.97%; H, 4.91%; N, 11.38%; found: C, 61.11%; H, 4.94%; N, 11.44%.

Methyl 6-amino-4-{3-[3-(3-chlorophenyl) ureido] phenyl}-5-cyano-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7c)**: Yield 73.1%, mp 202–205 ℃. ^{1}H–NMR spectrum (600 MHz, DMSO-d_6), δ: 8.83 s(1H), 8.78 s(1H), 7.71 s(1H), 7.38 d(1H, J=7.8 Hz), 7.33–7.17 m(4H), 7.09–6.92 m(3H), 6.83 d(1H, J=7.3 Hz), 4.30 s(1H), 3.93–3.73 m(2H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 398.6(NH_2), 2 182.2(CN), 1 743.7(C=O), 1 681.9(C=O), 1 595.1 (C=C_{arom}), 1 545.0(C=C_{arom}), 1 427.3(C=C_{arom}), 1 263.4, 1 197.8, 1 066.6. MS (ESI) m/z(%): 497.1[M+H]$^+$, 519.1[M+Na]$^+$. Anal. calcd for $C_{24}H_{21}ClN_4O_6$: C, 58.01%; H, 4.26%; N, 11.28%; found: C, 58.16%; H, 4.31%; N, 11.34%.

Methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-(3-{3-[2-(trifluoromethoxy)phenyl]ureido} phenyl)-4*H*-pyran-3-carboxylate(**7d**): Yield 76.4%, mp 197–200 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 9.32 s(1H), 8.45 s(1H), 8.28 dd(1H, J=8.1, 1.4 Hz), 7.44 dd(1H, J=8.1, 1.4 Hz), 7.40–7.31 m(2H), 7.29–7.19 m(2H), 7.09 td(1H, J=8.2, 1.5 Hz), 6.99 s(2H), 6.85 d(1H, J=7.7 Hz), 4.31 s(1H), 3.91–3.74 m(2H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 369.6(NH_2), 3 325.3(NH_2),

2 191.1(CN), 1 683.9(C=O), 1 600.9(C=C_{arom}), 1 546.9(C=C_{arom}), 1 437.0(C=C_{arom}), 1 352.1, 1 249.9, 1 057.0. MS(ESI) *m*/*z*(%): 547.1[M+H]$^+$, 569.1 [M+Na]$^+$. Anal. calcd for $C_{25}H_{21}F_3N_4O_7$: C, 54.95%; H, 3.87%; N, 10.25%; found: C, 55.12%; H, 3.90%; N, 10.32%.

Methyl 6-amino-5-cyano-2-(2-methoxy-2-oxoethyl)-4-(3-{3-[3-(trifluoromethyl)phenyl]ureido}phenyl)-4*H*-pyran-3-carboxylate**(7e)**: Yield 81.6%, mp 206–209 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 8.99 s(1H), 8.83 s(1H), 8.01 s(1H), 7.57 d(1H, *J*=8.4 Hz), 7.51 t(1H, *J*=7.6 Hz), 7.40 dd(1H, *J*=8.4, 1.1 Hz), 7.31 d(1H, *J*=7.6 Hz), 7.28–7.21 m(2H), 6.99 s(2H), 6.84 d(1H, *J*=7.7 Hz), 4.30 s(1H), 3.91–3.74 m(2H), 3.68 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 396.6(NH_2), 3 325.3(NH_2), 2 175.7(CN), 1 743.7 (C=O), 1 681.9(C=O), 1 599.0 (C=C_{arom}), 1 548.8 (C=C_{arom}), 1 442.8(C=C_{arom}), 1 325.1(CF_3), 1 267.2, 1 211.3, 1 157.3, 1 105.2, 1 064.7. MS (ESI) *m*/*z*(%): 531.1[M+H]$^+$, 553.1[M+Na]$^+$. Anal. calcd for $C_{25}H_{21}F_3N_4O_6$: C, 56.61; H, 3.99%; N, 10.56%; found: C, 56.73%; H, 4.04%; N, 10.62%.

Methyl 6-amino-5-cyano-4-{3-[3-(3,4-dimethoxyphenyl)ureido]phenyl}-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7f):** Yield 80.5%, mp 202–204 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 8.60 s(1H), 8.45 s(1H), 7.43–7.34 m(1H), 7.25–7.14 m(3H), 6.97 s(2H), 6.87 d(2H, *J*=0.8 Hz), 6.80 d(1H, *J*=7.7 Hz), 4.29 s(1H), 3.82–3.77 m(2H), 3.74 s(3H), 3.71 s(3H), 3.68 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 400.5(NH_2), 3 317.6(NH_2), 2 945.3, 2 181.5(CN), 1 676.1(C=O), 1 597.1(C=C_{arom}), 1 548.8(C=C_{arom}), 1 510.3(C=C_{arom}), 1 440.8, 1 213.2, 1 064.7. MS(ESI) *m*/*z*(%): 523.2 [M+H]$^+$, 545.1[M+Na]$^+$. Anal. calcd for $C_{26}H_{26}N_4O_8$: C, 59.77%; H, 5.02%; N, 10.72%; found: C, 59.91%; H, 5.06%; N, 10.81%.

Methyl 6-amino-4-(3-{3-[4-chloro-3-(trifluoromethyl)phenyl]ureido}phenyl)-5-cyano-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7g)**: Yield 85.1%, mp 223–225 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 9.12 s(1H), 8.89 s(1H), 8.09 d(1H, *J*=2.4 Hz), 7.69–7.55 m(2H), 7.39 dd(1H, *J*=8.1, 1.1 Hz), 7.25–7.16 m(2H), 6.99 s(2H), 6.85 d(1H, *J*=7.7 Hz), 4.30 s(1H), 3.90–3.73 m(2H), 3.68 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 375.4(NH_2), 2 943.4, 2 191.1(CN), 1 724.4(C=O), 1 674.2(C=O), 1 595.1(C=C_{arom}), 1 546.9(C=C_{arom}), 1 479.4(C=C_{arom}), 1 419.6, 1 317.4(CF_3), 1 219.0, 1 064.7. MS(ESI) *m*/*z*(%): 565.1[M+H]$^+$, 587.1[M+Na]$^+$. Anal. calcd for $C_{25}H_{20}ClF_3N_4O_6$: C, 53.16%; H, 3.57%; N, 9.92%; found: C, 53.25%; H, 3.63%; N, 10.00%.

Methyl 6-amino-5-cyano-4-(3-{3-[4-fluoro-3-(trifluoromethyl)phenyl]ureido}phenyl)-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7h)**: Yield 79.6%, mp 206–209 ℃. ^{1}H–NMR spectrum (600 MHz, DMSO-d_6), δ: 9.00 s(1H), 8.85 s(1H), 7.99 dd(1H, *J*=6.4, 2.6 Hz), 7.71–7.57 m(1H), 7.50–7.34 m(2H), 7.25–7.14 m(2H), 6.98 s(2H), 6.84 d(1H, *J*=7.7 Hz), 4.30 s(1H), 3.82–3.76 m(2H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 385.1(NH_2), 2 196.9(CN), 1 735.9(C=O), 1 680.0(C=O), 1 558.5 (C=C_{arom}), 1 433.1(C=C_{arom}), 1 319.3(CF_3), 1 222.9, 1 134.1, 1 072.4. MS(ESI) *m*/*z*(%): 549.1[M+H]$^+$, 571.1[M+Na]$^+$. Anal. calcd for $C_{25}H_{20}F_4N_4O_6$: C, 54.75%; H, 3.68%; N, 10.22%; found: C, 54.90%; H, 3.73%; N, 10.28%.

Methyl 6-amino-5-cyano-4-{3-[3-(2,4-dichlorophenyl)ureido]phenyl}-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7i)**: Yield 84.8%, mp 205–207 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 9.47 s(1H), 8.38 s(1H), 8.22 d(1H, *J*=9.0 Hz), 7.62 d(1H, *J*=2.5 Hz), 7.46–7.33 m(2H), 7.30–7.19 m(2H), 6.99 s(2H), 6.85 d(1H, *J*=7.7 Hz), 4.31 s(1H), 3.90–3.74 m(2H), 3.67 s(3H), 3.54 s(3H). IR spectrum, v, cm^{-1}: 3 398.6(NH_2), 3 336.9(NH_2), 2 196.9(CN), 1 718.6(C=O), 1 678.1(C=O), 1 545.0 (C=C_{arom}), 1 476.3(C=C_{arom}), 1 280.7, 1 209.4. MS (ESI) *m*/*z*(%): 531.0[M+H]$^+$, 553.0[M+Na]$^+$. Anal. calcd for $C_{24}H_{20}Cl_2N_4O_6$: C, 54.25%; H, 3.79%; N, 10.54%; found: C, 54.41%; H, 3.85%; N,10.60%.

Methyl 6-amino-5-cyano-4-{3-[3-(2,4-difluorophenyl)ureido]phenyl}-2-(2-methoxy-2-oxoethyl)4*H*-pyran-3-carboxylate**(7j)**: Yield 77.2%, mp 177–180 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO- d_6), δ: 9.05 d(1H, *J*=6.8 Hz), 8.47 d(1H, *J*= 4.8 Hz), 8.24–7.93 m(1H), 7.50–7.12 m(4H), 7.11–6.64 m(4H), 4.29 d(1H, *J*=7.1 Hz), 3.91–3.71 m(2H), 3.67 d(3H, *J*=7.2 Hz), 3.53 d(3H, *J*=7.2 Hz). IR spectrum, v, cm^{-1}: 3 400.5(NH_2), 2 196.9(CN), 1 689.6(C=O), 1 552.7 (C=C_{arom}), 1 436.9(C=C_{arom}), 1 273.0, 1 197.8, 1 070.5. MS(ESI) *m*/*z*(%): 499.1[M+H]$^+$, 521.1[M+Na]$^+$. Anal. calcd for $C_{24}H_{20}F_2N_4O_6$: C, 57.83%; H, 4.04%; N, 11.24%; found: C, 57.88%; H, 4.09%; N,11.30%.

Methyl 6-amino-5-cyano-4-{3-[3-(5-fluoro-2-methylphenyl)ureido]phenyl}-2-(2-methoxy-2-oxoethyl)-4*H*-pyran-3-carboxylate**(7k)**: Yield 73.9%, mp 153–155 ℃. ^{1}H–NMR spectrum(600 MHz, DMSO-d_6), δ: 9.19 s(1H), 8.02 s(1H), 7.86 dd(1H, *J*=12.1, 2.6 Hz), 7.44 d(1H, *J*=8.1 Hz), 7.33–7.11 m(3H), 6.99 s(2H), 6.84 d(1H, *J*= 7.7 Hz), 6.78–6.66 m(1H), 4.31 s(1H), 3.89–3.74 m(2H), 3.67 s(3H), 3.54 s(3H), 2.22 s(3H). IR spectrum, v, cm^{-1}:

3 628.1(NH_2), 3 394.7(NH_2), 3 188.3, 2 183.7(CN), 1 685.8(C=O), 1 599.0(C=C_{arom}), 1 445.0(C=C_{arom}), 1 271.1, 1 068.6. MS(ESI) *m*/*z*(%): 495.1$[M+H]^+$, 517.1$[M+Na]^+$. Anal. calcd for $C_{25}H_{23}FN_4O_6$: C, 60.73%; H, 4.69%; N, 11.33%; found: C, 60.89%; H, 4.78%; N, 11.43%.

2.2 *In vitro* anticancer activity

The anticancer activities of all the newly synthesized 4*H*-pyran derivatives bearing aryl urea(**7a**–**7k**) were evaluated against human lung cancer cell lines H460, human lung adenocarcinoma cell line A549 and human colon cancer cell line HT-29 using the standard MTT-based assay *in vitro*. Sorafenib was used as positive control. The IC_{50} of the compounds against these cancer cells were presented in Tab. 1. Results from Tab. 1 indicated that most of the synthesized compounds demonstrated good to moderate anticancer activity with the range of IC_{50}=(0.82±0.04) to (8.44±0.83)μmol·L^{-1}. Meanwhile, the cytotoxicities of three synthesized compounds were evaluated(**7a**, **7c** and **7g**) on the LO2 cell line(normal human hepatocytes cells) *in vitro*. Compared with sorafenib, no compounds showed obvious cytotoxicity against this cell line. Structure-activity relationship(SAR) studies indicated that the equipment of electron-withdrawing groups(EWGs) on the R part showed a positive effect on the antiproliferative activities, such as compound **7c**(R=3-Cl, IC_{50}=0.82 μmol·L^{-1} against H460), which was better than that of compound **7a**(R=H, IC_{50}=3.18 μmol·L^{-1} against H460). However, incorporation of other groups on the R part reduced the antiproliferative activities(R=4-OCH_3, 2,4-OCH_3 and 2-CH_3-5-F).

2.3 AO/EB staining

H460 and A549 cell lines were treated for 48 h with the most cytotoxicity of compound **7c** at three different concentrations 0.10, 1.00, 10.00 μmol·L^{-1}. The double staining was executed using equimolar mixture of AO/EB. The stained cells were analysed by fluorescent microscopy. It could be inferred from Fig. 2 that the control cells showed normal morphology and appeared green in color. Cells treated with different concentrations of compound **7c** clearly showed the morphological changes such as cell shrinkage, membrane blebbing, chromatin condensation and apoptotic body formation, suggesting that compound **7c** induced dose-dependent cell death in H460 and A549 cancer cells *via* apoptosis.

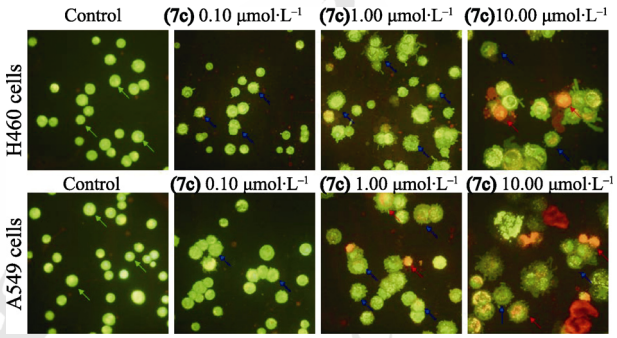


Fig. 2 AO/EB stained apoptosis of H460 and A549 cell lines with different concentrations of compound **7c** for 48 h

Green arrow indicated normal cells, the blue arrow indicated early apoptotic cells and the red arrow indicated later apoptotic cells.

图 2 AO/EB 染色法检测不同浓度的化合物 **7c** 作用 H460 和 A549 细胞 48 h 的诱导细胞凋亡情况

绿色箭头表示正常细胞，蓝色箭头表示早期凋亡细胞，红色箭头表示晚期凋亡细胞。

Tab. 1 *In vitro* antiproliferative activities of compounds **7a**–**7k**($\bar{x}\pm s$, *n*=3)

表 1 化合物 7a~7k 的体外抗增殖活性($\bar{x}\pm s$, *n*=3)

Compounds	Structure	IC_{50}/(μmol·L^{-1})			
	R	H460	A549	HT-29	LO2
7a	H	3.18±0.21	5.05±0.42	2.82±0.16	55.84±1.95
7b	4-methoxy	3.66±0.23	6.34±0.52	5.25±0.35	ND[a]
7c	3-chloro	0.82±0.04	0.98±0.06	2.15±0.19	62.69±2.32
7d	2-(trifluoromethoxy)	2.75±0.15	2.39±0.21	3.95±0.20	ND
7e	3-(trifluoromethyl)	2.94±0.19	2.83±0.24	4.10±0.17	ND
7f	3,4-dimethoxy	3.91±0.36	5.24±0.22	8.44±0.83	ND
7g	4-chloro-3-(trifluoromethyl)	2.14±0.12	2.37±0.14	1.37±0.08	45.36±3.88
7h	4-fluoro-3-(trifluoromethyl)	2.22±0.10	2.08±0.17	3.03±0.19	ND
7i	2,4-dichloro	4.71±0.38	2.60±0.24	2.91±0.12	ND
7j	2,4-difluoro	3.04±0.23	4.19±0.31	4.08±0.14	ND
7k	5-fluoro-2-methyl	5.94±0.29	7.11±0.18	3.24±0.26	ND
sorafenib	–	3.20±0.14	2.83±0.25	3.96±0.38	69.39±4.10

Note: [a]ND=not determined.

注释：[a]ND=未测试。

3 Conclusion

In summary, eleven novel 4*H*-pyran derivatives bearing arylurea moieties were designed, synthesized and evaluated for their biological activitives. The screening of anticancer activity led to the identification of a most promising compound **7c** with IC_{50} values of (0.82±0.04)μmol·L^{-1}, (0.98±0.06)μmol·L^{-1}, (2.15±0.19)μmol·L^{-1} and (62.69±2.32)μmol·L^{-1} against H460, A549, HT-29 and LO2 cell lines, respectively, representing a promising lead for further optimization. The initial SARs analysis disclosed that electron-withdrawing groups on the R part were more preferred. Meanwhile, the results AO/EB assays on H460 and A549 cells indicated of that compound **7c** could induce H460 and A549 cells apoptosis in a dose dependent manner. The above results demonstrated that pyran-arylurea was a promising skeleton for anticancer drug development and worth further study.

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收稿日期：2021-10-15

(本文责编：陈怡心)