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Design, synthesis and biological evaluation of 3,4-dihydronaphthalen-1(2*H*)-one derivatives as Bcl-2 inhibitors

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Abstract A series of new 3,4-dihydronaphthalen-1(2*H*)-one derivatives (**6a–f**) were designed, and synthesized by the Claisen–Schmidt condensation reaction. Their structures were characterized by NMR, FTIR, and MS spectroscopy. Their antitumor activities against human neoplastic cell lines Hela, Hepg2, K562, THP-1, SW1990, MIA PaCa-2, NCI-H460 and SK-BR-3 by the MTT method exhibited obvious anticancer activities and their cytotoxicities for LO2 cell lines were lower than DOX, especially for **6b** and **6d**. The inhibition activities against the Bcl-2 protein for **6b** was evaluated and the result shows that lipophilic 3,4-dimethoxybenzylidene and 3,4-dihydronaphthalen-1(2*H*)-one could bind slightly to the active pockets of the Bcl-2 protein.

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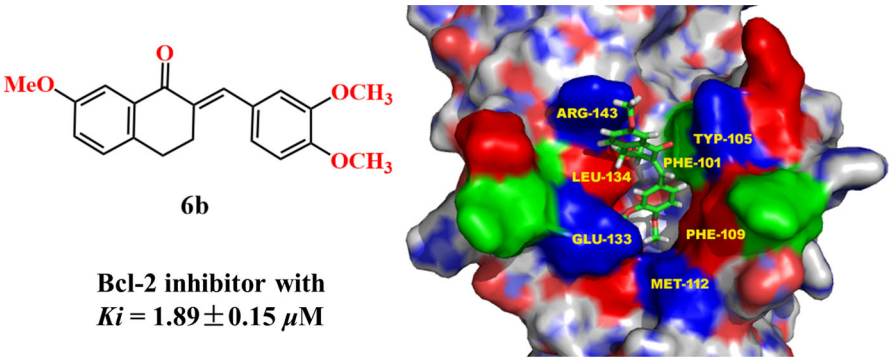
Graphical Abstract



Keywords Anticancer activity · Cytotoxicity · Bcl-2 inhibitors

Introduction

Malignant tumors seriously impact on human health. Currently, surgery and chemotherapy can prolong life for up to a few years in some patients [1–3]. Many chemotherapy drugs on the market are used for clinical applications. In the process of treatment, apoptosis is triggered by internal or external cellular stimuli, and the evasion of apoptosis is a hallmark of human cancer and contributes to the resistance to conventional therapies. Therefore, targeting key apoptosis regulators is a most promising strategy of the treatment for malignant tumors [4, 5].

The Bcl-2 (B cell lymphoma 2) family of proteins [6, 7] are very important therapeutic targets for malignant tumors and include Bcl-2, Bcl-xL, Mcl-1, Bfl-1/A1, Bcl-B and Bcl-w, and pro-apoptotic proteins, such as BAK, BAX, BID, BIM and BAD. The study and development of Bcl-2 inhibitors has become one of the important strategies for treating malignant tumors. Recently, some small-molecule Bcl-2 inhibitors, such as ABT-199 [8], Gossypol [9] and Obatoclax [10], have been used as antitumor agents in clinical trials [11]. However, these antitumor agents have some problems in clinical application, such as relatively low bioavailability, high toxicity, and so on.

Some 3,4-dihydronaphthalen-1(2*H*)-one derivatives have been investigated as novel modulators of allergic and inflammatory phenomena [12] and inhibitors of retinoic acid (RA)-metabolizing enzymes as potential agents for the treatment of skin conditions and cancer [13]. 3,4-dihydronaphthalen-1(2*H*)-one is a very potential active fragment in antitumor agents, but it is certainly rare for 3,4-dihydronaphthalen-1(2*H*)-one derivatives to be potent Bcl-2 family inhibitors. In addition, some antiproliferative drugs containing the pharmacophore of α,β-unsaturated keto can interact as the primary binding site to bio-thiols from susceptible neoplasms while having lower toxicity toward normal cells [14–16]. It will provide greater

antiproliferative activities against tumor cell lines when they can be combined between 3,4-dihydronaphthalen-1(2*H*)-one and α,β-unsaturated keto.

With this aim in mind, in this study, a series of new 3,4-dihydronaphthalen-1(2*H*)-one derivatives were designed and synthesized by the Claisen–Schmidt condensation reaction. Their antitumor activities against human neoplastic cell lines Hela, Hepg2, K562, THP-1, SW1990, MIA PaCa-2, NCI-H460, SK-BR-3 and against anti-apoptotic Bcl-2 proteins have been evaluated by the MTT method.

Experimental section

Materials and methods

Anisole, succinic anhydride, aluminum chloride, hydrazine hydrate, diethylene glycol (DEG), polyphosphoric acid (PPA), 2-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 2-fluorobenzaldehyde, 4-fluorobenzaldehyde, furfural, 2-thenaldehyde were purchased from Sinopharm Chemical Reagent (Shanghai, China). *R*(-)-gossypol (AT-101) was purchased from Selleckchem (Shanghai, China). They were used as obtained without further purification. NMR spectra were obtained on a Bruker Avance 400 MHz spectrometer (^{1}H NMR at 400 Hz, ^{13}C NMR at 100 Hz) in $CDCl_3$ using TMS as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Infrared (IR) spectra were obtained in the 400–4000 cm^{-1} range using a Bruker tensor-27 FTIR Spectrometer. All melting points were measured on a digital melting point apparatus and were uncorrected. The docking mode was simulated using the SYBYLX 2.0 software (Tripos; Certara, St. Louis, MO, USA).

Synthesis of 6a–f: general procedure [16]

7-Methoxy-3,4-dihydronaphthalen-1(2*H*)-one (5) was obtained following literature reports: 5- (1.76 g, 10.0 mmol) and 2-methoxybenzaldehyde (1.36 g, 10.0 mmol), or 3,4-dimethoxybenzaldehyde (1.66 g, 10.0 mmol), or 2-fluorobenzaldehyde (1.24 g, 10.0 mmol), or 4-fluorobenzaldehyde (1.24 g, 10.0 mmol), or furfural (0.96 g, 10.0 mmol), or 2-thenaldehyde (1.12 g, 10.0 mmol) were dissolved in 15 mL of methanol. Next, 5.0 mL 10% NaOH solution added, and the mixture was stirred for 3–4 h at ambient temperature (monitored by TLC). The solvent was removed through a pouring process, and the residues were purified on silica gel by a column using petroleum ether/EtOAc (4:1, v/v) as the eluent to afford light yellow powders **6a–f**.

*(E)-7-methoxy-2-(2-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (**6a**)* Yellow powder, yield: 87%; m.p. 132–134 °C. IR (cm^{-1}): 2944(m), 2839(m), 1662(s), 1595(s), 1483(s), 1423(s), 1292(s), 1234(s),1107(m), 1014(s), 979(m), 889(m), 809(m), 773(s). ^{1}H NMR: δ 8.00 (s, 1H), 7.65 (s, 1H), 7.30 (m, 2H), 7.18 (d, 1H), 7.01 (d, 1H), 6.98 (d, 1H), 6.94 (m, 2H), 3.88 (d, 6H), 3.04 (t, $J = 5.8$ Hz, 2H), 2.89 (t, $J = 5.8$ Hz, 2H). ^{13}C NMR: δ 187.82, 158.53, 158.19, 136.09, 135.41, 134.33,

132.73, 130.14, 130.09, 129.41, 124.85, 121.33, 119.98, 110.61, 110.23, 55.52, 55.44, 28.20, 27.73. HRMS: calcd for $C_{19}H_{19}O_3$ [M + H^+] 295.1334; found 295.1338.

(E)-2-(3,4-dimethoxybenzylidene)-7-methoxy-3,4-dihydronaphthalen-1(2H)-one ***(6b)*** Yellow solid, yield: 88%; m.p. 32–34 °C. IR (cm^{-1}): 2943(m), 2841(m), 1668(s), 1585(s), 1509(s), 1472(s), 1270(s), 1130(s), 1020(m), 887(m), 796(m), 744(s). ^{1}H NMR: δ 7.82 (s, 1H), 7.61 (s, 1H), 7.17 (d, 1H), 7.09 (m, 2H), 7.01 (d, 1H), 6.92 (m, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.14 (t, J = 5.9 Hz, 2H), 2.89 (t, J = 5.9 Hz, 2H). ^{13}C NMR: δ 187.62, 158.58, 149.51, 148.65, 136.88, 135.68, 134.33, 133.72, 129.31, 128.62, 123.25, 121.27, 113.16, 110.83, 110.24, 55.89, 55.88, 55.49, 27.89, 27.42. HRMS: calcd for $C_{20}H_{21}O_4$ [M + H^+] 325.1440; found 325.1443.

(E)-2-(2-fluorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-1(2H)-one ***(6c)*** Yellow powder, yield: 82%; m.p. 124–126 °C. IR (cm^{-1}): 2938(m), 2842(m), 1669(s), 1595(s), 1295(s), 1222(s), 1145(s), 1098(s), 1024(m), 972(m), 922(m), 775(s). ^{1}H NMR: δ 7.83 (s, 1H), 7.65 (s, J = 2.5 Hz, 1H), 7.37 (m, J = 7.2 Hz, 2H), 7.18 (m, 3H), 7.10 (m, 1H), 3.89 (d, 3H), 2.98 (t, J = 5.9 Hz, 2H), 2.91 (t, J = 5.9 Hz, 2H). ^{13}C NMR: δ 187.39, 161.84, 159.36, 158.62, 136.18, 135.83 (d, $^1J_{CF}$ = 362.0 Hz), 130.70 (d, $^3J_{CF}$ = 3.0 Hz), 130.28 (d, $^3J_{CF}$ = 8.3 Hz), 129.51, 129.35 (d, $^4J_{CF}$ = 2.7 Hz), 123.80 (d, $^2J_{CF}$ = 14 Hz), 123.79, 121.69, 115.81 (d, $^2J_{CF}$ = 22 Hz), 110.21, 55.54, 28.06, 27.80. HRMS: calcd for $C_{18}H_{16}FO_2$ [M + H^+] 283.1134; found 283.1131.

(E)-2-(4-fluorobenzylidene)-7-methoxy-3,4-dihydronaphthalen-1(2H)-one ***(6d)*** Yellow powder, yield: 87%; m.p. 123–125 °C. IR (cm^{-1}): 2938(m), 2836(m), 1665(s), 1595(s), 1496(s), 1429(s), 1295(s), 1228(s), 1149(m), 1027(s), 979(m), 915(m), 812(m), 734(s). ^{1}H NMR: δ 7.79 (s, 1H), 7.60 (s, J = 2.7 Hz, 1H), 7.39 (dd, J = 8.4, 5.5 Hz, 2H), 7.16 (d, J = 8.3 Hz, 1H), 7.08 (dt, J = 9.7, 5.7 Hz, 3H), 3.86 (d, 3H), 3.06 (t, J = 5.8 Hz, 2H), 2.88 (t, J = 5.8 Hz, 2H). ^{13}C NMR: δ 187.64, 163.81, 161.33, 158.64, 135.79, 135.54, 134.67 (d, $^1J_{CF}$ = 105.0 Hz), 131.89 (d, $^4J_{CF}$ = 4.0 Hz), 131.72 (d, $^3J_{CF}$ = 8.0 Hz), 129.43, 121.56, 115.52 (d, $^2J_{CF}$ = 22.0 Hz), 110.24, 55.53, 27.92, 27.27. HRMS: calcd for $C_{18}H_{16}FO_2$ [M + H^+] 283.1134; found 283.1129.

(E)-7-methoxy-2-(thiophen-2-ylmethylene)-3,4-dihydronaphthalen-1(2H)-one ***(6e)*** Yellow powder, yield: 85%; m.p. 98–100 °C. IR (cm^{-1}): 2954(m), 2839(m), 1659(s), 1586(s), 1496(s), 1426(s), 1289(s), 1226(s), 1094(m), 1027(s), 963(m), 825(m), 708(s). ^{1}H NMR: δ 8.05 (s, 1H), 7.61 (s, 1H), 7.52 (d, 1H), 7.41 (d, 1H), 7.20 (m, 1H), 7.15 (d, 1H), 7.07 (m, 1H), 3.88 (s, 3H), 3.19 (t, J = 5.9 Hz, 2H), 2.98 (t, J = 5.9 Hz, 2H). ^{13}C NMR: δ 187.07, 158.62, 139.11, 135.67, 134.41, 133.24, 131.75, 129.48, 129.34, 129.31, 127.54, 121.27, 110.25, 55.52, 27.26, 27.18. HRMS: calcd for $C_{16}H_{15}O_2S$ [M + H^+] 271.0793; found 271.0787.

(E)-2-(furan-2-ylmethylene)-7-methoxy-3,4-dihydronaphthalen-1(2H)-one ***(6f)*** Yellow powder, yield: 89%; m.p. 89–91 °C. IR (cm^{-1}): 2954(m), 2832(m), 1659(s),

1585(s), 1499(s), 1426(s), 1292(s), 1247(s), 1167(m), 1100(s), 1027(s), 935(m), 882(m), 822(m), 776(s). ^{1}H NMR: δ 7.58 (d, 2H), 7.54 (s, 1H), 7.05 (d, 1H), 7.02 (d, 1H), 6.68 (s, 1H), 6.50 (s, 1H), 3.83 (s, 3H), 3.26 (t, $J = 5.9$ Hz, 2H), 2.91 (t, $J = 5.9$ Hz, 2H). ^{13}C NMR: δ 187.12, 158.52, 152.41, 144.32, 136.15, 134.24, 131.76, 129.35, 122.81, 121.14, 116.50, 112.21, 110.22, 55.42, 27.42, 26.81. HRMS: calcd for $C_{16}H_{15}O_3$ [M + H^+] 255.1021; found 255.1026.

Anticancer testing with MTT method

Compounds **6a–f** were screened against human neoplastic cell lines: human cervical carcinoma cells (HeLa), human liver hepatocellular carcinoma cell line (HePG2), human chronic myelogenous leukemia cell line (K562), human acute mononuclear granulocyte leukemia (THP-1), human pancreatic cancer cells (SW1990 and MIA PaCa-2), human non-small cell lung cancer (NCI-H460) and human breast carcinoma cells (SK-BR-3) and human normal heptical cell line (LO2) by MTT assay (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide; MTT; Dojindo Laboratories, Tokyo, Japan). The cells were seeded in a 96-well plate at a density of 1×10^4 cells/well with 200 μL medium per well for 24 h. The cells were treated with the compounds (10, 8, 5, 3, 2, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL) and incubated for 24 h. Cells with only culture media were used as a negative control. After removal of the media, MTT (20 μL, 5 mg/mL) was added and the plates were incubated for 4 h at 37 °C. The media were removed and 150 μL DMSO was added to dissolve the dark-blue formazan crystals. The optical density was measured by a multi-well plate reader (TECAN, Männedorf, Switzerland) at 540 nm. The experiments were examined in triplicate, and the IC_{50} values are expressed in Table 1. Doxorubicin (DOX) was used as a positive control at the concentrations of 1.5, 1.2, 1.0, 0.8, 0.5, 0.3, 0.1, 0.05 and 0.01 μg/mL.

Binding assay for Bcl-2 protein [11]

A 26-residue Bid-BH3 peptide (QEDIIRNIARHLAQVGDSMDRSIPPG) was labeled at the *N*-terminus by 5-carboxyfluorescein succinimidyl ester (FAM) as the fluorescence-labeled peptide (5-FAMQEDIIRNIARHLAQVGDSMDRSIPPG). The 5-FAM-Bid-BH3 peptide had a Kd value of 58 nM to bind to the Bcl-2 protein [17]. In the competitive binding experiments, the Bcl-2 protein and compounds were preincubated in the PBS assay solution for 30 min at room temperature. Then, the 5-FAM-Bid-BH3 peptide was added into the solution and incubated for 20 min. The volume of the total solution was 200 μL. Finally, the solutions were transferred into 96-well plates with 60 μL per well and three wells per sample. The polarization values (milipolarization units, mpol) were measured at 540 nm using the multi-well plate reader (TECAN). In the binding experiments, compounds **6a–f** were prepared in DMSO at nine concentrations: 100, 50, 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL. Each value was reproduced in three independent assays and expressed with standard deviations. Doxorubicin (DOX) and *R*(-)-gossypol (AT-101) were used as a positive control at the concentrations of 100, 50, 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 μg/mL.

Table 1 Anticancer activity and cytotoxicity of **6a–f** and DOX

Compd	IC_{50} (μM)								
	Hela	Hepg2	K562	THP-1	SW1990	MIA PaCa-2	NCI-H460	SK-BR-3	LO2
6a	>20	12.43 ± 0.68	9.89 ± 0.31	9.78 ± 0.51	16.45 ± 0.72	11.09 ± 0.11	14.76 ± 0.29	15.65 ± 0.97	>20
6b	3.65 ± 0.68	6.12 ± 0.09	1.27 ± 0.89	0.56 ± 0.18	5.15 ± 0.34	5.49 ± 0.27	9.26 ± 0.56	8.37 ± 0.28	12.98 ± 0.15
6c	12.21 ± 0.43	>20	8.89 ± 0.29	8.17 ± 0.25	14.05 ± 0.29	>20	17.55 ± 1.57	12.25 ± 0.97	>20
6d	4.21 ± 0.12	6.54 ± 0.24	1.67 ± 0.27	0.61 ± 0.05	5.76 ± 0.48	6.84 ± 0.24	10.46 ± 0.47	10.54 ± 0.34	14.05 ± 0.43
6e	18.47 ± 0.87	>20	9.43 ± 0.25	11.02 ± 0.09	12.94 ± 0.38	>20	>20	13.40 ± 0.68	>20
6f	>20	>20	8.65 ± 0.31	9.69 ± 0.06	11.65 ± 0.68	>20	>20	15.63 ± 0.47	>20
DOX	4.62 ± 0.13	4.12 ± 0.07	1.78 ± 0.05	0.79 ± 0.18	2.21 ± 0.21	1.02 ± 0.12	2.42 ± 0.04	1.31 ± 0.11	10.30 ± 0.42

Molecular docking

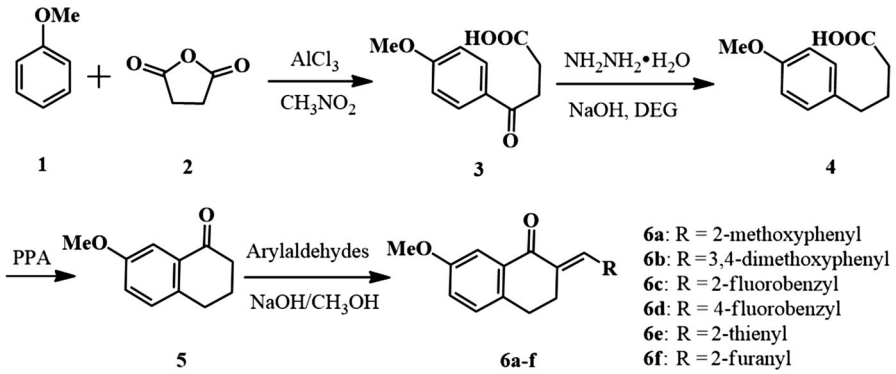
A molecular docking program using the SYBYLX 2.0 software was used with default values. The structures of **6b** and the Bcl-2 protein were optimized according to Ref. [11].

Results and discussion

Structural analysis

Our synthetic route to the target compounds is shown in Scheme 1. A vital intermediate 7-methoxy-3,4-dihydronaphthalen-1(2*H*)-one (5) as a white solid was prepared in an overall yield of 50% for the three steps based on the method in the literature [18, 19]. First, anisole and succinic anhydride were reacted by F–C acylation to generate 4-(4-methoxyphenyl)-4-oxobutanoic acid (3) by catalysis of Lewis acid ($AlCl_3$) in 92% yield. Secondly, after Wolff–Kishner–Huang–Minlon reduction, 4-phenoxybutanoic acid (4) was generated in 90% yield. Then, the key compound 5 was prepared for the cyclization in the presence of PPA in the lower yield of 61%. Lastly, 5 and arylaldehydes was subjected to Claisen–Schmidt condensation to yield a series of new 3,4-dihydronaphthalen-1(2*H*)-one derivatives (**6a–f**), which have been characterized by NMR, FTIR, and MS spectroscopy. Some selected spectral data are discussed below.

In the ^{1}H NMR spectra of **6a–f**, the chemical shifts of 8.00, 7.82, 7.83, 7.79, 8.05, and 7.58 ppm, respectively, appear as a singlet attributed to the proton of α,β-unsaturated ketone pharmacophore. All the compounds characteristically show two groups of triplets from the two intra-annular methylene groups at δ 3.19–2.88 ppm. From the FTIR spectra, the characteristic absorption bands in the range of 2954–2832 cm^{-1} correspond to the stretching vibration band of methyl and methylene of **6a–f**. The characteristic bands at around 1669–1659 cm^{-1} are attributed to –C=O group in α,β-unsaturated ketone. The strong bands at around



Scheme 1 Synthetic strategy of asymmetric compounds **6a–f**

1595–1585 cm^{-1} are attributed to the stretching vibration of –C=C bonds in α,β-unsaturated ketone pharmacophore. The strong stretching vibration bands of C-O can be found in the range of 1295 and 1222 cm^{-1}. Additionally, ^{13}C NMR, MS spectroscopy further confirm the correctness of their structures.

Anticancer activity and cytotoxicity

The anticancer activities against human carcinoma cell lines Hela, Hepg2, K562, THP-1, SW1990, MIA PaCa-2, NCI-H460 and SK-BR-3 were evaluated by the MTT method. Doxorubicin (DOX) was selected as the positive control. As shown in Table 1, their anticancer activities for **6a** and **6c** with the *o*-substituent group (–OCH_3 and –F) and **6e–f** with the heterocyclic substituent (thiophene and furan ring) are very poor against all the experimental cells, which can be proved from the high IC_{50} values (>8 μM). The results display weaker anticancer activity than the positive control DOX. Fortunately,the target compounds of **6b** with the 3,4-dimethoxy substituent group and **6d** with the *para*-fluoro substituent group against Hela, K562 and THP-1 cells were better than that against the others. Their IC_{50} values can be below 5.0 μM. Therefore, compounds **6b** and **6d** display better anticancer activity than DOX, and their IC_{50} values are only 0.56 ± 0.18 and 0.61 ± 0.05 μM, respectively. In order to evaluate the cytotoxicity of the target compounds, the LO2 cell line was selected as the experimental cells. The results suggested that their IC_{50} values were higher than 12 μM, which cytotoxicities are lower than DOX. Structure analysis shows that the 3,4-dihydronaphthalen-1(2*H*)-one scaffold substituted by *para*-substituents, such as **6b** and **6d**, are more beneficial for improved anticancer activity and decreased cytotoxicity toward human carcinoma cell lines.

Inhibition activities against Bcl-2 protein

The study of inhibiting anti-apoptotic Bcl-2 proteins was explored for 3,4-dihydronaphthalen-1(2*H*)-one derivatives. The binding affinities for Bcl-2 with K_i values are about 1.89 ± 0.15 μM of **6b** and 2.05 ± 0.11 μM of **6d**, respectively, while the binding affinities for other compounds are more than 10 μM (Table 2). Compounds **6b** and **6d** are close to the positive control DOX (1.84 ± 0.21 μM), but

Table 2 The binding affinities of **6a–f** to the Bcl-2 protein

Compd	K_i (μM)
6a	10.43 ± 0.71
6b	1.89 ± 0.15
6c	17.54 ± 1.03
DOX	1.84 ± 0.21
6d	2.05 ± 0.11
6e	46.21 ± 3.12
6f	37.18 ± 1.18
AT-101	0.41 ± 0.32

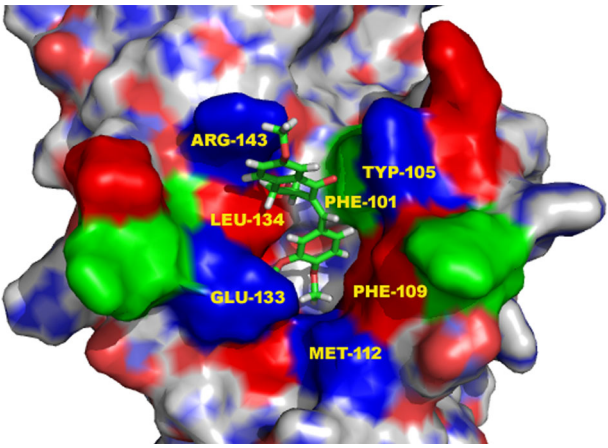


Fig. 1 The docking mode of compound **6b** in the active site of the Bcl-2 protein

significantly higher than AT-101 (0.41 ± 0.32 μM). To better study the interactions of 3,4-dihydronaphthalen-1(2*H*)-one derivatives with the Bcl-2 protein, compound **6b** with better anticancer activity and lower cytotoxicity was selected to dock with the Bcl-2 protein in the active site using the SYBYLX 2.0 software. As shown in Fig. 1, the 3,4-dimethoxybenzylidene group as the lipophilic group in **6b** could reasonably bind to the largest active pocket of the Bcl-2 protein, while the 3,4-dihydronaphthalen-1(2*H*)-one scaffold can also orient to the other active pocket of the Bcl-2 protein.

Conclusions

In this study, a series of new 3,4-dihydronaphthalen-1(2*H*)-one derivatives (**6a**–**f**) were designed, synthesized and evaluated for their antitumor activities against Hela, Hepg2, K562, THP-1, SW1990, MIA PaCa-2, NCI-H460 and SK-BR-3 by the MTT method. Compounds **6b** and **6d** exhibited obvious anticancer activities and lower cytotoxicities for LO2 than DOX. The inhibition activities against the Bcl-2 protein for **6b** shows that lipophilic 3,4-dimethoxybenzylidene and 3,4-dihydronaphthalen-1(2*H*)-one could bind to the active pockets of the protein.

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