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Synthesis and biological evaluation of tetrahydrobenzo[b]pyran derivatives as potential anti-ovarian cancer agents.

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Abstract

The synthesis and characterization of four novel pyran derivatives (1-4) have been done by means of IR, 1H NMR, HRMS, and single crystal X-ray crystallography. The antitumor activities of the four compounds were studied in order to inhibit three human ovarian cancer cells, which are OVCAR, HRA and COC1, growth by MTT assay. It was found that compound 4 exerted much more potent activities against all of these cell lines than other compounds did.

Keywords Pyran, Crystal, Ovarian cancer.

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Introduction

Oophoroma is one of the most common malignant tumours in the world [1]. Currently, the traditional therapies of surgery, chemotherapy and radiation therapy play an important role in the systemic treatment of ovarian cancer [2]. However, the treatment outcome is generally poor. Thus, it is very important to find an effective alternative treatment for oophoroma [3].

Much attention has been caught among many chemists in organic and medicinal filed on the synthesis of pyrans and their derivatives for many years as a great deal of natural and synthetic products contain this heterocyclic nucleus. Pyrans has a variety of pharmacological and biological activities including antitumor, analgesic and ulcerogenic, anti-inflammatory, anticoagulant, photo triggering, and fungicidal properties, moreover, it can be applied as anticoagulants in the production of pesticides [4-6]. In particular, the antitumor activity of pyran compounds has received considerable attention among researchers because of their cytotoxic activity against numerous types of cancers, including malignant melanoma, leukemia, renal cell carcinoma, prostate and breast cancer cell progression [7,8]. As mentioned above, we are here to report the synthesis of four new pyran derivatives (Figure 1) as well as their antitumor activity.

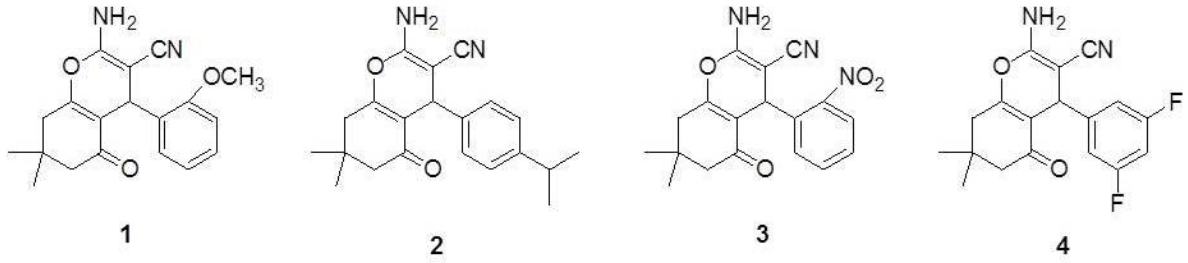

Figure 1. Chemical structures of compounds 1-4.

Experimental

Apparatus and materials

The way to obtain IR spectra (400-4000 cm^{-1}) is using a Brucker Equinox-55 spectrophotometer, 1H NMR spectra using a Varian Inova-400 spectrometer (at 400 MHz), Mass spectra using a micrOTOF-Q II mass spectrometer. What is more, a XT-4 micro melting apparatus plays a role as taking on the melting points, and the thermometer remained uncorrected.

Three human ovarian cancer cells (OVCAR, HRA and COC1) were obtained by paying to Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS) (Beijing, China).

Synthesis and characterization of compounds 1-4

The synthesis of compounds 1-4 were on the basis of a reported procedure [9]. A mixture of 1,1-dimethyl-3,5-cyclohexanedione (10 mmol), aromatic aldehyde (10 mmol), malononitrile (10 mmol) and 4-(dimethylamino) pyridine

(DMAP) (1 mmol) in ethanol (100 mL) was refluxed for 2-3 h and then cooled to room temperature. After filtering the sediments, they were orderly washed with ice-cooled water and ethanol finally becomes dried under a vacuum.

2-Amino-4-(2-methoxyphenyl)-3-cyano-7,7-dimethyl-5-oxo-4H-5,6,7,8-tetrahydrobenzo[b]pyran (1): 208-209°C. IR (KBr pellet cm^{-1}): 3406, 2155, 1639, 1530, 1323, 1041, 761 cm^{-1}. 1H NMR (DMSO-d6, δ, ppm): 7.143-7.186 (m, 1H), 6.944-7.008 (m, 2H), 6.819-6.873 (m, 3H), 4.484 (s, 1H), 3.755 (s, 3H), 2.431-2.574 (m, 2H), 2.233-2.274 (d, 1H), 2.049-2.089 (d, 1H), 1.050 (s, 3H), 0.974 (s, 3H). HRMS (ESI +): m/z: calcd for $C_{19}H_{20}N_2O_3$: 347.1366 [M^+Na^+]; found: 347.1327.

2-Amino-4-(isopropylphenyl)-3-cyano-7,7-dimethyl-5-oxo-4H-5,6,7,8-tetrahydrobenzo[b]pyran (2): 210-211°C. IR (KBr pellet cm^{-1}): 3370, 3330, 3181, 2962, 2187, 1650, 1601, 1373, 1241, 1134, 1034, 846 cm^{-1}. 1H NMR (DMSO-d6, δ, ppm): 7.149-7.169 (d, 2H), 7.037-7.057 (d, 2H), 6.964 (s, 2H), 4.135 (s, 1H), 2.822-2.856 (t, 1H), 2.503-2.521 (m, 1H), 2.237-2.277 (d, 1H), 2.139 (s, 1H), 2.092 (s, 1H), 1.191 (s, 3H), 1.174 (s, 3H), 1.046 (s, 3H), 0.977 (s, 3H). HRMS (ESI +): m/z: calcd for C21H24N2O2: 359.1730 [M^+Na^+]; found: 359.1788.

2-Amino-4-(2-nitrophenyl)-3-cyano-7,7-dimethyl-5-oxo-4H-5,6,7,8-tetrahydrobenzo[b]pyran (3): 239-240°C. IR (KBr pellet cm^{-1}): 3446, 2360, 1666, 1546, 1353, 1091, 761 cm^{-1}. 1H NMR (DMSO-d6, δ, ppm): 7.810-7.833 (q, 1H), 7.643-7.684 (m, 1H), 7.408-7.450 (m, 1H), 7.352-7.374 (q, 1H), 7.195 (s, 2H), 4.947 (s, 1H), 2.439-2.563 (m, 1H), 2.187-2.228 (d, 1H), 2.000-2.090 (t, 2H), 1.019 (s, 3H), 0.888 (s, 3H). HRMS (ESI^+): m/z: calcd for C18H17N3O4: 362.1111 [M^+Na^+]; found: 362.1267.

2-Amino-4-(3,5-difluorophenyl)-3-cyano-7,7-dimethyl-5-oxo-4H-5,6,7,8-tetrahydrobenzo[b]pyran (4): 230-241°C. IR (KBr pellet cm-1): 3398, 3211, 2198, 1683, 1659, 1620, 1601, 1373, 1251, 1037, 992, 734 cm^{-1}. 1H NMR (DMSO-d6, δ, ppm): 7.131 (s, 2H), 7.035-7.093 (m, 1H), 6.842-6.888 (m, 2H), 4.281 (s, 1H), 2.465-2.610 (m, 1H), 2.151-2.272 (q, 2H), 2.091 (s, 1H), 1.039 (s, 3H), 0.983 (s, 3H). HRMS (ESI^+): m/z: calcd for C18H16F2N2O2: 353.1072 [M^+Na^+]; found: 353.1099.

Crystal structure determination

Table 1. *Crystal data, data collection and structure refinement of compound 1.*

Formula	$C_{19}H_{20}N_2O_3$
Mr	324.37
Temperature/K	293 (2)
Crystal system	Monoclinic
Space group	$P2_1/n$
a/Å	8.8915 (4)
b/Å	17.5552 (9)
c/Å	11.0684 (5)
α/°	90
β/°	97.750 (4)
γ/°	90
V/Å3	1711.91 (14)
Z	4
Dcalc/g·cm^{-3}	1.259
μ(M_o $K_α$)/mm^{-1}	0.086
θ range/°	2.32 to 25.00
Reflections collected	6694
No. unique data [R(int)]	3020 [0.0227]
No. data with I ≥ 2σ(I)	2257
R_1	0.0445
ωR_2(all data)	0.1161
CCDC	1477404

According to the evaporation of chloroform solution, Suitable single crystals of compound 1 come to hand. The diffraction data were acquired on a Bruker Smart Apex CCD area detector using a graphite monochromated Mo Kα radiation (λ=0.71073 Å) at room temperature. The structure was solved by using the program SHELXL-97 [10] and Fourier difference techniques, and refined by full-matrix least-squares method on F2. All hydrogen atoms were added academically. Crystallographic data for compound 1 have been showed in Table 1.

Antitumor activity

Viability of human ovarian cancer cells (OVCAR, HRA and COC1) was determined with the help of the MTT assay. Once the confluency of cells reaches to 70-80% that was treated with all kinds of concentrations of the synthesized compounds with 1% dimethyl sulfoxide (DMSO) as a negative control. And then the cells were incubated for 48 h, another 4 h incubation were done after adding 20 μL of MTT solution (5 mg/mL in PBS). What after, the medium was aspirated carefully, and added with 150 μL of DMSO. After taking 15 min to incubate, the FlexStation 3 benchtop multi-mode microplate reader (Molecular Devices, USA) measured the optical density which is 490 nm. Data were recorded and analyzed for the procedure of the effects of the test substances on cell viability and growth inhibition.

Results and Discussion

Molecular structure

The 1H NMR, IR and MS for the products are suit for the title compounds. In the 1H NMR spectra of compounds 1-4, single peaks at δ 4.484, δ 4.135, δ 4.947 and δ 4.281 ppm

characteristic of the CH group respectively. The single crystals of compound 1 were cultured for X-ray diffraction analysis so that can make the confirmation of the configuration of the product.

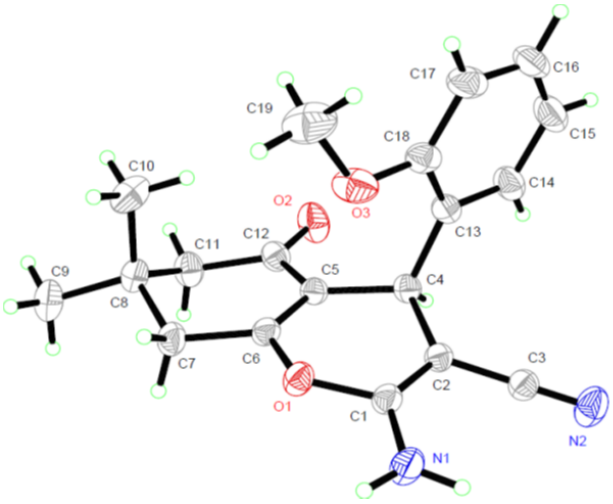

Figure 2. *Molecular structure of compound 1.*

Figure 2 presented us the crystal structure of compound 1. The 4H-pyran ring of compound 1 is nearly planar [maximum deviation=0.0685 Å] and the adjoining ketone ring takes a flattened chair conformation [dihedral angle (C5-C6-C7-C8)=15.654°]. The 4H-pyran ring is nearly in vertical direction to the benzene ring [dihedral angle=86.324°] and is almost coplanar with the mean plane of the ketone ring [dihedral angle=8.767°]. In addition, the molecules were collected to form a three-dimensional structure through π-π stacking together with no intermolecular hydrogen bonding interactions, as illustrated in Figure 3.

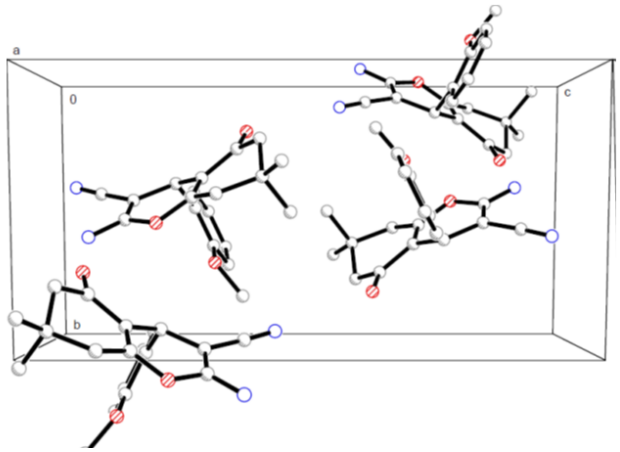

Figure 3. *Packing diagram in a unit cell of compound 1 (H atoms are omitted for clarity).*

Antitumor activity

Three human ovarian cancer cells (OVCAR, HRA and COC1) ,which is the representative three tumor types respectively, were used in the systematic analysis of the antitumor activities of the newly synthesized compounds 1-4 in vitro. For the sake of comparing, the evaluation of the cytotoxicity of cyclophosphamide, a standard antitumor drug, was set in the same condition. The results represent that the four compounds possessed a set number of antitumor activities against the three tumor cell lines and their inhibitory action become intensify with the corresponding higher concentration. The relevant half maximal inhibitory concentration (IC50) and IC90 values (dose of the compound which cause a different reduction of survival values, one is 50% and the other one is 90%) are listed in Table 2.

As can be seen in Table 2, there is great difference in the antitumor activity among the four compounds. Compound 4 represented much stronger antitumor activity against the three human ovarian cancer cells with IC_{50} and IC_{90} values of 23.44-71.23 μg/mL and 45.23-132.34 μg/mL, respectively, which is much lower than the IC_{50} and IC_{90} values (55.30-76.07 μg/mL and 98.45-145.67 μg/mL) of the standard antitumor drug cyclophosphamide. However, compounds 1-3 showed lower antitumor activity with relatively higher IC_{50} and IC_{90} values.

Table 2. *IC_{50} and IC_{90} values of compounds 1-4 and cyclophosphamide against three tumor cell lines (μg/mL).*

Drugs	SMMC-7721		Hep G2		HB611	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Compound 1	286.21	567.44	312.32	601.22	296.01	610.31
Compound 2	301.26	612.36	298.31	589.43	356.32	623.62
Compound 3	311.34	656.33	334.11	612.55	412.12	654.23
Compound 4	26.11	50.21	23.44	45.23	71.23	132.34
Cyclophosphamide	55.30	110.99	58.89	98.45	76.07	145.67

Conclusion

In this study, four new pyran compounds were synthesized and were found to exhibit cytotoxicity towards three human ovarian cancer cells (OVCAR, HRA and COC1). It showed that the potential of pyran compounds to be developed into the new antitumor agent. However, the exact target is still unknown, whether it has an exact target or is just a cytotoxic agent, the detailed mechanisms of the inhibitory effects need to be further investigated.

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