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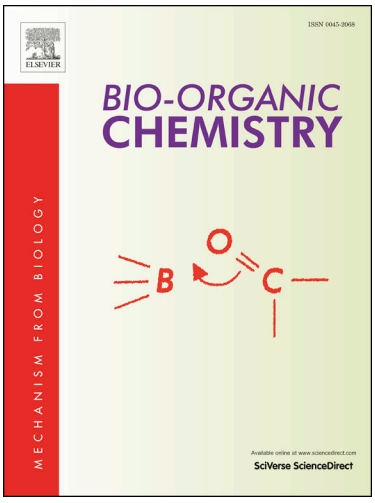

Design, synthesis and biological evaluation of novel 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one derivatives with potential anti-tumor activity

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Design, synthesis and biological evaluation of novel 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one derivatives with potential anti-tumor activity

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Abstract: In this study, a series of novel 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one derivatives were obtained by structural modification of the lead compounds with Fissitungfine B. A total 15 compounds were designed, synthesized and evaluated as inhibitors of tumor. These target compounds have the novel chemical structures that named three six-membered rings including one lactone six-membered ring. *In vitro* assay, the results showed that the target compounds have a broad spectrum and strong of anti-tumor activity. Such as the target compound **4n** to MCF-7 was IC_{50}=0.35 ± 0.01 μM, to A-549 was IC_{50}=0.37 ± 0.01 μM, to Hela was IC_{50}=0.56 ± 0.02 μM, to MDC-803 was IC_{50}=0.53 ± 0.02 μM and COLO-205 was IC_{50}=0.50 ± 0.02 μM *in vitro*. At the same time, *in vivo* anti-tumor activity assay results showed that the target compounds had a good inhibitory effect on tumor growth. Among them, the target compound **4n** had the best anti-tumor activity, it could inhibit tumor growth well at a low dose. The target compound **4n** could be used as a candidate drug for further research and development, in order to be used as early as application in the clinical treatment of tumors.

Keywords: Fissitungfine B; design; synthesis; anti-tumor activity

1. Introduction

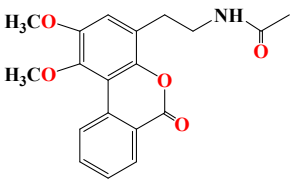
Malignant tumor was a disease caused by abnormal mechanism of controlling cell growth and proliferation (Birbrair, et al., 2014). It was a common disease that can seriously threaten human health. According to the world health organization (WHO), three out of five people in the world die of cancer, diabetes, cardiovascular disease and chronic respiratory diseases (Cunningham, et al.,

2011). In 2008, the cancer killed 7.6 million people, accounting for 13% of global deaths (Handa, et al., 2011). More than 70% of cancer deaths occurred in low and middle income countries, more than 13.2 million people were predicted to die of cancer by 2030. Anti-tumor drugs were a class of drugs for the treatment of tumor diseases. Simply speaking, there were chemotherapy drugs and biological agents (Katsurano, et al., 2012). In recent years, the development of molecular oncology and molecular pharmacology has gradually clarified the nature of tumor. The invention and application of large-scale rapid screening, combinatorial chemistry, genetic engineering and other advanced technologies have accelerated the process of drug development (Vogelstein, et al., 2013). The research and development of anti-tumor drugs has entered a new era. At present, drug therapy has become one of the important means for the clinical treatment of cancer (Murphy, 2014). Due to the high incidence and mortality of cancer, the sales of anti-tumor drugs were also increasing year by year. After years of development, many important advances have been made in the research and development of anti-tumor drugs (Eyers & Murphy, 2016). However, in the face of the most serious threat to human life and health, solid tumors, which account for more than 90% of malignant tumors. The still lack effective and specific drugs, which on the one hand reflects the difficulty in the development of anti-tumor drugs (Linden, et al., 2013). On the other hand, it also means that the development of anti-tumor drugs still needs the application of new ideas, new technologies and new methods.

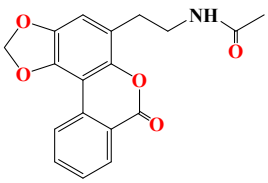
With the development of modern medicine, natural drugs have been gradually recognized and developed as anti-tumor drugs. In recent years, there have been more and more researches on the anti-tumor effect of calm drugs. The researches on the anti-tumor active ingredients and anti-tumor mechanism of natural drugs have begun to take shape in different parts of the world (Cho, et al., 2010). The discovery of many potent anticancer compounds in plants could be attributed directly or indirectly to the history of plant anticancer in traditional medicine. The first plant-derived drugs to enter the clinic were the alkaloids of vinblastine and vincristine. *Catharanthus roseus* has been used to treat type 2 diabetes in many countries (Gangjee, et al., 2013). At first, the oral hypoglycemic effect of *Catharanthus roseus* was mainly studied. Later, it was discovered by chance that *Catharanthus roseus* could reduced white blood cell count and inhibit bone marrow regeneration in mice. However, there have been adverse events related to the drug's ability to reduce white blood cell count and prevent bone marrow regeneration, which may result in limited clinical use of the

drug. Another example of a more direct anticancer ingredient derived from traditional medicine was the acetabulum toxin isolated from the root of the malus (Shi & Guang, 2018). Taxol was a natural secondary metabolite isolated from the bark of gymnosperms of taxus cuspidate. In the biological activity screening, it was found that the extract containing paclitaxel had high biological activity on the inhibition of mouse tumor cells *in vitro*, further studies were conducted to isolate the anti-tumor active ingredients.

As a Chinese endemic medicinal plant, only distributed in Hainan Island (Hu, et al., 2007), up to now, there is only a preliminary investigation on the chemical composition of *Fissistigma tungfangense* (Zhang, et al., 2007). Previous chemical studies of *Fissistigma tungfangense* have led to the separation of a number of compounds (Hwangs et al., 2009), including oxymorphines, aporphines, phenanthrenes, morphinandienones, flavonoids, protoberberines, and chalcones. Early pharmacological studies have shown that these compounds have anti-tumor, anti-platelet aggregation anti-inflammatory and immunosuppressive activities (Ge, et al., 2013). Fissitungfine A and Fissitungfine B (**Figure 1**) were extracted from the tropical medicinal plant *Fissistigma tungfangense* (Qi, et al., 2016). It was a natural product with strong anti-tumor activity, but its content in plants was very low. On the basis of the structure of the lead compound Fissitungfine B, a series of 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one derivatives were designed and synthesized by using the bioisostere and modification with alkyl groups (**Figure 2 (a)**). In terms of spatial structure, the designed compounds has a similar spatial structure to Fissitungfine B. In terms of drug structure-activity relationship (SAR), the anti-tumor activity (Hwangs et al., 2009; Hong C, et al. 2019 ; Hong C, Y, et al. 2020) of the designed compounds may be better than that of the lead compound Fissitungfine B. Because the substituents designed on the benzene ring could better bind to the receptor, thus achieving a better effect of inhibiting the growth of tumor cells. In the process of synthesis, target compounds were synthesized by acyl chloride reaction, esterification reaction and heck reaction (**Scheme 1**). The synthetic route was characterized by simple operation and high total yield. In the process of screening the anti-tumor activity *in vitro* and *in vivo*, the experimental results showed that these compounds had a good anti-tumor activity. These compounds could be used as the potential anti-tumor drug candidates and their value remains to be further studied.



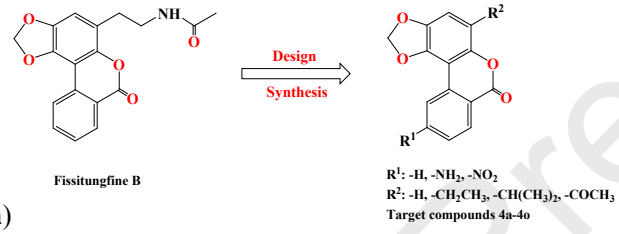
Fissitungfine A



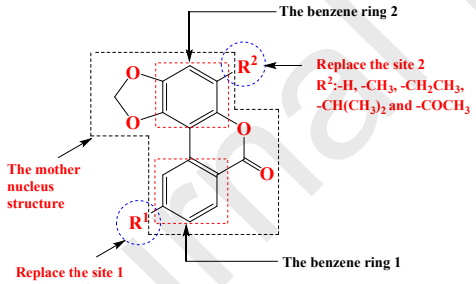
Fissitungfine B

Compounds	IC_{50} (μM)				
	Hela	MCF-7	A-549	MGC-803	COLO-205
Fissitungfine A	2.30	2.92	3.19	3.01	3.21
Fissitungfine B	4.01	5.21	6.31	4.98	6.70

Figure 1 The chemical structure of Fissitungfine A and B.



(a)



(b)

Figure 2 The design and structure-activity relationship (SAR) of target compounds **4a-4o**.

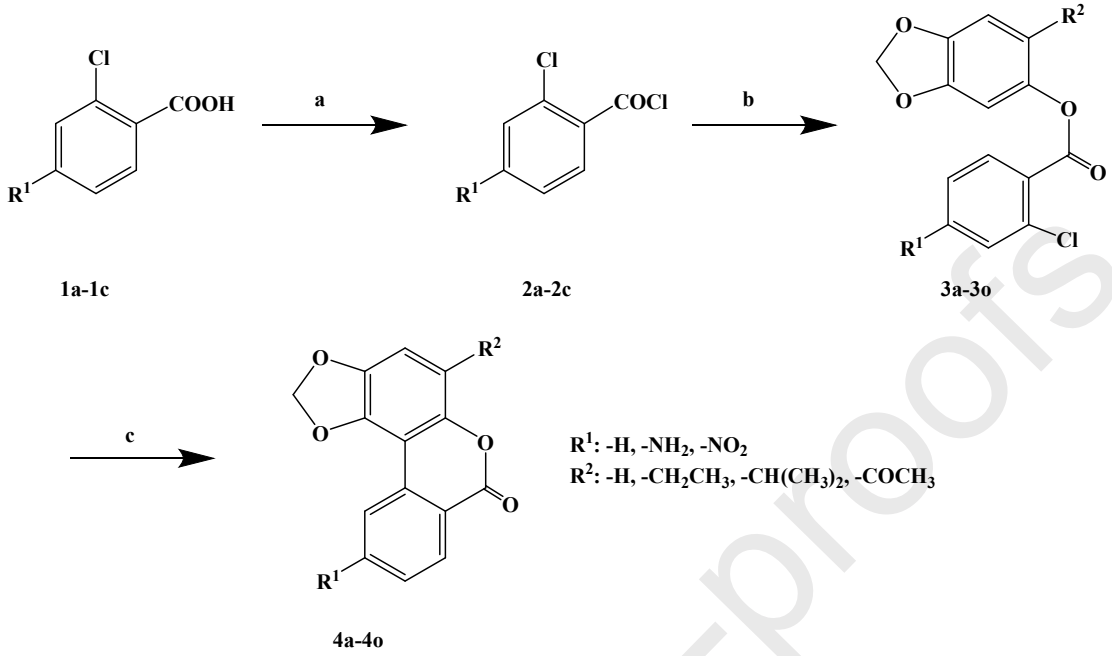
2. Results and discussion

*2.1. Design and synthesis of the target compounds **4a-4o***

Fissitungfine A and Fissitungfine B were extracted from the tropical medicinal plant *Fissistigma tungfangense*. They were natural product that showed a broad spectrum and strong of anti-tumor activity (MCF-7, A-549, Hela, MDC-803 and COLO-205), and IC_{50} were at the μM level *in vitro* (**Figure 1**). In this study, Fissitungfine B was used as the lead compound to replace the substituents on the benzene ring 1 and benzene ring 2 on the basis of retaining the basic skeleton

(**Figure 2** and **Figure 4**). During the structural design of the target compounds, a total of 15 target compounds **4a-4o** were designed. In the design of the target compounds, we chose -H, -NH_2 and -NO_2 as substituents for R^1 on the benzene ring 1, the -H, -CH_3, -CH_2CH_3, -$CH(CH_3)_2$ and -$COCH_3$ as substituents for R^2 on the benzene ring 2. This kind of drug design, which retains the parent nucleus, provides certain guarantee for the success of new drug research and development, and also shortens the cycle and reduces the cost of research and development. The structure was designed to alter the physical and chemical properties of the target compounds, including log*P* and pK_a and the toxicity of the target compounds. The anti-tumor activity *in vitro* and *in vivo* were screened to obtain the target compounds with a good anti-tumor activity and low toxicity. On this basis, the further pharmacokinetic, clinical and other related studies were conducted. In the synthetic route design process, we chose an efficient and simple synthetic route (**Scheme 1**). Using substituted benzoic acid (compounds **1a-1c**) as starting material, the target compounds **4a-4o** were synthesized by acyl chloride reaction, esterification reaction and heck reaction in three steps. The acyl chloride reaction was conducted with substituted benzoic acid (compounds **1a-1c**) as starting material. In this step, the methylene chloride (CH_2Cl_2) as solvent, *N*, *N*'-dimethyl formamide (DMF) used as catalyst and thionyl chloride ($SOCl_2$) as chloride reagent. In the first step of reaction, it only takes 2 hours for refluxing reaction to complete the acyl chloride reaction, and the acyl chloride reaction yield was relatively high, up to more than 98%. The esterification reaction, the substitutedchlorobenzoyl chloride (compounds **2a-2c**) and substituted sesamol as reactants, methylene chloride (CH_2Cl_2) as solvent. During the esterification reaction process, there was no need to add any catalyst and heat the reaction. The esterification reaction could be completed at room temperature and stirring 2 hours. In the process of esterification reaction, a good yield could also be obtained, and the yield of intermediate **3a-3o** were more than 95%. The heck reaction, tetrahydrofuran (THF) as solvent, anhydrous potassium carbonate as acid applying agent and palladium acetate ($Pd(OAc)_2$) used as catalyst for the reaction. The heck reaction could be completed after 5 hours of reflux reaction of intermediate **3a-3o**. In the heck reaction, the yield of the synthesized target compounds **4a-4o** reached 74% to 88%. According to the design of synthesis route, we successfully synthesized the target compounds **4a-4o**. In the process of synthesis, the conditions of each steps of the reaction were optimized, and the yield of the target compounds were a good under the optimal reaction conditions. The synthesis route has few reaction steps and only three steps to complete the synthesis

of the target compounds. Each steps of the synthesis operation was relatively simple, the reaction conditions was mild and the total yield was high.



Scheme 1 The synthetic route of target compounds **4a-4o**. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, DMF, reflux 2 hours; (b) substituted sesamol, CH_2Cl_2, stirring 2 hours; (c) THF, K_2CO_3, $Pd(OAc)_2$, reflux 5 hours.

*2.2. Biological activities of the target compounds **4a-4o***

2.2.1. Biological activity screening in vitro

Anti-tumor drugs was a class of drugs that could directly kill or inhibit the growth or proliferation of tumor cells. The mechanisms include inhibition of nucleic acid or protein synthesis, interference with macromolecular metabolism, interference with microtubule system and inhibition of topoisomerase. *In vitro* tests were mainly used to screen candidate compounds, to preliminarily understand the mechanism of action, the type and intensity of sensitive tumor, and to provide references for subsequent *in vivo* tests. *In vitro* anti-tumor activity screening of the target compounds **4a-4o** were performed by MTT method to determine the proliferation inhibitory activity on a variety of human tumor cell lines. Determine the anti-tumor activity selectivity of the target compounds among different tumor lines, thus providing a basis for *in vivo* nude mouse model experiment. In the anti-tumor tests *in vitro*, human breast cancer cells (MCF-7), lung adenocarcinoma cells (A-549), cervical cancer cells (Hela), gastric adenocarcinoma cells (MDC-803) and colon cancer cancer

cells (COLO-205) were used as the inhibitory targets. The human fibroblast (Hum. Fib.) cell line was used as control for toxicity. The inhibitory activity was measured by half maximal inhibitory concentration (IC_{50}), it's commonly used as a measure of drug effectiveness. The adriamycin was used as positive drug and dimethylsulfoxide (DMSO) was used as the blank control. The anti-tumor *in vitro* results of biological activity tests were shown in **Table 1**. The data of this study were the statistical software was versions SPSS13.0, the analyzed by linear regression and the results of data analysis show a linear fit. In **Table 1**, the test results showed that target compounds **4d**, **4i**, **4m** and **4n** had better inhibitory effect on breast cancer cells (MCF-7), lung adenocarcinoma cells (A-549), cervical cancer cells (Hela), gastric adenocarcinoma cells (MDC-803) and colon cancer cancer cells (COLO-205). In addition to the target compounds **4d**, **4i**, **4m** and **4n**, other target compounds also showed good anti-tumor activity in vitro, with IC_{50} at the level of one to tens of micromoles. Among them, the inhibitory activity of the target compound **4n** to breast cancer cells (MCF-7) was IC_{50}=0.35 ± 0.01 μM, to lung adenocarcinoma cells (A-549) was IC_{50}=0.37 ± 0.01 μM, to cervical cancer cells (Hela) was IC_{50}=0.56 ± 0.02 μM, to gastric adenocarcinoma cells (MDC-803) was IC_{50}=0.53 ± 0.02 μM and to colon cancer cancer cells (COLO-205) was IC_{50}=0.50 ± 0.02 μM. It could be seen from the data in **Table 1** that these target compounds had a broad spectrum and strong of anti-tumor activity *in vitro*. At the same time, most of these compounds showed no obvious cytotoxicity effects on human fibroblast, except compounds **4f** and **4j** (**Table 1**). By analyzing the preliminary anti-tumor activity data *in vitro*, the target compounds **4d**, **4i, 4m** and **4n** could be used to evaluate the anti-tumor activity *in vivo*. According to the results of *in vitro* activity and substituents (R^1 and R^2), the structure-activity relationship (SAR) of drugs could be established. At the same time, the physical chemical constants (log*p* and pK_a) of the target compounds were determined (**Table 1**). From the data of physical and chemical constants, it could be seen that these target compounds were fat-soluble compounds, which were conducive to the absorption of compounds. Meanwhile, the pK_a of the target compounds were 7.0-9.4, and its absorption site was concentrated in the intestinal tract. These physical and chemical constants were helpful for further study of pharmacokinetics.

Table 1 The target compounds **4a-4o** of inhibitory activity *in vitro*.

Compounds	R^1	R^2	log*p*	pK_a	IC_{50} (μM)[a] ± *SD*					
					MCF-7	A-549	Hela	MDC-803	COLO-205	Hum. Fib.[b]
4a	H	H	2.91	8.3	5.34 ± 0.28	8.13 ± 0.35	12.36 ± 0.34	11.23 ± 0.31	10.23 ± 0.30	＞100[c]
4b	H	CH_3	3.41	8.2	2.57 ± 0.08	2.65 ± 0.09	4.31 ± 0.12	4.11 ± 0.12	3.59 ± 0.10	＞100
4c	H	CH_2CH_3	3.80	8.2	1.03 ± 0.04	0.89 ± 0.03	2.12 ± 0.06	2.01 ± 0.06	1.56 ± 0.05	＞100
4d	H	$CH(CH_3)_2$	4.13	8.1	0.61 ± 0.02	0.42 ± 0.02	0.74 ± 0.03	0.78 ± 0.03	0.60 ± 0.03	＞100
4e	H	$COCH_3$	2.24	7.8	7.24 ± 0.36	10.66 ± 0.36	14.33 ± 0.42	13.26 ± 0.40	12.04 ± 0.36	＞100
4f	NH_2	H	2.15	9.4	11.24 ± 0.62	17.63 ± 0.81	23.67 ± 0.92	21.09 ± 0.88	19.34 ± 0.86	76.32 ± 4.02
4g	NH_2	CH_3	2.60	9.3	5.67 ± 0.30	5.88 ± 0.34	8.56 ± 0.37	8.11 ± 0.31	7.55 ± 0.32	＞100
4h	NH_2	CH_2CH_3	3.03	9.3	2.10 ± 0.08	1.69 ± 0.06	4.33 ± 0.11	4.13 ± 0.11	3.24 ± 0.19	＞100
4i	NH_2	$CH(CH_3)_2$	3.36	9.0	0.81 ± 0.02	0.79 ± 0.02	1.10 ± 0.04	0.81 ± 0.04	0.90 ± 0.04	＞100
4j	NH_2	$COCH_3$	1.46	8.5	23.13 ± 0.92	35.27 ± 1.33	42.03 ± 1.89	40.37 ± 1.78	38.96 ± 1.76	94.26 ± 4.51
4k	NO_2	H	1.63	7.6	3.06 ± 0.13	3.84 ± 0.16	5.34 ± 0.30	5.04 ± 0.28	4.80 ± 0.25	＞100
4l	NO_2	CH_3	2.05	7.5	1.22 ± 0.05	1.06 ± 0.04	2.10 ± 0.08	2.03 ± 0.08	1.24 ± 0.05	＞100
4m	NO_2	CH_2CH_3	2.50	7.5	0.62 ± 0.03	0.44 ± 0.02	0.79 ± 0.03	0.88 ± 0.03	0.74 ± 0.04	＞100
4n	NO_2	$CH(CH_3)_2$	2.71	7.3	0.35 ± 0.01	0.37 ± 0.01	0.56 ± 0.02	0.53 ± 0.02	0.450 ± 0.02	＞100
4o	NO_2	$COCH_3$	1.14	7.0	4.78 ± 0.02	5.00 ± 0.02	7.24 ± 0.42	7.01 ± 0.40	6.57 ± 0.38	＞100
Adriamycin	___	___	___	___	0.81 ± 0.03	0.86 ± 0.03	1.45 ± 0.08	1.38 ± 0.08	1.09 ± 0.07	＞100
DMSO	___	___	___	___	None[d]	None	None	None	None	None

[a] Data represent the mean values from at least eight independent experiments each in triplicate (n≥8).

[b] Human fibroblast (Hum. Fib.) cell line was used as control for toxicity.

[c] Without any noticeable toxicity against human fibroblast.

[d] None=No biological activity.

2.3. Anti-tumor in vivo

In vivo anti-tumor activity test was used to confirm the target compounds killing or inhibiting effect on specific types of tumor cells, and to explore the dosage, route, frequency and cycle of the target compounds pharmacological effect. The Kunming mice were subcutaneously inoculated with sarcoma S180 and lung cancer H22 tumor strains according to the standard methods of anti-tumor activity test *in vivo*. *In vivo* anti-tumor activity tested results were important indicators to evaluate the efficacy of target compounds. The relative tumor proliferation rate T/C (%) was used as the evaluation index, and the evaluation criteria were: T/C (%) >40 % was invalid, T/C (%) ≤40% and $P < 0.05$ was considered effective by statistical analysis. In the process of screening for anti-tumor activity *in vitro*, the target compounds **4d**, **4i**, **4m** and **4n** were screened for breast cancer cells (MCF-7), lung adenocarcinoma cells (A-549), cervical cancer cells (Hela), gastric adenocarcinoma cells (MDC-803) and colon cancer cancer cells (COLO-205) showed good inhibitory effect and a broad spectrum anti-tumor activity. On the basis of anti-tumor activity *in vitro*, the anti-tumor activity of target compounds **4d**, **4i**, **4m** and **4n** *in vivo* was further evaluated. In the process of *in vivo* anti-tumor activity testing, the low, medium and high doses (10 mg. kg^{-1}. d^{-1}, 50 mg. kg^{-1}. d^{-1} and 100 mg. kg^{-1}. d^{-1}) of the target compounds **4d**, **4i**, **4m** and **4n** were respectively used for relevant tests (**Figure 3**). *In vivo* anti-tumor evaluation method, we chose to measure the change of tumor volume after direct administration. The data of this study were the statistical software was versions SPSS13.0, the analyzed by linear regression and the results of data analysis show a linear fit. In **Figure 3**, the *in vivo* anti-tumor activity test data showed that the target compounds **4d**, **4i**, **4m** and **4n** had a good inhibitory effect on tumor growth in *vivo*. The low, medium and high doses (10 mg. kg^{-1}. d^{-1}, 50 mg. kg^{-1}. d^{-1} and 100 mg. kg^{-1}. d^{-1}) , the relative tumor proliferation rates T/C (%) of these target compounds on sarcoma S180 and lung cancer H22 were both lower than 40%, which was considered as effective tests. In **Figure 3**, it could be found that the compounds **4d**, **4i**, **4m** and **4n** showed a good inhibitory effect on tumor growth at low dose (**Figure 3a**, 10 mg .kg^{-1}. d^{-1}), medium dose (**Figure 3b,** 50 mg. kg^{-1}. d^{-1}) and high dose (**Figure 3c,** 100 mg. kg^{-1}. d^{-1}). At the same dose, the anti-tumor activity of the target compounds were comparable to or better than that of the positive control (adriamycin). For example, the anti-tumor activity of the target compound **4n** was significantly higher than that of the positive control at the same dose. The target compounds **4d**, **4i**, **4m** and **4n** showed good activity *in vivo* anti-tumor of evaluation, which may be related to

itsphysical chemical constants (log*p* and pK_a). The good physical and chemical constants were conducive to the digestion and absorption of drugs, and improve the efficacy of drugs. In addition, these target compounds may have better spatial structure and substituents, which was beneficial for the target compounds to bind to receptor proteins better, thus showing good anti-tumor activity *in vivo*. At the same time, we also investigated the weight changes of the target compound **4n** in nude mice (**Figure 4**). The results showed that there was little change in body weight between the two models, suggesting that compound **4n** was active and had relatively few side effects. Could be seen from the test data *in vivo*, the significance of these target compounds should be further, particularly as target compound **4n** can be used as an anti-tumor drug candidate for mechanism and pharmacokinetic study. It is also the job next to further more research direction, for an early into clinical trials make necessary experimental basis and theoretical basis.

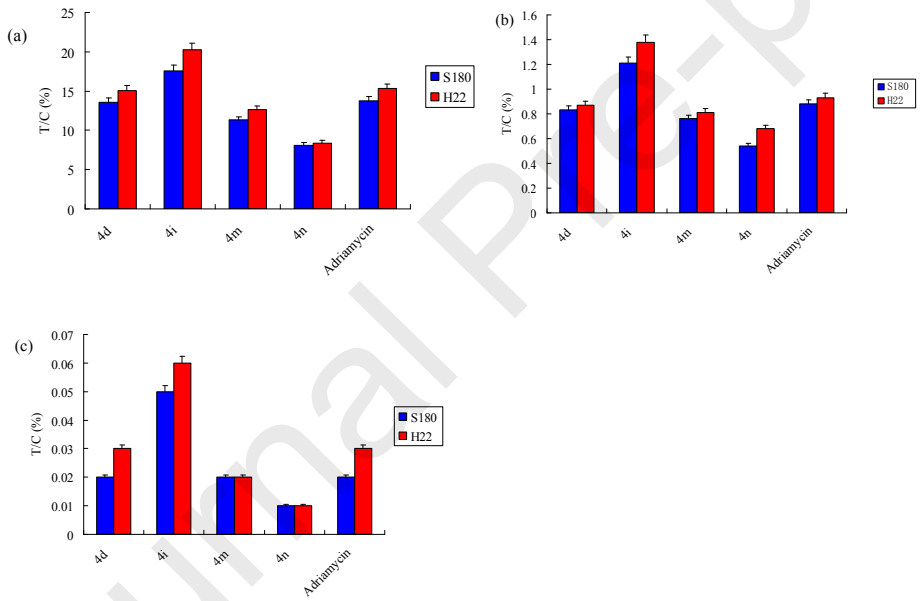


Figure 3 *In vivo* anti-tumor activity test. The data show the mean±SEM (n=8). (a): low dose (10 mg. kg^{-1}. d^{-1}); (b) medium dose (50 mg .kg^{-1}. d^{-1}); (c) high dose (100 mg. kg^{-1}. d^{-1}).

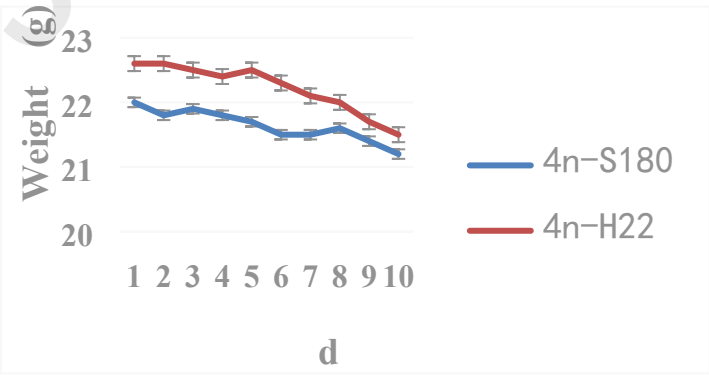


Figure 4 The body weight change of nude mice after compound **4n** treatment.

2.4. Structure-activity relationship (SAR) of study

By combining the results of *in vitro* and *in vivo* anti-tumor activity tests and analyzing the relationship between the structures of the new compounds. We could obtain some useful key structural information characteristics that affect the anti-tumor activity of the target compounds. The parent structure of the synthesized new compound remained unchanged and the sites of the difference were different substituents on benzene ring 1 and benzene ring 2 (**Figure 2 (b)**). The structure-activity relationship (SAR) of study were summarized as follows: (1) The anti-tumor activity of the target compounds whose substituent group on benzene ring 1 was the electron-absorbing group was better than that of the electron-donating group ($-NO_2$ > -H > $-NH_2$). (2) The anti-tumor activity of the target compounds with the substituent of benzene ring 2 as the electron-donor group was better than that of the electron-sucking group. (3) With the increase of carbon chain the anti-tumor activity of the electron-donor group in benzene ring 2 increases continuously (-H < $-CH_3$ < $-CH_2CH_3$ < $-CH(CH_3)_2$). The study of SAR provides necessary theoretical guidance for further structural optimization in the future. To provides guarantee for the rapid and convenient acquisition of target compounds with better anti-tumor activity, lower toxic effects and better pharmacokinetic properties.

2.5. Lethal dose 50% (LD_{50}) of study

Acute toxicity refers to the toxic effect caused by a single (or multiple within 24 hours) exposure of the organism (human or laboratory animals) to foreign compounds, or even death. In toxicology, the lethal dose (LD_{50}) was the dose of hazardous substances, toxic substances, or ionizing radiation that can kill half of the test population. The lower the LD_{50} value was the stronger the toxicity of the exogenous chemical. Conversely, the higher the LD_{50} value, the lower the toxicity. Determination of LD_{50} to understand the toxicity intensity, nature and possible target organs of the tested substance, to provide a basis for further toxicity test dose and toxicity observation index selection, and according to LD_{50} toxicity grading. As could be seen from the LD_{50} test results in **Table 2**, the acute toxicity of the target compounds were tested by intragastric administration and the toxicity of the target compounds **4d**, **4i**, **4m** and **4n** were lower than that of the positive control group (adriamycin). According to the classification of acute toxicity, this kind of target compounds

belongs to low toxicity compound, which meets the standard of new drug development. The results of *in vitro* anti-tumor activity data and acute toxicity data showed that there was a certain linear relationship between them. The better the anti-tumor activity of the target compound, the higher the toxicity. This linear relationship was consistent with the basic characteristics of general anti-tumor drugs, which was necessary to further study the mechanism of action of such drugs.

Table 2 The LD_{50} test result.

Compounds	Method of administration	$LD_{50} \pm SD$ (mg. kg^{-1})[a]
4d	i.g.	821.4 ± 4.2
4i	i.g.	846.7 ± 4.6
4m	i.g.	786.5 ± 3.7
4n	i.g.	763.6 ± 3.3
Adriamycin	i.g.	568.4 ± 2.3

[a] LD_{50} values are displayed as means±standard deviations, n≥8.

3. Conclusion

We reported that a novel series of 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one derivatives were designed, synthesized and evaluated as inhibitors of tumor. The Fissitungfine B as the lead compound, using the principle of modifying by bioisostere formation and modification with alkyl groups. In the process of synthesis, target compounds were synthesized by acyl chloride reaction, esterification reaction and heck reaction. *In vitro* assay, the results showed that the target compounds have a broad spectrum and strong of anti-tumor activity, such as the target compounds **4d**, **4i, 4m** and **4n**, especially of target compound **4n** to human breast cancer cells (MCF-7) was IC_{50}=0.35 ± 0.01 μM, to lung adenocarcinoma cells (A-549) was IC_{50}=0.37 ± 0.01 μM, to cervical cancer cells (Hela) was IC_{50}=0.56 ± 0.02 μM, to gastric adenocarcinoma cells (MDC-803) was IC_{50}=0.53 ± 0.02 μM and to colon cancer cancer cells (COLO-205) was IC_{50}=0.50 ± 0.02 μM. *In vivo* anti-tumor activity test results showed that the target compounds **4d**, **4i**, **4m** and **4n** had a good inhibitory effect on tumor growth. At the same time, the results of acute toxicity tests showed that the target compounds **4d**, **4i**, **4m** and **4n** were of low toxicity. In general, the target compound **4n** could inhibit tumor growth well at a low dose and could be used as a candidate drug for further research and development. At the same time, the results of SAR research and analysis indicated that

these target compounds have the potential of further structural optimization to find more effective anti-tumor activity target compounds.

4. Experimental section

4.1. Chemistry section

Chemistry materials and general methods. The reagents (chemicals) were purchased and used without further purification. The chemical reagent used was purchased from Aladdin (Shanghai), with a purity ≥ 97%. Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker AMX-400 spectrometer (IS as TMS). The melting point (m.p.) was determined by SGW X-4B micro melting point instrument. Mass spectroscopy was performed with an Agilent 6460 system. HPLC analysis of all final biologically test compounds was carried out using an Agilent 1260 series HPLC system. The purity was determined by reversed-phase HPLC and is ≥95% for all biologically tested compounds.

*4.1.1. A general method for synthesis of compounds **2a** to **2c***

The 2-chlorobenzoic acid (compound **1a**, 15.66 g, 0.10 mol) was putted into 250 mL round bottom flask, then 50 mL methylene chloride (CH_2Cl_2) as solvent and 5 drops of *N*, *N*'-dimethyl formamide (DMF) as the catalyst for the reaction were added. The flask was placed in the magnetic mixer, stirring until the reaction liquid became clarified. The thionyl chloride (18.75 mL, 0.20 mol) was constantly dropped into the flask under stirring, at the same time the drop speed rate and reaction temperature were controlled (≤25 ℃ and ≥ 20 min). While the reactants were added, the reaction lasted 2 hours under refluxing. The methylene chloride and excessive thionyl chloride were removed under vacuum. The mixture was dried ,which can get the crude product of 2-chlorobenzoyl chloride (compound **2a**). The crude product was atmospheric distillation, collect the product of 238 ℃, and give pure product of 2-chlorobenzoyl chloride (compound **2a**) as a colorless transparent liquid. The general method was used to synthesis of compounds **2b** to **2c** and all were of colorless transparent liquid.

The 2-chlorobenzoyl chloride (compound **2a**, 12.66 mL, 0.10mol) was putted into 250 mL round bottom flask, then 50 mL methylene chloride (CH_2Cl_2) as solvent for the reaction were added. The flask was placed in the magnetic mixer (≤25 ℃), and a magnetic mixer was used to stir until the reaction liquid became balanced. Dissolve the sesamol (13.81 g, 0.10 mol) in 20 mL of

methylene chloride (CH_2Cl_2) and constantly dropped into the flask under stirring, and the drop speed rate and reaction temperature were controlled (≤25 ℃ and ≥20min). After the reactants were added, the reaction needs stir for 2 hours. After the stirring reaction was completed, the solvent methylene chloride (CH_2Cl_2) was removed by vacuum distillation. The mixture was dried, which could get the crude product of benzo [*d*] [1, 3] dioxol-5-yl-2-chlorobenzoate (compound **3a**). The crude product was recrystallised with toluene, filtered, and dried in vacuum to give pure product of compound **3a** as a white powder. The general method was used to synthesis of compounds **3b** to **3o** and all were of white powder.

*4.1.3. A general method for synthesis of the target compounds **4a** to **4o***

The benzo [*d*] [1, 3] dioxol-5-yl-2-chlorobenzoate (compound **3a**, 27.67 g, 0.10 mol) was putted into 250 mL round bottom flask. Then 50 mL tetrahydrofuran (THF) as solvent, anhydrous potassium carbonate (K_2CO_3, 6.89 g, 0.05 mol) as acid applying agent and 0.03% palladium acetate ($Pd(AcO)_2$) used as catalyst for the reaction were added. After the reactants were added, the reaction lasted for 5 hours under refluxing. When the reflux reaction was completed, the mixture was filtered at a high temperature, removing unreacted anhydrous potassium carbonate (K_2CO_3) and catalyst palladium acetate ($Pd(AcO)_2$), the filtrate was collected, cooled and white powder were precipitated. The white powder was filtered and vacuum dried to obtain the crude product of 7*H*-benzo [*c*] [1, 3] dioxolo [4, 5-*f*] chromen-7-one (compound **4a**). The crude product compound **4a** was recrystallised from petroleum ether (boiling range 60-90℃), filtered, and vacuum dried to give the pure product of compound **4a** as white powder. The general method was used to synthesis of compounds **4b** and **4o** as all were of white powder.

compound **4a** white powder, yield 84%, m.p. 168-170℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.26 (2H, d, J = 15.5 Hz, -CH_2-), 6.98 (1H, d, J = 8.5 Hz, Ph-H), 7.13 (1H, d, J = 8.5 Hz, Ph-H), 7.40-7.60 (2H, 7.45 (ddd, J = 8.1, 7.4, 1.4 Hz, Ph-H), 7.55 (ddd, J = 8.0, 7.4, 1.4 Hz, Ph-H)), 7.76 (1H, ddd, J = 8.1, 1.4, 0.5 Hz, Ph-H), 8.30 (1H, ddd, J = 8.0, 1.4, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 107.8, 113.6, 117.3, 118.0, 127.0, 129.7, 132.7, 132.9, 139.0, 139.4, 149.4, 149.9, 155.2, 167.8; HR-ESI-MS *m/z*: calcd for $C_{14}H_8O_4$ {［M+H］$^+$} 240.0424, found 240.2145; Anal. calcd for $C_{14}H_8O_4$: C, 70.00; H, 3.36; O, 26.64; found: C, 70.02; H, 3.35; O, 26.63%.

compound **4b** white powder, yield 83%, m.p. 165-167℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 2.35 (3H, s, -CH_3), 6.26 (2H, d, J = 15.5 Hz, -CH_2-), 6.72 (1H, s, Ph-H), 7.41 (1H, ddd, J = 8.1, 7.6, 1.3

Hz, Ph-H), 7.50-7.66 (2H, 7.53 (ddd, *J* = 8.2, 7.6, 1.4 Hz, Ph-H), 7.62 (ddd, *J* = 8.1, 1.4, 0.5 Hz, Ph-H)), 7.69 (1H, ddd, *J* = 8.2, 1.3, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 15.9, 101.6, 108.1, 111.8, 121.5, 122.8, 124.5, 126.8, 127.0, 132.4, 133.1, 144.6, 145.7, 162.1; HR-ESI-MS *m/z*: calcd for $C_{15}H_{10}O_4$ {［M+H］$^+$}254.0573, found 254.2416; Anal. calcd for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96; O, 25.17; found: C, 70.85; H, 3.96; O, 25.18%.

compound **4c** white powder, yield 81%, m.p. 163-165℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.16 (3H, s, -CH_3), 2.83 (2H, q, *J* = 7.2 Hz, CH_2-), 6.24 (2H, d, *J* = 15.5 Hz, CH_2-), 6.43 (1H, s, Ph-H), 7.44 (1H, ddd, *J* = 8.1, 7.6, 1.3 Hz, Ph-H), 7.50-7.62 (2H, 7.53 (ddd, *J* = 8.2, 7.6, 1.4 Hz, Ph-H), 7.60 (ddd, *J* = 8.1, 1.4, 0.5 Hz, Ph-H)), 7.69 (1H, ddd, *J* = 8.2, 1.3, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 16.2, 21.8, 101.6, 109.8, 110.6, 121.4, 124.0, 125.4, 126.8, 126.9, 131.8, 133.1, 143.6, 144.2, 146.0, 161.5; HR-ESI-MS *m/z*: calcd for $C_{16}H_{12}O_4$ {［M+H］$^+$} 268.0732, found 268.2689; Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51; O, 23.86; found: C, 71.65; H, 4.51; O, 23.84%.

compound **4d** white powder, yield 80%, m.p. 160-162℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.16 (6H, *s*, -CH_3), 3.31 (1H, d, *J* = 6.9 Hz, -CH-), 6.22 (2H, d, *J* = 15.5 Hz, -CH_2-), 6.29 (1H, s, Ph-H), 7.44 (1H, ddd, *J* = 8.1, 7.6, 1.3 Hz, Ph-H), 7.50-7.63 (2H, 7.53 (ddd, *J* = 8.2, 7.6, 1.4 Hz,Ph-H), 7.61 (ddd, *J* = 8.1, 1.4, 0.5 Hz, Ph-H)), 7.67 (1H, ddd, *J* = 8.2, 1.3, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 22.9, 27.4, 101.6, 109.3, 110.4, 121.6, 125.4, 128.7, 128.8, 132.5, 133.1, 143.8, 145.0, 147.1, 161.6; HR-ESI-MS *m/z*: calcd for $C_{17}H_{14}O_4$ {［M+H］$^+$} 282.0898, found 282.2954; Anal. calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00; O, 22.67; found: C, 72.35; H, 5.00; O, 22.65%.

compound **4e** white powder, yield 78%, m.p. 166-168℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 2.32 (3H, s, -CH_3), 6.43 (2H, d, *J* = 12.5 Hz, -CH_2-), 7.49-7.60 (2H, 7.51 (ddd, *J* = 7.9, 7.4, 1.3 Hz, Ph-H), 7.58 (ddd, *J* = 7.7, 7.4, 1.4 Hz, Ph-H)), 7.65 (1H, ddd, *J* = 7.9, 1.4, 0.5 Hz, Ph-H), 7.74 (1H, s, Ph-H), 7.98 (1H, ddd, *J* = 7.7, 1.3, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 30.9, 101.6, 110.2, 110.4, 120.7, 122.1, 125.5, 126.7, 126.8, 132.7, 133.1, 144.0, 146.2, 150.6, 162.0, 201.3; HR-ESI-MS *m/z*: calcd for $C_{16}H_{10}O_5$ {［M+H］$^+$} 282.0525, found 282.2513; Anal. calcd for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57; O, 28.34; found: C, 68.07; H, 3.58; O, 28.35%.

compound **4f** white powder, yield 88%, m.p. 156-158℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.12 (2H, d, *J* = 15.5 Hz, -CH_2-), 6.64 (1H, dd, *J* = 8.5, 1.2 Hz, Ph-H), 6.81 (1H, d, *J* = 8.5 Hz, Ph-H),

7.12 (1H, d, J = 8.5 Hz, Ph-H), 7.56-7.61 (2H, 7.58 (dd, J = 1.2, 0.4 Hz, Ph-H), 7.60 (dd, J = 8.5, 0.4 Hz, Ph-H)); ^{13}C NMR (100MHz, DMSO-d_6) δ: 101.9, 104.8, 106.6, 112.5, 114.9, 118.3, 130.3, 136.3, 143.9, 144.0, 148.4, 150.4, 162.0; HR-ESI-MS *m/z*: calcd for $C_{14}H_9NO_4$ {［M+H］$^+$} 255.0536, found 255.2296; Anal. calcd for $C_{14}H_9NO_4$: C, 65.88; H, 3.55; N, 5.49; O, 25.07; found: C, 65.86; H, 3.57; N, 5.47; O, 25.09%.

compound **4g** white powder, yield 85%, m.p. 154-156℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 2.31 (3H, s, -CH_3), 6.13 (2H, d, J = 15.5 Hz, -CH_2-), 6.54 (1H, dd, J = 8.5, 1.2 Hz, Ph-H), 6.64 (1H, s, Ph-H), 7.53 (1H, dd, J = 1.2, 0.4 Hz, Ph-H), 7.60 (1H, dd, J = 8.5, 0.4 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 15.9, 101.6, 104.8, 107.3, 111.8, 115.0, 119.1, 123.0, 130.3, 135.2, 144.2, 144.8, 145.2, 150.4, 161.4; HR-ESI-MS *m/z*: calcd for $C_{15}H_{11}NO_4$ {［M+H］$^+$} 269.0683, found 269.2568; Anal. calcd for $C_{15}H_{11}NO_4$: C, 66.91; H, 4.12; N, 5.20; O, 23.77; found: C, 66.90; H, 4.13; N, 5.21; O, 23.76%.

compound **4h** white powder, yield 83%, m.p. 150-152℃; ^{1}H NMR (400MHz, DMSO) δ: 1.15 (3H, s, -CH_3), 2.87 (2H, q, J = 7.2 Hz, -CH_2-), 6.15 (2H, d, J = 15.5 Hz, -CH_2-), 6.42 (1H, s, Ph-H), 6.54 (1H, dd, J = 8.5, 1.2 Hz, Ph-H), 7.56 (1H, dd, J = 1.2, 0.4 Hz, Ph-H), 7.63 (1H, dd, J = 8.5, 0.4 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO) δ: 16.2, 21.8, 101.6, 104.7, 108.8, 110.6, 115.0, 118.7, 124.5, 130.3, 134.7, 140.5, 143.7, 145.3, 150.5, 161.4; HR-ESI-MS *m/z*: calcd for $C_{16}H_{13}NO_4$ {［M+H］$^+$}283.0841, found 283.2836; Anal. calcd for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94; O, 22.59; found: C, 67.83; H, 4.64; N, 4.95; O, 22.58%.

compound **4i** white powder, yield 80%, m.p. 148-150℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.17 (6H, s, -CH_3), 3.16 (1H, d, J = 6.9 Hz, -CH-), 6.13 (2H, d, J = 15.5 Hz, -CH_2-), 6.25 (1H, s, Ph-H), 6.54 (1H, dd, J = 8.5, 1.2 Hz, Ph-H), 7.52-7.61 (2H, 7.53 (dd, J = 1.2, 0.4 Hz, Ph-H), 7.60 (dd, J = 8.5, 0.4 Hz, Ph-H)); ^{13}C NMR (100MHz, DMSO-d_6) δ: 22.9, 27.4, 101.6, 104.9, 108.6, 109.4, 115.0, 119.1, 129.2, 130.3, 135.3, 143.2, 143.5, 146.7, 150.4, 161.5; HR-ESI-MS *m/z*: calcd for $C_{17}H_{15}NO_4$ {［M+H］$^+$} 297.1007, found 299.1065; Anal. calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71; O, 21.53; found: C, 68.67; H, 5.09; N, 4.73; O, 21.52%.

compound **4j** white powder, yield 77%, m.p. 162-164℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 2.32 (3H, s, -CH_3), 6.34 (2H, d, J = 12.5 Hz, -CH_2-), 6.60 (1H, dd, J = 8.5, 1.2 Hz, Ph-H), 7.55 (1H, dd, J = 8.5, 0.4 Hz, Ph-H), 7.65 (1H, s, Ph-H), 7.71 (1H, dd, J = 1.2, 0.4 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 30.9, 101.6, 105.7, 110.5, 115.0, 118.9, 120.6, 130.3, 135.5, 143.9, 146.1,

150.0, 150.4, 162.0, 201.3; HR-ESI-MS *m/z*: calcd for $C_{16}H_{11}NO_5$ {［M+H］$^+$} 297.0633, found 297.2661; Anal. calcd for $C_{16}H_{11}NO_5$: C, 64.65; H, 3.73; N, 4.71; O, 26.91; found: C, 64.64; H, 3.76; N, 4.70; O, 26.90%.

compound **4k** white powder, yield 80%, m.p. 182-184℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.31 (2H, d, J = 15.5 Hz, -CH_2-), 7.02 (1H, d, J = 8.6 Hz, Ph-H), 7.12 (1H, d, J = 8.6 Hz, Ph-H), 7.99 (1H, dd, J = 8.5, 0.5 Hz, Ph-H), 8.06 (1H, dd, J = 8.5, 1.8 Hz, Ph-H), 8.36 (1H, dd, J = 1.8, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 101.9, 107.7, 111.6, 112.2, 119.4, 124.7, 127.5, 129.3, 135.9, 143.5, 144.5, 149.3, 151.0 161.1; HR-ESI-MS *m/z*: calcd for $C_{14}H_7NO_6$ {［M+H］$^+$} 285.0271, found 285.2113; Anal. calcd for $C_{14}H_7NO_6$: C, 58.96; H, 2.47; N, 4.91; O, 33.66; found: C, 58.97; H, 2.46; N, 4.90; O, 33.67%.

compound **4l** white powder, yield 79%, m.p. 180-182℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 2.35 (3H, s, -CH_3), 6.31 (2H, d, J = 15.5 Hz, -CH_2-), 6.75 (1H, s, Ph-H), 7.99-8.07 (2H, 8.00 (dd, J = 8.5, 0.5 Hz, Ph-H), 8.06 (dd, J = 8.5, 1.8 Hz, Ph-H)), 8.32 (1H, dd, J = 1.8, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 15.9, 101.6, 107.7, 111.8, 119.3, 123.0, 124.7, 128.2, 129.3, 134.9, 143.7, 144.7, 146.0, 151.2, 159.9; HR-ESI-MS *m/z*: calcd for $C_{15}H_9NO_6$ {［M+H］$^+$} 299.0434, found 299.2382; Anal. calcd for $C_{15}H_9NO_6$: C, 60.21; H, 3.03; N, 4.68; O, 32.08; found: C, 60.20; H, 3.04; N, 4.67; O, 32.09%.

compound **4m** white powder, yield 77%, m.p. 179-181℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.19 (3H, t, J = 7.2 Hz, -CH_3), 2.82 (2H, q, J = 7.2 Hz, -CH_2-), 6.33 (2H, d, J = 15.5 Hz, -CH_2-), 6.49 (1H, s, Ph-H), 7.99-8.07 (2H, 8.00 (dd, J = 8.5, 0.5 Hz, Ph-H), 8.06 (dd, J = 8.5, 1.8 Hz, Ph-H)), 8.32 (1H, dd, J = 1.8, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 16.3, 21.8, 101.6, 109.3, 110.6, 119.4, 123.7, 124.7, 127.8, 129.3, 134.4, 143.7, 144.2, 147.2, 151.2, 159.9; HR-ESI-MS *m/z*: calcd for $C_{16}H_{11}NO_6$ {［M+H］$^+$} 313.0587, found 313.2653; Anal. calcd for $C_{16}H_{11}NO_6$: C, 61.35; H, 3.54; N, 4.47; O, 30.64; found: C, 61.34; H, 3.53; N, 4.48; O, 30.63%.

compound **4n** white powder, yield 75%, m.p. 177-179℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.21 (6H, s, -CH_3), 3.27 (1H, d, J = 7.0 Hz, -CH-), 6.30 (2H, d, J = 15.5 Hz, -CH_2-), 6.46 (1H, s, Ph-H), 7.99-8.07 (2H, 8.00 (dd, J = 8.5, 0.5 Hz, Ph-H), 8.06 (dd, J = 8.5, 1.8 Hz, Ph-H)), 8.32 (1H, dd, J = 1.8, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 22.9, 27.3, 101.6, 109.4, 110.2, 119.5, 124.7, 126.2, 129.2, 123.0, 135.0, 143.5, 144.5, 147.4, 151.2, 160.1; HR-ESI-MS *m/z*: calcd for $C_{17}H_{13}NO_6$ {［M+H］$^+$} 327.0746, found 327.2921; Anal. calcd for $C_{17}H_{13}NO_6$: C, 62.39; H, 4.00; N, 4.28;

O, 29.33; found: C, 62.38; H, 4.01; N, 4.27; O, 29.34%.

compound **4o** white powder, yield 74%, m.p. 191-193℃; ^{1}H NMR (400MHz, DMSO-d_6) δ: 1.21 (6H, s, -CH_3), 3.27 (1H, d, J = 7.0 Hz, -CH-), 6.30 (2H, d, J = 15.5 Hz, -CH_2-), 6.46 (1H, s, Ph-H), 7.99-8.07 (2H, 8.00 (dd, J = 8.5, 0.5 Hz, Ph-H), 8.06 (dd, J = 8.5, 1.8 Hz, Ph-H)), 8.32 (1H, dd, J = 1.8, 0.5 Hz, Ph-H); ^{13}C NMR (100MHz, DMSO-d_6) δ: 30.9, 101.6, 109.9, 110.5, 119.3, 120.6, 124.7, 126.3, 129.3, 135.2, 144.0, 146.3, 150.2, 151.2, 161.1, 200.9; HR-ESI-MS *m/z*: calcd for $C_{16}H_9NO_7$ {［M+H］$^+$} 327.0375, found 327.2482; Anal. calcd for $C_{16}H_9NO_7$: C, 58.72; H, 2.77; N, 4.28; O, 34.22; found: C, 58.71; H, 2.78; N, 4.29; O, 34.21%.

4.2. Biological activities section

4.2.1. Biological activity screening in vitro

Cells in logarithmic phase were collected (human breast cancer cells (MCF-7), lung adenocarcinoma cells (A-549), cervical cancer cells (Hela), gastric adenocarcinoma cells (MDC-803) and colon cancer cancer cells (COLO-205) and cell suspension concentration was adjusted. Then inoculated into 96-well plates, 200 μL for each hole and cells density was adjusted to 10^4 holes by paving plates. The culture plates were placed at 37 ℃ in 5% CO_2 incubator to make the cells stick to the wall for 24 hours. The prepared concentration gradients of the samples to be tested were repeated 6 times for each gradient. The 20 μL was taken to each hole of the experimental group, and a blank group and a control group were set for further cultivation for 48 hours. When the culture was completed, the supernatant was carefully aspirated, the 80 μL fresh RPMI 1640 was added, then the 20 μL MTT solution (5 mg.ml^{-1}, 0.5% MTT) was added and the incubation for 4 hours. The culture solution was centrifuged and then the supernatant was sucked off 150 μL of dimethyl sulfoxide (DMSO) was added into each hole, and low-speed oscillations were set on the shaker for 10 min to fully dissolve the crystals. The optical density (OD) of each hole were measured by enzyme linked immunosorbent assay (ELISA) at 490 nm. At the same time, the zeroing holes (medium, MTT, DMSO) were set and the control holes (cells, the same concentration of drug solvent, culture medium, MTT, DMSO) were set, and each group was set to have 3 multiple holes. The data processing: The cell inhibition rate was calculated by formula 1-1. The data of this study were analyzed by linear regression, and the statistical software was versions SPSS13.0.

$$\text{Cell inhibtion rate} = \frac{OD_{\text{control group}} - OD_{\text{specimen}}}{OD_{\text{control group}}} \times 100\% \quad \textbf{1-1}$$

4.2.2. Animals

The Kunming type nude mice weighing approximately 200-250 g at the beginning of the experimental procedure were used. Four rats were housed per cage (size 26×41 cm^2); animals were fed with standard laboratory diet and tap water ad libitum, kept at 23±1 °C with a 12 h light/dark cycle, light at 7 a.m. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Chongqing Normal University and approved by the Animal Ethics Committee of Chongqing Normal University.

4.2.3. Biological activity evaluation in vivo

According to the standard method of anti-tumor activity test *in vivo*, the Kunming type nude mice were selected. The logarithmic growth phase of tumor cells (sarcoma S180 and lung cancer H22 tumor strains) was taken and cell suspensions were prepared for subcutaneous inoculation. The animals were randomly assigned to the drug after the tumor grew to 100-300 mm^3. There were three dose treatment groups of 10 mg. kg^{-1}. d^{-1}, 50 mg. kg^{-1}. d^{-1} and 100 mg. kg^{-1}. d^{-1}, a positive control group (50 mg. kg^{-1}. d^{-1}) and a blank control group, and take it orally once a day for 10 days. The anti-tumor effect of the sample was observed dynamically by measuring tumor diameter. The measurement frequency of tumor diameter depends on the growth of transplanted tumor, usually 2-3 times per week. The trial will also look at indicators related to drug safety, such as animal weight gain and mortality, and comparing these data between the treatment group and the standard treatment control group is important for determining drug safety and development prospects.The relative tumor proliferation rate (T/C, %) was used as the evaluation index of anti-tumor activity *in vivo*. The relevant formulas were shown in equations **1-2**, **1-3** and **1-4**. The data of this study were analyzed by linear regression, and the statistical software was versions SPSS13.0.

$$T/C\ (\%) = \frac{T_{RTV}}{C_{RTV}} \times 100\% \quad \textbf{1-2}$$

T_{RTV}: The treatment group RTV.

C_{RTV}: The blank control group RTV.

Tumor volume， $V = \frac{1}{2} \times a \times b^2 or \frac{\prod}{6} \times a \times b \times c$ ……**1-3**

a: Length.

b: Width.

c: Height.

Relative tumor volume， $RTV = \frac{V_t}{V_0}$ ……**1-4**

V_0：Tumor volume was measured during general administration.

V_t：Tumor volume at each measurement.

4.2.4. Acute toxicity test

The Kunming mice were kept in the laboratory animal room for observation for 3-5 days, so that they could adapt to the environment. After it was proved that they were healthy animals, they were randomly divided into half males and half females, and the weight of the mice was 18-22 g. Mice were fasted on an empty stomach for 16 hours, during the time, drinking water could be properly fed. Intragastric administration was used, and the gavage volume of each dose group and positive control group were the same, with a volume of 0.40 mL (20 mL. kg^{-1}). The tested compounds was prepared into different dose groups with dimethylsulfoxide (DMSO) as solvent, adriamycin as positive control group. After oral administration of the subject, the subject shall be observed for 7 or 14 days. If death continues on the 4th day after administration, the patient shall be observed for 14 days, or extended to 28 days if necessary. Record the number of deaths, refer to the table to obtain lethal dose 50% (LD_{50}), and record the time of death and poisoning manifest.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

Birbrair A, Zhang T, Wang Z, et al. (2014) Type-2 pericytes participate in normal and tumoral angiogenesis. Am. J. Physiol. Cell. Ph. 307: 25–38.

Cunningham F, Fiebelkorn S, Johnson M, et al. (2011) A novel application of the Margin of Exposure approach: segregation of tobacco smoke toxicants. Food. Chem. Toxicol. 49: 2921–2933.

Handa O, Naito Y, Yoshikawa T. (2011) Redox biology and gastric carcinogenesis: the role of Helicobacter pylori. Red. Rep. 16: 1–7.

Katsurano M, Niwa T, Yasui Y, et al. (2012) Early-stage formation of an epigenetic field defect in a mouse colitis model, and non-essential roles of T-cells and B-cells in DNA methylation induction. Oncogene. 31: 342–351.

Vogelstein B, Papadopoulos N, Velculescu V, et al. (2013) Cancer genome landscapes. Science. 339: 1546–1558.

Murphy J. (2014) A robust methodology to subclassify pseudokinases based on their nucleotide-binding properties. J. Biochem. 457: 323–334

Eyers P, Murphy J. (2016) The evolving world of pseudoenzymes: proteins, prejudice and zombies. BMC Biology. 14: 98.

Linden O, Kooistra A, Leurs R, et al. (2013) KLIFS: A knowledge-based structural database to navigate kinase-ligand interaction space. J. Med .Chem. 57: 249–277.

Cho T, Dong S, Jun F, et al. (2010) Novel potent orally active multitargeted receptor tyrosine kinase inhibitors: synthesis, structure-activity relationships, and anti-tumor activities of 2-indolinone derivatives. J. Med. Chem. 53: 8140-8149.

Gangjee A, Zaware N, Raghavan S, et al. (2013) Synthesis and biological activity of 5-chloro-N-4-substituted phenyl-9H-pyrimido [4, 5-b] indole-2, 4-diamines as vascular endothelial growth factor receptor-2 inhibitors and antiangiogenic agents. Bioorg. Med. Chem. 21: 1857-1864.

Shi Z, Guang C. (2018) Design, synthesis and bioactivity evaluation of tumor Sorafenib analogues. RSC adv. 8: 37643-37651.

Hu X, Zhong X, Zhang X, et al. (2007) 7′-(3′, 4′-dihydroxyphenyl)-N-[(4-methoxyphenyl) ethyl] propenamide (Z23), an effective compound from the Chinese herb medicine Fissistigma oldhamii (Hemsl.) Merr, suppresses T cell-mediated immunity in vitro and in vivo. J. P. Life. Sci. 81: 1677–1684.

Zhang Y, Zhong X, Zheng Z, et al. (2007) Discovery and synthesis of new immunosuppressive alkaloids from the stem of Fissistigma oldhamii (Hemsl.) Merr. Bioorg. Med. Chem. 15: 988–996.

Hwang T, Li G, Lan Y, et al. (2009) Potent inhibition of superoxide anion production in activated human neutrophils by isopedicin, a bioactive component of the Chinese medicinal herb Fissistigma oldhamii. Free. Radical. Bio. Med. 46: 520–528.

Ge Y, Zhu S, Shang M, et al. (2013) Aristololactams and aporphines from the stems of Fissistigma oldhamii (Annonaceae). Phytochem. 86: 201–207.

Qi Z, Yan H, Yu Q, et al. (2016) Fissitungfines A and B, two novel aporphine related alkaloids from Fissistigma tungfangense. Tet. Lett. 57: 4162–4164.

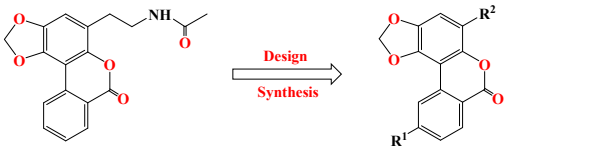
Hong C, Jing Z, Pei H, et al. (2019) Synthesis, biological evaluation and molecular docking of 4-Amino-2H-benzo[h]chromen-2-one (ABO) analogs containing the piperazine moiety. Bioorg. Med. Chem. 27: 115081.

Hong C, Yu Q, Hui J, et al. (2020) Synthesis and pharmacological evaluation of naftopidil-based arylpiperazine derivatives containing the bromophenol moiety. Pharmacolog. Rep. 72: 1058-1068.

Highlights

1. A series of derivatives were obtained by structural modification of Fissitungfine B.
2. The target compounds had a good inhibitory effect on tumor growth.
3. The target compound **4n** could be used as a candidate drug.

Graphical abstract



Fissitungfine B

R^1: -H, -NH_2, -NO_2
R^2: -H, -CH_2CH_3, -$CH(CH_3)_2$, -$COCH_3$
Target compounds 4a-4o

Declaration of interests

☑ The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: