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Antitumor Activities and DNA-Binding Studies of Some Resveratrol Bioisosteres

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Several bioisosteres of resveratrol (**1-7**) were designed and synthesized. The *in vitro* antitumour activities screening revealed that compound **1** exhibited better inhibition activities than the commercial anticancer drug 5-fluorouracil on MGC80-3 cell lines, with IC_{50} of 35.57 ± 1.49 μM and compound **2** showed higher cytotoxicity than 5-fluorouracil on A-375 cells, with IC_{50} of 44.09 ± 1.58 μM, while compound **3** displayed preferable inhibition than 5-fluorouracil on Hep G2 cells, with IC_{50} of 15.52 ± 0.86 μM, respectively. In addition, the binding properties of compounds **1-7** to DNA were investigated by fluorescence emission titration and the result shows that seven compounds have medium binding with DNA, with quenching constant values in the range of 10^2-10^3 L mol^{-1}.

Keywords: Resveratrol, Antitumor activity, DNA-binding studies, Bioisosterism.

INTRODUCTION

Bioisosterism is a tactics of pharmaceutical chemistry for the rational design of new drugs, applied with a lead compound as a special process of molecular modification and it is of momentous significance and has been widely used in the discovery of new drugs.

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) is a naturally occurring polyphenol mainly present in grapes, red wine and other plants. Previous work has demonstrated that resveratrol inhibit cancer promotion and possess cancer chemopreventive activity[1-4]. Therefore, design and synthesis of resveratrol analogs with better medicinal virtue have greatly attracted bioinorganic chemists' interest and become a hot research topic in bioinorganic chemistry. The aim of the present work was to synthesize some resveratrol bioisosteres by replacing CH= group in resveratrol skeleton with N atom and to evaluate their *in vitro* antitumor activities. Preliminary investigation on whether compounds **1-7** interact with deoxyribonucleic acid (DNA) to exert their antitumor activities is also investigated.

EXPERIMENTAL

The seven resveratrol bioisosteres (Fig. 1) were synthesized according to the literature[5]. All chemicals and solvents used were of analytical grade. The fluorescence spectroscopy was scanned by the RF-5301 spectrophotometer.

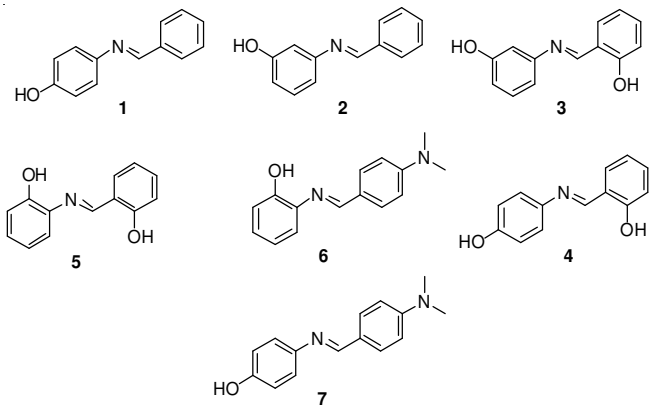

Fig. 1. Structure of compounds **1-7**

in vitro Cytotoxicity

General procedure for cytotoxic evaluation *in vitro*: A-375, HepG2 and MGC-803 cells were seeded into 96-well microculture plates and allowed to adhere for 24 h, respectively. After cells were exposed to compounds at concentrations from 100 to 0.01 μM for 48 h, medium was aspirated and replenished with complete medium. IC_{50} was evaluated by MTT tetrazolium dye assay[6]. Each experiment was performed three times.

DNA-binding studies: The DNA-binding property of the seven compounds were investigated by fluorescence quenching spectroscopy. At room temperature, the nine compounds and ct-DNA were dissolved in DMF-water (7:3, v:v) to the proper

concentration. After respective addition of 25 µL ct-DNA solution into 500 µL solution of the compound **1-7**, the mixtures were settled to the volume of 1 mL and allowed to stand for 0.5 h (until the stable) with intermittent shaking and the fluorescence spectroscopy was scanned in the RF-5301 fluorospectrophotometer.

RESULTS AND DISCUSSION

***in vitro* Cytotoxicity:** The *in vitro* cytotoxic potency of compounds **1-7** were evaluated by MTT assay against A375, HepG2 and MGC-803 tumour cell lines (with 5-fluorouracil as the positive control). As showed in Table-1, after incubation of tumour cells with each compound at the concentration of 2×10^{-5} M for 48 h as identical conditions, these compounds exhibit different antitumour activity and certain selectivity. It is important to note that compound **1** exhibited better inhibition activities than the commercial anticancer drug 5-fluorouracil on MGC80-3 cell lines, with IC_{50} of 35.57 ± 1.49 µM. Moreover, compound **2** showed higher cytotoxicity than 5-fluorouracil on A-375 cells, with IC_{50} of 44.09 ± 1.58 µM, while compound **3** displayed preferable inhibition than 5-fluorouracil on Hep G2 cells, with IC_{50} of 15.52 ± 0.86 µM, respectively. The above results demonstrate that the rational design of resveratrol bioisosteres as novel antitumor leads is feasible.

TABLE-1
IC_{50} (µM) VALUES OF COMPOUNDS **1-7** TOWARDS THE SELECTED TUMOUR CELL LINES

Compounds	A375	Hep G2	MGC80-3
1	>100	>100	35.57±1.49
2	44.09±1.58	>100	>100
3	>100	15.52±0.86	>100
4	>100	>100	>100
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
5-Fluorouracil	46.25±2.35	29.98±2.37	46.93±2.09

DNA binding studies: Although there are other kinds of biological targets in tumour cells, containing RNA, enzyme or proteins, it is popularly considered that DNA is the primary target for many anticancer drugs[7,8]. Analogously, interactions between small molecules and DNA belonged the primary action mechanisms of antitumour activity. In order to investigate the binding properties of compounds **1-7** to DNA, fluorescence-quenching spectroscopy studies was carried out.

The fluorescence-quenching spectroscopy was employed to study DNA-binding property of seven compounds. Upon addition of ct-DNA into the DMF-H_2O (v:v = 7:3) solution of the seven compounds, the fluorescence intensity decreased gradually (Fig. 2).

In the presence of DNA at different concentration, the fluorescences of compounds were quenched and the spectral information were analysed by the well-known Stern-Volmer model[9]:

$$\frac{F_0}{F} = 1 + K_q \tau [Q] = 1 + K_{SV}[Q] \quad (1)$$

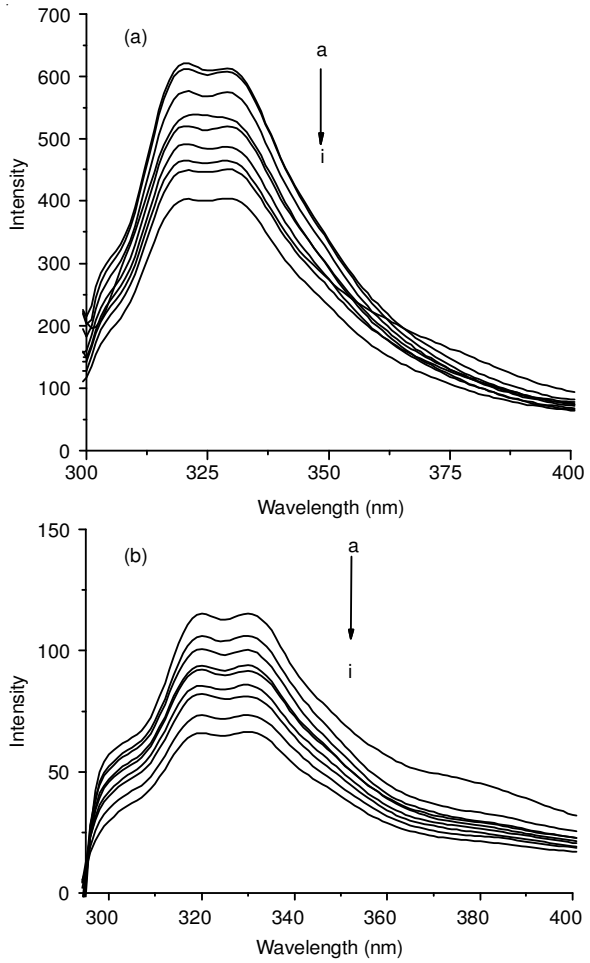

Fig. 2. Fluorescence spectra of compounds **2** (A) and **7** (B) quenched by DNA (The concentration of **2** and **7** were both 1×10^{-3} mol L^{-1}; the concentrations of DNA a→i(a→i) were: 0, 4.45, 6.81, 9.08, 11.35, 13.62, 15.89, 20.43, 24.97×10^{-5} (0, 4.45, 6.81, 7.95, 9.08, 11.35, 15.89, 20.43, 22.7×10^{-5}) mol L^{-1}, respectively)

where F_0 and F were the steady-state fluorescence intensities in the absence and presence of quencher (ct-DNA), respectively, K_q is the bimolecular quenching constant; t is the lifetime of the fluorophore. K_{sv} is the Stern-Volmer quenching constant and [Q] is the concentration of quencher (DNA in this work). Accordingly eqn. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q] and the Stern-Volmer plots were shown in Fig. 3 (compounds **2** and **7**). As can be seen in Table-2, K_q were in the range of 5.47×10^{10} to 4.86×10^{11} M^{-1} s^{-1}, were greater than the value of the maximum scatter collision quenching constant 2×10^{10} L mol^{-1} s^{-1}[10], which indicated that the fluorescence quenching was caused by a specific interaction. When fluorescent material and fluorescence quenching body formed the complex happened static destroyed, type (1) can become Lineweaver-Burk eqn. 11

$$\frac{F_0}{F_0 - F} = 1 + \frac{1}{K_A C(Q)} \quad (2)$$

Accordingly eqn. 2 was applied to determine K_A by linear regression of a plot of $(F_0\text{-}F)^{-1}$ against $[Q]^{-1}$. The value of binding constants (K_A) for the nine compounds can be determined (Table-2).

TABLE-2
BINDING CONSTANTS K_A AND BINDING NUMBERS OF THE COMPOUNDS

Entry	1	2	3	4	5	6	7
K_A/M^{-1}	9.67×10^3	1.04×10^3	6.14×10^3	3.02×10^3	3.07×10^3	1.59×10^2	9.08×10^3
K_{SV}/M^{-1}	9.41×10^2	2.47×10^3	1.81×10^3	3.58×10^3	6.71×10^2	5.47×10^2	4.86×10^3
$kq/M^{-1}\ s^{-1}$	9.41×10^{10}	2.47×10^{11}	1.81×10^{11}	3.58×10^{11}	6.71×10^{10}	5.47×10^{10}	4.86×10^{11}
n	1	1	1	1	1	1	1

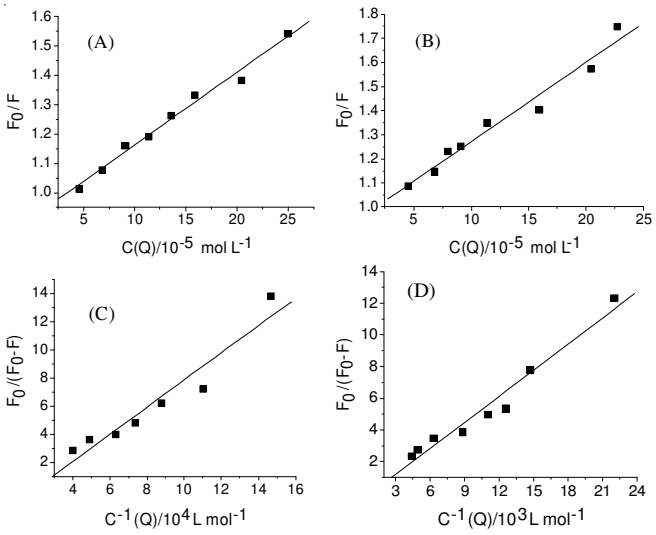

Fig. 3. Stern-Volmer (A (**2**) and B (**7**)) and Lineweaver-Burk (C (**2**) and D (**7**)) plots of the fluorescence quenching of complex **2** and **7**, respectively

Table-2 showed that the binding constants K_A were in the range of 1.59×10^2 to 9.67×10^3 M^{-1}, compounds **1** and **7** showed good DNA-binding abilities with the binding constant of 9.08×10^3 and 9.67×10^3 M^{-1}, respectively. Compounds **6** showed much worst DNA-binding abilities than other eight compounds, with the binding constant of 1.59×10^2 M^{-1}. In addition, all the binding numbers of compounds **1-7** with DNA have been found to be **1**, suggesting that each unit of compound is bond with a unit of ct-DNA. The above binding constant data suggested a moderate DNA-binding of these seven compounds, indicating DNA may be a possible primary target of compounds **1-7**. The present work is expected to be much useful in the design of new drugs with novel biomedical functions and DNA-binding properties.

Conclusion

In conclusion, we have designed and synthesized some resveratrol bioisosteres as antitumor drug leads. Of all the compounds, compound **1** exhibited better inhibition activities than the commercial anticancer drug 5-fluorouracil on MGC80-3 cell lines and compound **2** showed higher cytotoxicity than 5-fluorouracil on A-375 cells, while compound **3** displayed preferable inhibition than 5-fluorouracil on Hep G2 cells. In addition, DNA-binding studies indicated that DNA may be a possible primary target of compounds **1-7**.

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REFERENCES

1. M.S. Jang, E.N. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W.W. Beecher, H.H.S. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon and J.M. Pezzuto, *Science*, **275**, 218 (1997).
2. A. Damianaki, E. Bakogeorgou, M. Kampa, G. Notas, A. Hatzoglou, S. Panagiotou, C. Gemetzi, E. Kouroumalis, P.-M. Martin and E. Castanas, *J. Cell. Biochem.*, **78**, 429 (2000).
3. M. Jang and J.M. Pezzuto, *Drugs Exp. Clin. Res.*, **25**, 65 (1999).
4. L.A. Stivala, M. Savio, P. Carafoli, F. Perucca, L. Bianchi, G. Maga, L. Forti, U.M. Pagnoni, A. Albini, E. Prosperi and V. Vannini, *J. Biol. Chem.*, **276**, 22586 (2001).
5. Y. Zhang, B.Q. Zou, K. Wang, Y.M. Pan, H. Liang, X.H. Yi and H.S. Wang, *Med. Chem. Res.*, **21**, 1341 (2012).
6. X.C. Huang, M. Wang, Y.M. Pan, X.Y. Tian, H.S. Wang and Y. Zhang, *Bioorg. Med. Chem. Lett.*, **23**, 5283 (2013).
7. C.X. Zhang and S.J. Lippard, *Curr. Opin. Chem. Biol.*, **7**, 481 (2003).
8. C.H. Wu, D.H. Wu, X. Liu, G. Guoyiqibayi, D.D. Guo, G. Lv, X.M. Wang, H. Yan, H. Jiang and Z.H. Lu, *Inorg. Chem.*, **48**, 2352 (2009).
9. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, p. 280 (1999).
10. M.R. Eftink and C.A. Ghiron, *Anal. Biochem.*, **114**, 199 (1981).
11. X.J. Chang, Y. Huang and Q. He, *Acta Chim. Sin.*, **63**, 223 (2005).