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Pyran Derivatives: Anti-Breast Cancer Activity and Docking Study[1]

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Abstract—Four novel pyran derivatives **1**–**4** are synthesized and characterized by IR, ^{1}H NMR, HRMS, and single crystal X-ray data. Anticancer activity of the compounds is tested against four human breast cancer cells (HCC1937, HCC70, MDA-MB-436, and BT474) by MTT assay. Molecular docking study supports the biological assay data and suggests that compound **2** has a potential as an anticancer agent.

Keywords: pyran, anticancer activity, molecular docking study

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Dihydropyrans represent an active class of compounds demonstrating a wide range of biological activities, including anticoagulant, insecticidal, anthelminthic, hypnotic, antifungal, and HIV protease inhibiting [1, 2]. Such activities made dihydropyrans to be attractive targets of organic synthesis [3]. In the current study four new pyran derivatives **1**–**4** (Fig. 1) were synthesized and their potential anti-tumor activity tested.

EXPERIMENTAL

IR spectra (KBr discs) were recorded on a Brucker Equinox-55 spectrophotometer. ^{1}H NMR spectra were measured on a Varian Inova-400 spectrometer using DMSO-d_6 as a solvent. Mass spectra were measured on a micrOTOF-Q II mass spectrometer. Melting points were determined on a XT-4 micro melting apparatus.

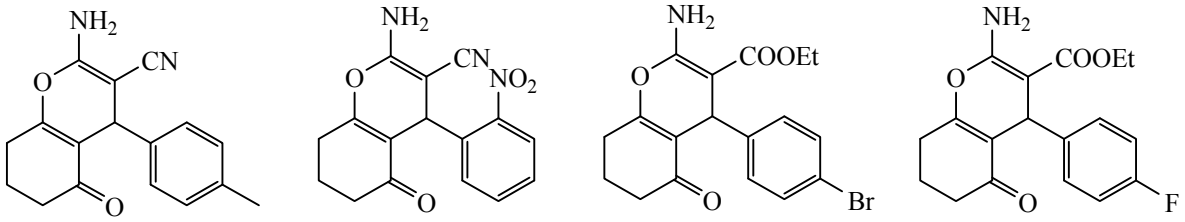
Synthesis of compounds 1–4. The compounds **1**–**4** were synthesized according to a developed earlier procedure [4]. A mixture of 3,5-cyclohexanedione (10 mmol) with aromatic aldehydes (10 mmol), malononitrile (10 mmol) and 4-(dimethylamino) pyridine (DMAP) (1 mmol) in ethanol (100 mL) was refluxed for 2–3 h, then cooled down to room temperature. The precipitated products were filtered off and washed with ice-cold water and ethanol, and then dried under vacuum.

2-Amino-4-(4-methylphenyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1). mp 221–222°C. IR spectrum, ν, cm^{-1}: 3021, 2634, 2421, 1712, 1601, 985. ^{1}H NMR spectrum, δ, ppm: 6.989–7.073 m (6H), 4.138 s (1H), 2.585–2.623 m (2H), 2.250–2.305 m (5H), 1.869–1.978 m (2H). HRMS (ESI$^+$): *m*/*z*: calculated for $C_{17}H_{16}N_2O_2$: 303.1104 [*M* + Na]$^+$; found: 303.1123.

2-Amino-4-(2-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (2). mp 234–235°C. IR spectrum, ν, cm^{-1}: 3066, 2711, 2533, 1710, 1601, 980. ^{1}H NMR spectrum, δ, ppm: 7.800–7.823 q (1H), 7.634–7.656 q (1H), 7.374–7.451 m (2H), 7.202 s (2H), 4.933 s (1H), 2.578–2.608 t (2H), 2.133–2.255 m (2H), 1.824–1.953 m (2H). HRMS (ESI$^+$): *m*/*z*: calculated for $C_{16}H_{13}N_3O_4$: 334.0798 [*M* + Na]$^+$; found: 334.0783.

Ethyl 2-amino-4-(4-bromophenyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (**3**). mp 198–199°C. IR spectrum, ν, cm^{-1}: 3712, 2612, 2319, 1723, 1612, 972. ^{1}H NMR spectrum, δ, ppm: 7.592 s (2H), 7.386–7.400 q (2H), 7.106–7.120 d (2H), 4.505 s (1H),

[1] The text was submitted by the authors in English.



1 **2** **3** **4**

Fig. 1. Formulas of compounds **1–4**.

3.945–3.959 q (2H), 2.602–2.619 t (2H), 2.276–2.331 m (1H), 2.200–2.245 m (1H), 1.932–1.972 m (1H), 1.810–1.842 m (1H), 1.070–1.093 t (3H). HRMS (ESI^+): *m*/*z*: calculated for $C_{18}H_{18}BrNO_4$: 414.0317 $[M + Na]^+$; found: 414.0321.

Ethyl 2-amino-4-(4-fluorophenyl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carboxylate (4). mp 200–201°C. IR spectrum, ν, cm^{-1}: 3371, 2712, 2541, 1821, 1611, 921. ^1H NMR (DMSO-d_6, δ, ppm): 7.568 s (2H), 7.161–7.185 m (2H), 7.000–7.030 t (2H), 4.535 s (1H),

Table 1. Crystal data and structure refinement for compounds **1** and **2**

Parameter	Value	
	1	**2**
Formula	$C_{17}H_{16}N_2O_2$	$C_{16}H_{13}N_3O_4$
M_r	280.32	311.29
Temperature, K	293(2)	293(2)
Crystal system	Triclinic	Triclinic
Space group	*P*-1	*P*-1
a, Å	8.5783 (8)	8.8933 (6)
b, Å	8.7427 (8)	9.3992 (9)
c, Å	11.0298 (9)	10.9991 (9)
α, deg	72.652 (8)	73.190 (8)
β, deg	69.856 (8)	85.811 (6)
γ, deg	80.054 (8)	78.133 (7)
V, $Å^3$	739.01 (11)	861.23 (12)
Z	2	2
D_{calc}, g/cm^3	1.260	1.200
μ(Mo$K_α$), mm^{-1}	0.084	0.088
θ range, deg	2.45–24.99	2.56–25.00
Reflections collected	4709	5674
Number of unique data (R_{int})	2600 (0.0221)	3032 (0.0291)
Number of data with $I \geq 2\sigma(I)$	1889	2169
R_1	0.0506	0.0619
ωR_2 (all data)	0.1714	0.2028
CCDC	1559701	1559700

Table 2. Hydrogen-bond geometry (Å) for compounds **1** and **2**

Comp. no.	D–H···A	D–H	H···A	D···A	∠D–H···A, deg	Symmetry code
1	N^1–H^{1A}···N^2	0.8600	2.3100	3.167(3)	176.00	$1-x, 1-y, 1-z$
	N^1–H^{1B}···O^2	0.8600	2.1600	2.997(2)	164.00	$-1+x, y, z$
	C^{12}–H^{12A}···N^2	0.9300	2.5100	3.258(3)	138.00	$2-x, 1-y, 1-z$
2	N^1–H^{1A}···N^2	0.8600	2.2000	3.057(3)	172.00	$-x, 1-y, 2-z$
	C^3–H^{3A}···O^3	0.9800	2.2000	2.920(3)	129.00	$-x, 1-y, 2-z$
	C^3–H^{3A}···N^3	0.9800	2.5900	3.041(3)	108.00	$-x, 1-y, 2-z$

3.950–3.962 d (2H), 2.596–2.624 m (2H), 2.214–2.305 m (2H), 1.935–1.966 m (1H), 1.808–1.848 m (1H), 1.066–1.090 t (3H). HRMS (ESI^+): *m*/*z*: calculated for $C_{18}H_{18}FNO_4$: 354.1118 $[M + Na]^+$; found: 354.1129.

Crystal structure. Suitable single crystals of compounds **1** and **2** were crystallized from chloroform (Table 1). Diffraction data were acquired on a Bruker Smart Apex CCD area detector using a graphite monochromated MoK_α radiation (λ = 0.71073 Å) at room temperature. The structure was solved by using the program SHELXL-97 [5]. All hydrogen atoms were added academically.

MTT assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [6] was used in determination of anticancer activity of compounds **1** and **2** and doxorubicin. First 1×10^4 cells per well were seeded in Dulbecco's modified Eagle's medium (DMEM, 100 μL) supplemented with 10% fetal bovine serum in each well of 96-well micro-culture plates and incubated for 24 h at 37°C in a CO_2 incubator. Compounds were deliquated to the particular concentrations in culture medium and added to the wells with respective vehicle control. After 48 h of incubation, MTT (10 μL, 5 mg/mL) was replenished into each well and the plates continued to be incubated for 4 h more. Then the supernatant from each well was carefully removed, compounds were dissolved in DMSO (100 μL) and absorbance at 570 nm was recorded.

Simulation details. Autodock Vina v1.2 was used in the study of the binding mode of compounds **1** and **2** with tubulin [7]. The geometry structures of the compounds were optimized by quantum chemistry calculations by Gaussian 09, B3LYP theory (6-31g* basic set). AutoDockTools v1.5.6 were used to transfer 1AS0 and optimize the structures. Only polar hydrogens of the structures were considered. The center coordinates of search grid of tubulin were set to

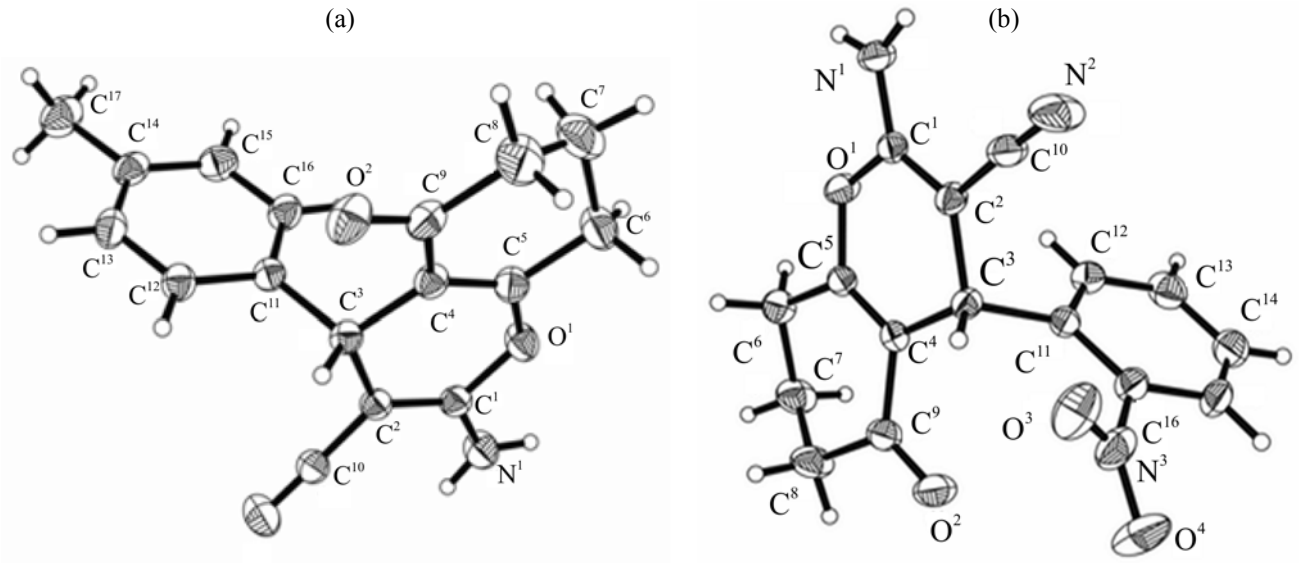


Fig. 2. Molecular structures of compounds (a) **1** and (b) **2**.

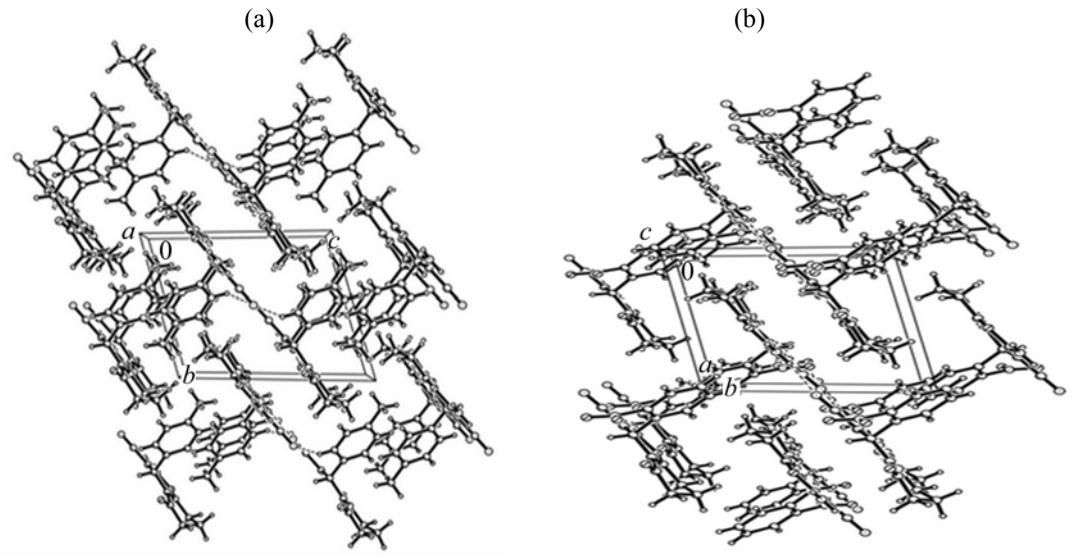


Fig. 3. The crystal packing structure of (a) compound **1** along the *a* axis and (b) compound **2** along the *c* axis.

38.15, –26.99, and 6.18, length of the search grid was 15. All parameters needed by autodock vina were used as default if not mentioned specifically. The calculated data were analyzed and visualized by PyMoL v1.8.6.

RESULTS AND DISCUSSION

Crystal structures of compounds **1** and **2** were studied by X-ray diffraction single-crystal structural analysis. Due to similarity of two structures, the structure of compound **1** was selected as the sample in this study. The method demonstrated that the compound **1** belonged to the triclinic crystal system with the space group of *P*-1. The compound **1** two-dimensional supra-molecular structure was determined by three types of H-bond: N–H···N, N–H···O and C–H···N (Table 2). The molecular structural units of compounds **1** and **2** are presented in Fig. 2 and their crystals packing in Fig. 3. The estimated bond distances were in their normal ranges and could be comparable with the reported compounds.

***In vitro* anticancer screening.** Evaluation of compounds **1–4** for their *in vitro* cytotoxic activity against four human breast cancer cells (HCC1937, HCC70, MDA-MB-436, and BT474) was carried out using doxorubicin as the reference drug. Cytotoxicity was assessed at concentrations of 0, 0.01, 0.1, 10 and 100 μg/mL. The relation between remaining fraction and drug concentration was plotted to obtain survival curves of the three tumor cell lines after accretion of the specified compounds.

Comparison of IC_{50} values determined for the compounds **1–4** and the reference drug are listed in Table 3. According to the accumulated data, compound **2** demonstrated the highest cytotoxic effect against the four human cancer cells, and it was close to that of the reference drug.

Table 3. Results of *in vitro* cytotoxic activity of **1**–**4** and doxorubicin on four human breast cancer cells

Compound	IC_{50}, μM			
	HCC1937	HCC70	MDA-MB-436	BT474
1	30	25	25	25
2	15	10	10	8
3	>100	>100	>100	>100
4	>100	>100	>100	>100
Doxorubicin	8	8	10	10

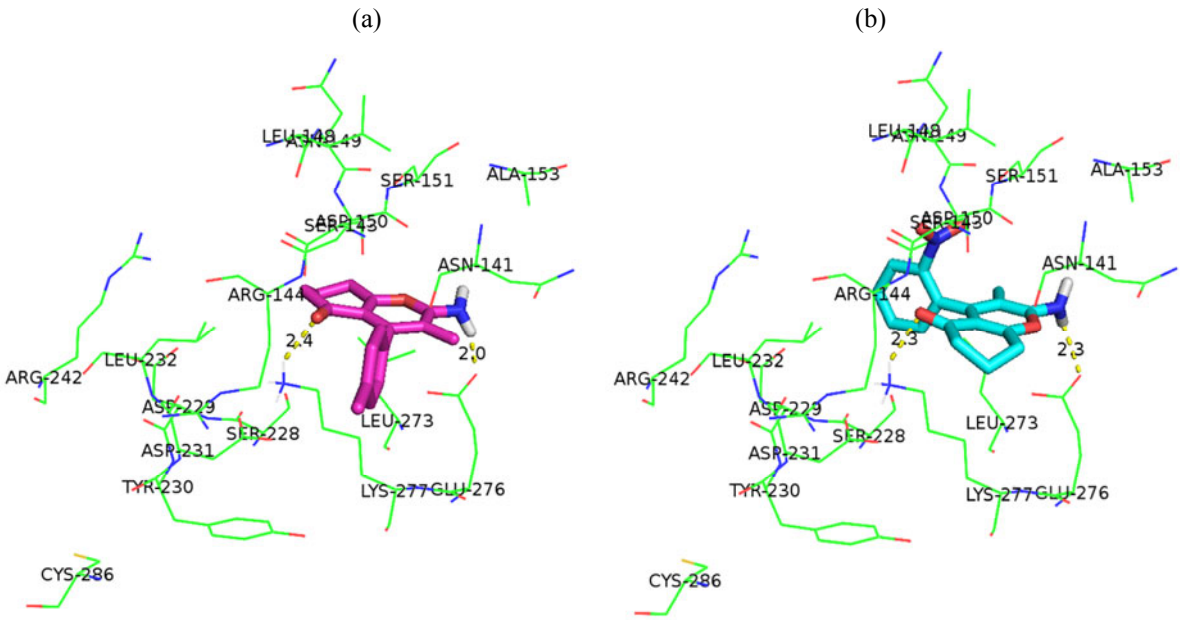


Fig. 4. The interaction mode of compounds (a) **1** and (b) **2** with tubulin at the colchicine binding site.

Molecular docking. The possible binding modes of compounds **1** and **2** with receptor 1AS0 were studied by autodocking. As shown in Fig. 4, the fused pyran and cyclohexanone rings of compounds **1** and **2** interacted with the surrounding protein molecule. Two hydrogen bonds were formed by oxygen atom of carbonyl group and hydrogen atom of the amino groups of proteins (GLU-276 and LYS-277). For compound **1**, the bond length values were determined to be 2.4 and 2.0 nm respectively; for compound **2**, the lengths of both H-bonds were 2.3 nm. The hydrogen bonds between compound **2** and the protein was determined to be stronger than that of compound **1**, which could be supported by the lowest free binding energy values for compounds **1** and **2** (–7.5 and –8.8 kcal/mol respectively).

CONCLUSIONS

Novel pyran derivatives **1–4** are synthesized and their anti-tumor activity is studied. The compound **2** demonstrates the most potent anti-cancer activity, supported by molecular docking simulation which indicates its stronger H-bonding with the protein.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Du, X., Zhu, Y., Peng, Z., Cui, Y., Zhang, Q., Shi, Z., Guan, Y., Sha, X., Shen, T., Yang, Y., Li, X., Wang, Z., Li, X., and Liu, G., *J. Dairy Sci*., 2018, vol. 101, p. 3476. doi 10.3168/jds.2017-13234
2. Li, J., Lv, C.W., Li, X.J., Qu, D., Hou, Z., Jia, M., Luo, X.X., Li, X., and Li, M.K., *Molecules,* 2015, vol. 20, p. 17469. doi 10.3390/molecules200917469
3. Li, J., Xue, X.Y., Li, X., Hou, Z., Yang, X.H., Qu, D., Zhou, Y., Zhang, Z.D., Luo, X.X., and Li, J.T., *Arch. Pharm. Res.*, 2016, vol. 39, p. 1349. doi 10.1007/s12272-015-0614-7
4. Li, J., Sui, Y.P., Xin, J.J., Du, X.L., Li, J.T., Huo, H.R., Ma, H., Wang, W.H., Zhou, H.Y., Zhan, H.D., Wang, Z.J., Li, C., Sui, F., and Li, X., *Bioorg. Med. Chem. Lett.,* 2015, vol. 25, p. 5520. doi 10.1016/j.bmcl.2015.10.063
5. Sheldrick, G.M., *SHELXL-97*, Program for Solution Crystal Structure and Refinement, Göttingen, Germany: University of Göttingen, 1997.
6. Kupcsik, L., *Methods Mol. Biol*., 2011, vol. 740, p. 13. doi 10.1007/978-1-61779-108-6_3
7. Trott, O. and Olson, A.J., *J. Comput. Chem*., 2010, vol. 31, p. 455. doi 10.1002/jcc.21334