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Synthesis, antitumor activity evaluation of some new N-aroyl- α , β -unsaturated piperidones with fluorescence

Jufeng Sun¹*, Suwen Wang²*, Hongjuan Li¹, Wenguo Jiang¹, Guige Hou¹, Feng Zhao¹, and Wei Cong¹

¹School of Pharmacy, Binzhou Medical University, Yantai, Shandong, P.R. China and ²Yantai Yuhuangding Hospital Emergency, Yantai, Shandong, P.R. China

Abstract

Novel *N*-aroyl-*a*, β -unsaturated piperidones, series **1**, series **2** and series **3** (featuring 2-bromo-4,5-dimethoxybenzylidene, 4-dimethylaminobenzylidene and 4-trifluoromethylbenzylidene, respectively), were synthesized as candidate cytotoxins. Most of the compounds displayed potent cytotoxicity against the human neoplastic cell lines SK-BR-3, PG-BE1, NCI-H460, MIA PaCa-2 and SW1990 *in vitro*, and approximately 64% of the IC₅₀ values were lower than 5 μ M. Among those tested, compound **1b** of series **1**, **3a**, **3d** and **3e** of series **3** proved to be the most active. Importantly, **1b** displayed marked inhibitory effects on tumor growth *in vivo* and had no apparent toxicity to mice; this was evaluated by a nude mouse PG-BE1 xenograft model. In addition, the fluorescent properties of compounds series **1–3** were investigated. The interesting fluorescence exhibited by these compounds could be useful for their visualization in tumor cells, permitting further studies on these α , β -unsaturated piperidones as candidates for novel fluorescent antitumor agents.

Introduction

Piperidone derivatives are frequently encountered in natural products and drug candidates because of their interesting biological activities and are therefore important synthetic targets¹. Many piperidone derivatives are used widely in the synthesis of new drugs and fine chemicals because of both their pharmaceutical importance and the presence of a keto functionality with its adjacent active methylene center that can easily accommodate the introduction of other substituted groups². To date, some piperidone derivatives³⁻⁷ have been shown to possess antitumor, antidepression, antimycobacterium, antithrombotic, sedation, cholesterol-lowering and proteasome-inhibiting activities. In particular, some α,β -unsaturated piperidones, 3,5-bis(arylidene)-4-piperidones, have been reported to display cytotoxic activities⁸. Therefore, a number of novel N-aroyl-3,5-bis(benzylidene)-4piperidones were designed and evaluated for their cytotoxic and antitumor activities (Figure 1). These compounds feature 2-bromo-4,5-dimethoxy, 4-dimethylamino or 4-trifluoromethyl functionality in the phenyl ring appendages, as well as various substituents at the piperidone nitrogen.

An additional consideration inspired the synthesis and evaluation of antitumor effects for these 4-piperidones. Molecules carrying one or more α,β -unsaturated keto groups show a greater preference, or sometimes an exclusive affinity, for bio-thiols over both amino and hydroxy groups. As thiols are not found in nucleic acids, these benzylidene piperidones may avoid the genotoxic effects associated with a number of currently available

Keywords

Antitumor agents, cytotoxicity, fluorescence, α , β -unsaturated piperidones, xenograft model

History

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anticancer drugs⁹⁻¹¹. Moreover, one or more thiol groups are found in a multitude of cellular constituents, for example, glutathione, cysteine and thioredoxin. Thus, thiol alkylators have the potential to covalently bond with multiple biological targets. The pharmacophore, a 1,5-diaryl-3-oxo-1,4-pentadienyl group with alicyclic unsaturated enones and cyclic piperidone, can react at each primary binding site A (Figure 2) and may allow successive chemical attacks by cellular thiols. The electronwithdrawing or electron-donating properties of different groups in the benzene rings could influence the binding $effect^{12,13}$. In addition, the nature of the N-substituents at binding site B could affect the cytotoxic properties of the molecules. The effect could increase cytotoxic activity by facilitating the approach of the piperidone to a specific binding site or reduce the cytotoxic activity by hindering this interaction. Malignant cells are more susceptible than normal cells to this type of sequential chemical attack, so these antitumor drugs possess preferential toxicity for malignant cells over normal tissues¹⁴. Thus, this kind of compounds may have lower toxic side effect which is the main drawback of the current antitumor drugs to the normal cells. Moreover, they might activate caspase-3 to induce cell apoptosis or cause cell autophagy¹⁵. These reasons illustrate the importance of synthesizing and studying new 3,5-bis(benzylidene)-4-piperidone derivatives as antitumor drugs, which possess variable substitution on the aryl groups in the main binding site A and a range of N-substituents in the additional binding site B.

The noteworthy fluorescent properties of these molecules are another reason to synthesize and study them. In the case of cytotoxic molecules, natural fluorescence could permit tracking of the target organelle in cells. Recent investigations showed interesting fluorescent properties of a series of 3,5-bis(arylidene)-4-piperidone derivatives; some of these could be potential twophoton sensitizers because of the donor- π -acceptor- π -donor

^{*}Jufeng Sun and Suwen Wang contributed equally to this article.

Address for correspondence: Jufeng Sun, School of Pharmacy, Binzhou Medical University, Yantai, Shandong 264003, P.R. China. Tel: +86 5356913406. Fax: +86 5356913719. E-mail: sjf.xzy@163.com

Figure 1. The structures of 3,5-bis(benzylidene)-4-piperidones synthesized are shown.



Main binding site A



Figure 2. Binding sites A and B and designation of the torsion angles θ_1 and θ_2 of the compounds synthesized.

structure of the molecules, and their fluorescent properties may be affected by the substituents in the benzene $ring^{16-19}$. As these types of compounds demonstrate both marked antitumor activities and fluorescent properties, they have been recognized as promising antineoplastic drug candidates.

As substituted groups in the benzene ring might affect both the cytotoxicity and fluorescent properties of these compounds, 2-bromo-4,5-dimethoxy (having both electron-withdrawing and electron-donating properties), 4-dimethylamino (electron-donating) and 4-trifluoromethyl (electron-withdrawing) functionality was introduced into these derivatives. In this study, 15 new *N*-aroyl-3,5-bis(benzylidene)-4-piperidones are reported. Their cytotoxic and antitumor activities were evaluated by a CCK-8 method and xenograft model, and their fluorescent properties were investigated.

Experimental

Materials and methods

Samples for ultraviolet (UV) studies were prepared as 10^{-4} M in a mixture of ethanol and dimethyl sulfoxide (V_{ethanol} - $V_{\text{dimethyl sulfoxide}} = 4:1$), and spectra were obtained with a Beckman Du-70 ultraviolet spectrophotometer (Beckman, Reja, FL). Elemental analyses were performed on a Perkin-Elmer Model 240c analyzer (Perkin-Elmer, Wellesley, MA). ¹H NMR data of **1a**, **1e**, **2a** and **3a** were collected using a Bruker Avance-500 MHz spectrometer (Bruker, Billerica, MA), and others were collected using a Bruker Avance-400 MHz spectrometer (Bruker, Billerica, MA). All ¹³C NMR data were collected at 150 MHz

using a Bruker Avance-600 MHz spectrometer (Bruker, Billerica, MA). Chemical shifts are reported in δ relative to TMS. All fluorescence measurements were carried out on a Cary Eclipse Spectrofluorimeter (Varian, Australia) equipped with a xenon lamp and quartz carrier at room temperature. Biological evaluation was performed using a Microplate Reader 450 (Bio-Rad, Hercules, CA) enzyme-labeled instrument.

Synthesis of 1a, 2a, 3a

4-Piperidone hydrochloride monohydrate 2 (0.01 mol) and the appropriate aryl aldehyde (0.02 mol) were mixed into glacial acetic acid (50 mL). The mixture was piped into dry hydrogen chloride gas for 0.5 h, until a clear solution was obtained. After stirred for about 24 h at room temperature, the precipitate was collected and washed with 40% sodium hydroxide in water. The free base was collected and washed with water until pH7, then dried under the vacuum at 50–65 °C for 2 h. The compounds were recrystallized from methanol (1a); methanol–ethanol–chloroform (2a) and methanol–chloroform (3a). The melting points (°C) and yields (%) of 1a–3a were as follows: 1a: 246–247, 48%; 2a: 270–272, 72%; 3a: 227–228, 59%. ¹H NMR, ¹³C NMR and elemental analysis data for compounds 1a, 2a, and 3a are available in supplementary material.

Synthesis of 1b, 2b, 3b

Two milliliters of a 25% (w/v) aqueous sodium hydroxide solution containing a catalytic amount of tetrabutylammonium bromide and a suspension of 1a, 2a or 3a (1 mmol) in 1,2-dichloroethane (5 mL) were combined and stirred for 15 min at 0-5 °C. 4-Nitrobenzoyl chloride (1.5 mmol) in 1,2-dichloroethane (5 mL) was then added to the flask. The resulting mixture was stirred for approximately 6-24 h at room temperature and detected by TLC (dichloromethane-methanol = 25:1). The solvent was removed under vacuum. Then, 10% potassium carbonate was added to the residue, and this was stirred for 2 h. The precipitate was collected and dried in vacuo. The compounds were recrystallized from methanol (1b), dimethylformamide (2b), or methanol-chloroform (3b). The melting points (°C) and yields (%) of 1b-2b were as follows: 1b: 236-238, 40%; 2b: 256-259, 73%; **3b**: 209–212, 78%. ¹H NMR, ¹³C NMR and elemental analysis data for compounds 1b, 2b, and 3b are available in supplementary material.

General synthesis of 1c-1e, 2c-2e, 3c-3e

Two milliliters of a 25% (w/v) aqueous sodium hydroxide solution was mixed with a suspension of **1a**, **2a** or **3a** (1 mmol) in

1,2-dichloroethane (5 mL). A catalytic amount of tetrabutylammonium bromide was added to the mixture, and this was stirred for 10 min at 0-5 °C. To the mixture was added alternatively 4-methylbenzenesulfonyl chloride or 4-acetaminobenzenesulfonyl chloride or 4-nitrobenzenesulfonyl chloride (1.5 mmol) in 1,2-dichloroethane (5 mL). The resulting solution was stirred for approximately 7-24 h at room temperature and detected by TLC (chloroform–methanol = 25:1). After the reaction was complete, glacial acetic acid was added to the mixture until achieving pH 3. The precipitate was collected, washed with water and dried in vacuo. The compounds were recrystallized from methanol or methanol-chloroform (1c-1e), dimethylformamide or acetone (2c-2e), or methanol, 95% ethanol or chloroform (3c-3e). The melting points (°C) and yields (%) of 1c-1e, 2c-2e, 3c-3e were as follows: 1c: 229-231, 68%; 1d: 255-256, 81%; 1e: 238-239, 49%; 2c: 239-240, 63%; 2d: 255-256, 47%; 2e: 248-249, 75%; 3c: 182–183, 79%; **3d**: 216–219, 45%; **3e**: 235–236, 74%. ¹H NMR, ¹³C NMR and elemental analysis data for compounds 1c-1e, 2c-2e, and 3c-3e are available in supplementary material.

A chloroform solution of 1e (7.4 mg, 0.01 mmol) was kept at room temperature. Upon slow evaporation of the solvent about 15 days, bright yellow crystals of 1e were obtained.

Biological evaluation

Cell cytotoxicity assay by cell counting kit-8

Compounds belonging to series 1-3 were screened against five tumor cell lines (Peking union medical college), namely SW1990 and MIA PaCa-2 (human pancreatic carcinoma), PG-BE1 and NCI-H460 (human lung carcinoma) and SK-BR-3 (human breast cancer) using a cell counting kit-8 (WST-8, Dojindo Laboratories, Tokyo, Japan) method. Cells were cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT), 2 mM Lglutamine and 50 µg/mL gentamycin. Cytotoxicity for each individual compound was measured after 48h of cocultivation with each cell line. Briefly, the different cell lines were seeded into 96-well plates at a concentration of 1×10^4 cells/100 µL/well. The cells were allowed to attach overnight at 37 °C in a humidified atmosphere containing 5% CO₂. The tested compounds were initially dissolved in DMSO (Sigma-Aldrich), and the working solutions were added to a FBS free culture medium. The final DMSO concentration in each well was 1%. The compounds were added to wells with increasing drug concentrations. After a 48-h incubation period, 10 µL of CCK-8 reagent was added, and the plate was incubated for 4 h at 37 °C. The optical density (OD) was measured by a multi-well plate reader (TECAN, Männedorf, Switzerland) at 450 nm. The results are expressed as a decrease in the cell viability (%) in comparison to untreated controls. The concentration of each compound was examined in triplicate, and the IC₅₀ values are expressed graphically. The concentrations of the compounds used were 200, 100, 10, 1, 0.1 and 0.01 µg/mL. 5-Fluorouracil (5-FU, Sigma-Aldrich) was used as a positive control. The concentrations of 5-FU used were 250, 25, 2.5, 0.25 and 0.025 µg/mL.

Tumor xenograft model

The human lung carcinoma PG-BE1 xenograft model was set up. The BALB/c female athymic nude mice (BALB/c, nu/nu) were purchased from the Institute for Experimental Animals, Chinese Academy of Medical Sciences & Peking Union Medical College. The study protocols were according to the regulations of Good Laboratory Practice for non-clinical laboratory studies of drugs issued by the National Scientific and Technologic Committee of People's Republic of China. The treatment and use of animals during the study was permitted by the Animal Ethics Committee of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College (permission number: c1-2011-1121).

PG-BE1 cells growing in exponential phase were implanted into the 6-8-week-old female athymic nude mice by subcutaneous injection of 10×10^6 cells on the right flank. After 3 weeks, the tumors were dissected aseptically, and pieces of tumor tissue (2 mm³ in size) were transplanted into athymic mice subcutaneously. When the size of tumors reached approximately 100 mm³, the mice were divided into groups at random (n=6 per group)and treated with 5-FU, compound 1b at different doses twice weekly for 3 weeks, respectively. Control group was injected with saline. The tumor diameter was measured with a caliper, and tumor volumes were calculated with the following formula: $V=0.5a \times b^2$, where a and b are the long and the perpendicular short diameters of the tumor, respectively. Typically, the experiments were concluded when tumors in the control animals were close to an average size of 2000 mm³. The tumor inhibitory rate was calculated as $IR\% = \{1 - [(tumor volume_{final} - tumor volume$ volume_{initial} for the treated group)/(tumor volume_{final} - tumor volume_{initial} for the control group)] $\times 100\%$.

Single-crystal structure determination

X-Ray intensity data were measured at 298(2) K on a Bruker SMART APEX CCD-based diffractometer (Mo K α radiation, $\lambda = 0.71073$ Å) using a SMART and SAINT programs (Bruker, Billerica, MA). The structures were solved by direct methods and refined on F^2 by full-matrix least-squares methods with SHELXTL version 6.1 (Göttingen, Germany). Crystal data of **1e**: C₃₁H₂₈Br₂Cl₆N₂O₉S, M = 977.13, triclinic, space group *P*-1, yellow block, a = 10.322(9)), b = 11.353(10) Å, c = 18.561(16) Å, $a = 87.462(13)^\circ$, $\beta = 86.197(11)^\circ$, $\gamma = 68.646(10)^\circ$, V = 2021(3) Å³, Z = 2, $D_c = 1.606$ g cm⁻³, μ (Mo K α) = 2.505 mm⁻¹, T = 298(2) K; 7168 unique reflections [$R_{int} = 0.0282$]. Final R_1 [with $I > 2\sigma(I)$] = 0.0580, wR_2 (all data) = 0.1457.

CCDC 918752 (1e) contains the supplementary crystallographic data for this article. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223336033; e-mail: deposit@ccdc.cam.ac.uk).

Results and discussion

Synthesis and structures

Fifteen new compounds 1a-1e (series 1), 2a-2e (series 2) and 3a-3e (series 3) were prepared by the synthetic route shown in Scheme 1. Dry hydrogen chloride was used to catalyze the reaction of various aryl aldehydes with 4-piperidone via a Claisen-Schmidt condensation, leading to the preparation of 1a, 2a and 3a. Treatment of these compounds with each of 4-nitrobenzoyl chloride, 4-methylbenzenesulfonyl chloride, 4-acetamidobenzenesulfonyl chloride or 4-nitrobenzenesulfonyl chloride produced the corresponding N-acylated or N-sulfonylated derivatives 1b-1e, 2b-2e and 3b-3e. The yields of the compounds were between 40% and 81%. Their structures were fully characterized by ¹H NMR, ¹³C NMR and elemental analysis. ¹H NMR spectroscopy indicated that molecules in series 1–3 were stereoisomerically pure. The NMR signals of CH₂ groups in the central piperidone ring were split due to the influence of noncoplanarity between two aryl rings of the parent NH-3,5bis(benzylidene)-4-piperidones and the adjacent olefinic linkages for some compounds, most notably for 1b, 2b, 3b and 3e. Some carbon signals in the ¹³C NMR spectra for the two aryl rings (attached to the olefinic bonds) were similarly influenced

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Scheme 1 Synthetic chemical pathway of compounds in series 1–3. The reagents used in the syntheses were as follows: (I) 2-bromo-4,5-dimethoxy-benzaldehyde/CH₃COOH/dry HCl; (II) 4-dimethylamino-benzaldehyde/ CH₃COOH/dry HCl; (III) 4-trifluoromethylbenzaldehyde/CH₃COOH/dry HCl; (IV) 4-nitrobenzoyl chloride/NaOH/ClCH₂CH₂Cl/ K₂CO₃/tetrabutylammonium bromide or 4-methylbenzene sulfonyl chloride/ 4-acetamidobenzenesulfonyl chloride/ 4-nitrobenzenesulfonyl/NaOH/ClCH₂CL₂Cl/ tetrabutylammonium bromide/CH₃COOH.



Table 1. Cytotoxicity of **1a–1e**, **2a–2e** and **3a–3e** against human SK-BR-3, PG-BE1, NCI-H460, MIA PaCa-2 and SW1990 cell lines.

Compound	IC ₅₀ (μM)					
	SK-BR-3	PG-BE1	NCI-H460	MIA PaCa-2	SW1990	
1a	4.38 ± 0.48	1.31 ± 0.04	0.960 ± 0.108	0.377 ± 0.027	1.91 ± 0.37	
1b	3.19 ± 0.53	0.382 ± 0.098	0.402 + 0.056	0.167 ± 0.017	0.978 ± 0.496	
1c	10.8 ± 1.9	3.16 ± 0.73	3.44 ± 0.67	1.24 ± 0.35	3.66 ± 0.28	
1d	2.86 ± 0.35	1.77 ± 0.28	1.39 ± 0.51	0.29 ± 0.06	1.72 ± 0.37	
1e	23.1 ± 1.1	2.27 ± 0.54	1.41 ± 0.30	1.33 ± 0.07	4.74 ± 0.76	
2a	64.0 ± 10.5	11.3 ± 2.77	11.8 ± 3.6	8.23 ± 0.33	36.7 ± 5.0	
2b	>100	>100	>100	>100	>100	
2c	13.6 ± 1.5	23.4 ± 2.4	9.66 ± 3.79	8.82 ± 1.48	12.6 ± 3.4	
2d	>100	>100	>100	>100	>100	
2e	>100	>100	>100	>100	>100	
3a	0.843 ± 0.092	0.661 ± 0.046	1.04 ± 0.21	0.417 ± 0.094	1.94 ± 0.24	
3b	3.70 ± 0.14	4.08 ± 0.32	3.38 ± 0.11	0.230 ± 0.016	1.51 ± 0.48	
3c	4.40 ± 0.15	1.75 ± 0.44	0.544 ± 0.243	0.593 ± 0.132	3.69 ± 0.21	
3d	1.12 ± 0.24	0.820 ± 0.085	0.321 ± 0.081	0.353 ± 0.016	0.755 ± 0.553	
3e	0.417 ± 0.080	0.565 ± 0.042	0.126 ± 0.031	0.107 ± 0.006	0.365 ± 0.109	
5-FU	1297 ± 330	94.3 ± 23.9	35.8 ± 11.6	102 ± 9	474 ± 96	

and split, for example in compound $2b^{8,13,20-22}$. An X-ray crystallographic structure of **1e** showed that it adopted an *E*,*E* configuration.

The UV spectra of the molecules were inspected at a concentration of 10^{-4} mol L⁻¹ in a mixture of ethanol and dimethyl sulfoxide. From the spectra, two λ_{max} values could be seen for almost all the derivatives except **3a** and **3e**. The main λ_{max} values ranged from 374 to 383 nm for series 1, 461–472 nm for series 2 and 303–311 nm for series 3. The λ_{max} values increased from series 2 to series 1 to series 3 due to the extension of the π -conjugation system in the molecules. This was caused mainly by the change of substituents in the benzene rings of the parent NH-3,5-bis(4-dimethylaminobenzylidene)-4-piperidone for series 2, NH-3,5-bis(2-bromo-4,5-dimethoxybenzylidene)-4-piperidone for series 1 and NH-3,5-bis(4-trifluoromethylbenzylidene)-4piperidone for series 3. The electron-withdrawing effects of the substituents strengthened gradually from series 2, to series 1, to series 3; thus, the π -conjugation system lengthened in the order of series 3, series 1 and series 2. These differing properties caused a

change in the appearance of the compounds. Series 3 compounds are mainly pale yellow crystals, series 1 compounds are generally yellow or bright yellow crystals and series 2 compounds are a red or red-orange powder. The melting points for compounds in these series decrease according to the following: 2>1>3 when comparing three compounds having identical substituents attached to the piperidyl nitrogen atom. This is related to increases in electron-donating effects of aryl substituents in the parent benzene rings.

Antitumor activity

Cell cytotoxicity

The cytotoxic activities of the synthetic compounds were evaluated against five human carcinoma cell lines: SW1990, MIA PaCa-2, PG-BE1, NCI-H460 and SK-BR-3. The results are shown in Table 1, which summarizes the corresponding IC_{50} values (the IC_{50} represents the drug concentration resulting in 50% growth inhibition).



Figure 3. The inhibitory effect of compounds 1a-1e and 3a-3e against five human carcinoma cell lines.





As shown in Table 1, the biological evaluation data showed that 96% of the IC_{50} values of the compounds in series 1 and 3 against SW1990, NCI-H460, PG-BE1, MIA PaCa-2 and SK-BR-3 cells were lower than 5μ M, while 44% of these values were submicromolar. These data revealed that almost all the compounds in series 1 and 3 were potent inhibitors of the growth of various malignant cells; the especially high potencies of 1b, 3a, 3d and 3e should be noted. In the case of series 2, only 2a and 2c showed moderate potency, while the IC_{50} values of 2b, 2d and 2e were all greater than 100 μ M. As displayed in Figure 3, among compounds 1a–1e and 3a–3e, the SW1990, MIA PaCa-2, NCI-H460 and SK-BR-3 cell lines were the most sensitive toward compound 1b.

To verify the discrepancy between potencies, some investigations were conducted. As mentioned previously, biological potencies may be influenced by the nature of the aryl substituents in rings A and B (Figure 2). It can be seen in Table 1 that compounds 2a-2e, which have strong electron-donating dimethylamino groups in the para-position of the parent benzene rings, were the least active among the three series. Comparing compounds 3a-3e, with only electron-withdrawing trifluoromethyl groups in the parent benzene rings, and compounds 1a-1e, bearing both electron-withdrawing bromine groups and electron-donating methoxy groups in the parent benzene rings, more than 80% of the IC₅₀ values for 3a-3e were higher than or similar to those of the corresponding compounds of 1a-1e. Compound **3b** was the only compound in series **3** that exhibited lower IC₅₀ values against all five human carcinoma cell lines than compound 1b in series 1. In addition, the nature of N-substituted groups may also be one of the factors that can affect the cytotoxic potencies. As presented in Table 1, the potencies of N-aroyl analogs 1b, 1d, 3b, 3d and 3e against SW1990 were higher than those of the parent NH-4-piperidones 1a and 3a. This difference demonstrated that about half of the N-aroyl analogs had potencies that were intensified in series 1 and 3. In particular, the IC_{50}



Figure 5. (A) The tumor growth inhibition of compound **1b** against nude mice with PG-BE1 xenograft (in the light of tumor volume). (B) The effect of compound **1b** on the body weight of nude mice with PG-BE1 xenograft.



Figure 6. (A) The fluorescence spectra of 1a-1e at room temperature. (B) The fluorescence spectra of 2a-2e at room temperature. (C) The fluorescence spectra of 3a-3e at room temperature.

values of compounds **1b** and **3e** against all five carcinoma cell lines were higher than those of **1a** and **3a** separately.

Moreover the noncoplanarity between the two aryl rings of the parent NH-3,5-bis(benzylidene)-4-piperidones and the adjacent olefinic linkages might allow a favorable topography for the alignment of these compounds with their targets at cellular binding sites (Figure 2), thereby increasing the cytotoxicity of the molecules²². To confirm this, the X-ray crystallographic structure of the representative compound N-(4-

nitrobenzensulfonyl)-3,5-bis(2-bromo-4,5-dimethoxybenzylidene)-4-piperidone (1e) was investigated. The ORTEP diagram of 1e is presented in Figure 4. The X-ray crystallographic structure revealed that compound 1e crystallizes in the triclinic space group *P*-1 with two CHCl₃ molecules. As shown in Figure 4, a singlecrystal structure of 1e shows the *E* stereochemistry of the olefinic double bonds (*E*,*E* isomer), which is confirmed by the C12–C15– C16–C17 torsion angle value ($\theta_1 = 28.915(7)^\circ$) and C10–C9–C7– C8 torsion angle value ($\theta_2 = -30.15(8)^\circ$). It is noteworthy that, in this structure, the 4-nitrophenylsulfonyl group is almost parallel to the C14–C10–C11–C12–C13 plane and the two aryl groups in 3,5-bis(benzylidene)-4-piperidones. The corresponding dihedral angles are ca. $25.9(2)^{\circ}$, *ca*. $6.52(2)^{\circ}$ and ca. $9.35(2)^{\circ}$. In addition, the N1–S1–C24 angle is only $108.0(2)^{\circ}$, which demonstrates that the olefinic double bonds are close to the 4-nitrophenylsulfonyl group. The corresponding C12···C24, C15···C29, C10···C24 and C9···C25 distances are 3.372(2), 3.612(2), 3.541(3) and 3.777(3) Å. Such short distances may result in inter-conjugatedsystem interactions between the 4-nitrophenylsulfonyl groups and 3,5-bis(benzylidene)-4-piperidones, further influencing the fluorescent properties of the molecules.

Antitumor activity in vivo

The antitumor activity in vivo is the most important factor to evaluate for new antitumor agents. The sensitive PG-BE1 xenograft model was used to investigate the antitumor activity in vivo of compound 1b, which had higher potency. The diameters of tumors were measured two times per week after treatment of **1b** in athymic nude mice with PG-BE1 xenografts (Figure 5A). With doses of 60, 30 and 15 mg/kg, the corresponding tumor inhibitory rates were 58.8%, 38.3% and 31.0%, respectively, which demonstrated that compound 1b could inhibit the growth of the tumor. Moreover, toxicity is one of the metrics to judge antitumor agents, and the body weight changes of nude mice posttherapy can be influenced by the toxicity of the drugs indirectly. From Figure 5(B), it was shown that the body weight of mice did not decreased obviously compared with the control group, indicating that compound 1b has no apparent toxicity to nude mice bearing PG-BE1 xenografts.

Fluorescent properties

Lengthening the conjugated spacer by inserting additional substituted groups into 3,5-bis(arylidene)-4-piperidone may result in increasing the electron-transporting effects and fluorescent properties of the molecules^{23,24}. Taking this into account, the *N*-aroyl-3,5-bis(arylidene)-4-piperidone derivatives, discussed above, were designed and synthesized. Their fluorescent properties were then explored (Figure 6A–C).

The compounds were excited at 435 nm for series 1, 365 nm for series 2 and 373 nm for series 3 to obtain the fluorescence spectra. The solutions $(10^{-4} \text{ mol L}^{-1})$ were prepared in a mixture of ethanol and dimethyl sulfoxide ($V_{\text{ethanol}}-V_{\text{dimethyl sulfoxide}} = 4:1$). The fluorescence emission was measured at room temperature from 460 to 650 nm for series 1, 480–700 nm for series 2 and 390–590 nm for series 3. Figure 6(A)–(C) displays contrasts in the emission spectra from series 1 to series 3, respectively. Compounds of series 1 exhibit an emission maximum at approximately 521 nm, compounds of series 2 at approximately 572 nm and compounds of series 3 at approximately 424 nm.

The results of Figure 6(A)-(C) revealed that compounds 2a-2e, with strong electron-donating dimethylamino groups, possessed the strongest fluorescence. The fluorescence intensities of compounds 1a-1e and 3a-3e were weaker due to the influence of the heavy atom effect from bromine and the electron-withdrawing effects of trifluoromethyl groups, respectively. In addition, the emission intensity of the parent NH-3,5-bis(benzylidene)-4-piperidones (1a, 2a, 3a) is stronger than that of *N*-(4-nitrobenzoyl) derivatives (1b, 2b, 3b) and *N*-(4-nitrobenzenesulfonyl) derivatives (1c, 2c, 3c).

At the same time, the results indicated that the fluorescent properties of these compounds were correlated with their cytotoxic activities. It can be seen from Table 1 and Figure 6(A)-(C) that compounds 2a-2e, with stronger fluorescence intensity, demonstrated lower cytotoxic activities, while compounds 1a-1e and 3a-3e, with weaker fluorescent intensity, demonstrated higher cytotoxic activities. Further investigation into the structure-cytotoxicity-fluorescence relationships of the compounds is warranted.

Conclusions

In summary, we synthesized 15 new α,β -unsaturated piperidones (*N*-aroyl-3,5-bis(benzylidene)-4-piperidones), **1a–1e** (series **1**), 2a-2e (series 2) and 3a-3e (series 3), and investigated their cytotoxic and fluorescent properties. In addition, we assessed the antitumor activity *in vivo* of compound **1b** (having better potency) using a nude mouse PG-BE1 xenograft model. Among the derivatives assayed, the majority of compounds 1a-1e (possessing strong electron-withdrawing trifluoromethyl groups in the aryl rings of A and B) and 3a-3e (possessing both electronwithdrawing bromine and electron-donating methoxy groups in aryl rings A and B) displayed significant cytotoxic properties, but their fluorescent activities were a little weaker. Alternatively, molecules 2a-2e (bearing strong electron-donating dimethylamino groups in the aryl rings A and B) illustrated poor cytotoxic properties, but their fluorescent activities were stronger than those of compounds 1a-1e and 3a-3e. Nevertheless, all the compounds that were investigated had interesting fluorescent properties overall. Compounds 1b, 3a, 3d and 3e displayed remarkable cytotoxic activities towards five human carcinoma cell lines. Importantly, the results of a PG-BE1 xenograft model showed that compound 1b possessed marked inhibitory effects on tumor growth and had no obvious toxicity toward the nude mice. Compounds 1b, 3a, 3d and 3e represent possible lead molecules for synthesizing new derivatives with the aim to increase their cytotoxic potencies, while producing low-toxicity antitumor drug candidates that undergo fluorescence.

Declaration of interest

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Supplementary material available online Supplementary information