



Cite this: *RSC Adv.*, 2024, **14**, 20339

Received 15th April 2024
 Accepted 19th June 2024

DOI: 10.1039/d4ra02797c
rsc.li/rsc-advances

Synthesis and bioactivity investigation of benzophenone and its derivatives†

Chun Lei,^{abc} Wanjing Yang,^{abc} Ziyu Lin,^{abc} Yuyan Tao,^{bc} Renping Ye,^{bc} Yucai Jiang,^d Yuli Chen^{bc} and Beidou Zhou  ^{*abc}

Four benzophenones, three dihydrocoumarins, and two coumarins were synthesised by a 1–3 step reaction, with yields ranging from 6.2 to 35%. Next, we investigated the *in vitro* antitumour activity of these compounds. Compounds **1**, **8**, and **9** exhibited strong antitumour activity and were considered promising candidates in this field. In particular, compound **1** exhibited very strong inhibitory activity against HL-60, A-549, SMMC-7721, and SW480 cells, with IC₅₀ values of 0.48, 0.82, 0.26, and 0.99 μ M, respectively. Finally, the antitumour mechanism of compound **1** was investigated through network pharmacology and molecular docking analyses, which identified 22 key genes and 21 tumour pathways. AKT1, ALB, CASP3, ESR1, GAPDH, HSP90AA1, and STAT3 were considered as potential target hub genes for compound **1**. These results will enable the future development of benzophenone and its derivatives.

1 Introduction

Two aryl groups are connected to both sides of a carbonyl group to produce benzophenone. These two aryl and carbonyl groups form a conjugated system.¹ Dihydrocoumarin and coumarin are derivatives of benzophenone (Fig. 1). Their basic structure consists of a benzene ring and a carbonyl group, which are linked to other groups in different ways to form various types of compounds. The two aryl groups of dihydrocoumarin are linked by a chiral carbon. In addition, the chemical name for coumarin is benzopyranone, which is the parent nucleus of a large class of coumarin compounds. These factors affect their physicochemical properties and biological activity.

The unique structure of natural products and their attractive effects are of great significance for the discovery and development of new drugs. Inspired by the structure and efficacy of natural products, chemists can synthesise new medicinal molecules. For example, morphine was isolated from opium poppy and diacetylated to produce heroin, which was more analgesic and addictive. For another example, camptothecin was extracted from plants and exhibited good inhibitory activity against several tumor cells, but its human metabolite 10-hydroxycamptothecin exhibited better inhibitory activity and

less toxic side effects than it. Therefore, 10-hydroxycamptothecin was synthesised by some chemists as a medicinal molecule.

The benzophenone scaffold is one of structures in medicinal chemistry, found in several synthetic or naturally occurring molecules that exhibit a variety of biological activities, such as anticancer, antimicrobial, anti-inflammatory, and antiviral effects (Fig. 2). The benzophenone scaffold is the antitumor parent nucleus, and 3-hydroxy-4-methoxyphenyl and 3,4,5-trimethoxyphenyl are important antitumor groups, so phenstatin exhibits good antitumor activity.² Khanum *et al.* integrated

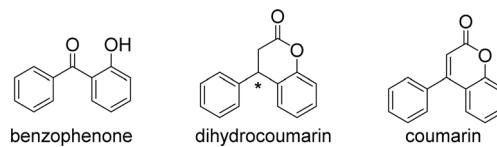


Fig. 1 The structure of benzophenone and its derivatives.

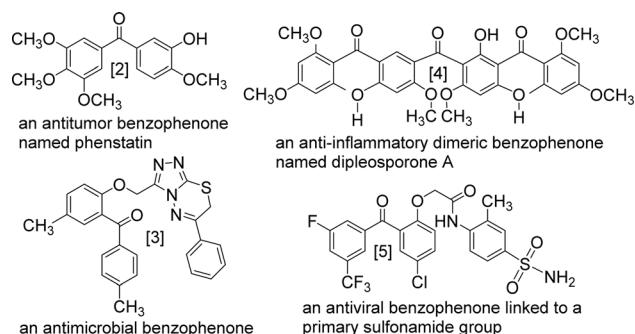


Fig. 2 Four benzophenone compounds with different biological activities.

^aSchool of Pharmacy, Fujian Medical University, Fuzhou 350108, China. E-mail: 491986737@qq.com; beidouzhou@ptu.edu.cn; Tel: +8613205940072

^bSchool of Pharmacy and Medical Technology, Putian University, Putian 351100, China

^cKey Laboratory of Pharmaceutical Analysis and Laboratory Medicine (Putian University), Fujian Province University, Putian 351100, China

^dThe Affiliated Hospital (Group) of Putian University, Putian 351100, China

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4ra02797c>



1,3,4-oxadiazole-2-(3*H*)thione and triazolothiadiazine moieties into benzophenone framework to obtain compounds with good antimicrobial activity.³ These two heterocycles are key groups in antimicrobial activity. Zeng *et al.* reported three dimeric benzophenones inhibited lipopolysaccharide-induced NO production in RAW 264.7 cells, with half-maximal inhibitory concentration (IC_{50}) values ranging from 8.8 to 18.1 μ M, being more potent than the positive control, dexamethasone.⁴ Which means that dimeric benzophenones is the anti-inflammatory parent nucleus. Benzophenone derivatives with amino-sulfanilamide are non-nucleoside reverse transcriptional inhibitors, which seems to be effective against human immunodeficiency virus (HIV).⁵ This amino-sulfanilamide moiety is a key group that inhibits HIV. In addition, various synthetic benzophenone motifs are present in marketed drugs, such as ketoprofen, tolcapone, and fenofibrate (Fig. 3).⁶ Our team previously synthesised the benzophenone compound **s3** using isovanillic acid (**s1**) and 2,4-dimethylphenol (**s2**) in Eaton's reagent (Fig. 4); this compound exhibited strong inhibitory activity against promyelocytic leukemia cell line HL-60 and hepatocarcinoma cell line SMMC-7721 with IC_{50} values of 0.122 and 0.111 μ M, respectively.⁷ Therefore, **s3** is an excellent candidate for further modification.

In addition, some representative studies on the synthesis of dihydrocoumarins and coumarins are discussed below. Jeon *et al.* reported the selective preparation of dihydrocoumarin derivatives using intramolecular cyclisation of cinnamic acid and substituted phenol, catalysed by *p*-toluene sulfonic acid (PTSA) or trifluoroacetic acid (TFA). A selective method for preparing dihydrocoumarin from aryl cinnamates using PTS, MeSO₃H, TiCl₄, or SnCl₄ was also reported.⁸ Jagdale *et al.* reported the hydroarylation of cinnamic acid with *p*-cresol mediated by PTS to give dihydrocoumarin, followed by dehydrogenation of the dihydrocoumarin product with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to afford coumarin. Coumarin could be obtained by oxidation of dihydrocoumarin by DDQ,⁹ and also by reaction of benzophenones with sodium acetate and acetic anhydride.¹⁰

Advances in modern bioinformatics and computing techniques greatly facilitate the development of pharmaceutical molecules into drugs. Network pharmacology studies the mechanism of action of drugs in a multi-component, multi-

target, and multi-pathway manner, and has important reference value for the development of novel compounds.

In short, **s3** is a good lead compound that can be used for further modification. Some of its analogues and derivatives can be synthesised by the related methods and produce similar or better biological activities. Therefore, network pharmacology and molecular docking are used to explore the mechanism of action of the synthesized compounds.

2 Results and discussion

2.1 Synthesis

Our team previously reported that **s3** had a yield of only 9.0%, so its synthesis process was explored and optimized.⁷ The optimum synthesis conditions of **s3** were found to be an isovanillic acid/2, 4-dimethylphenol/phosphorus pentoxide molar ratio of 2:2:1 and a reaction time of 2.5 h. Under these conditions, its yield could reach 29.5%. Compound **1** was obtained by the reaction of **s3** with 4-dimethylaminopyridine, triethylamine, and 2-chloroacetyl chloride in anhydrous 1,4-dioxane (Fig. 5). As a result, the hydroxyl at the *ortho*-position of the methoxy group reacted with 2-chloroacetyl chloride. Owing to the steric hindrance of the methyl group, the hydroxyl at the *ortho*-position of the carbonyl group could not be esterified.

Using anhydrous potassium carbonate, **s3** reacted with ethyl 2-chloroacetate in acetone to yield compound **2**. The hydroxyl group adjacent to the methoxy group in **s3** underwent a nucleophilic substitution reaction with ethyl 2-chloroacetate. Also due to steric hindrance, the hydroxyl group adjacent to the carbonyl group in **s3** could not undergo nucleophilic substitution reaction with ethyl 2-chloroacetate. Next, compound **2** was refluxed in tetrahydrofuran and 24 equivalents of 1 M hydrochloric acid; then, its ester group was hydrolysed to yield compound **3** (Fig. 6). Compound **2** was initially refluxed in hydrochloric acid and ethanol; then, both ether and ester bonds were hydrolysed to give **s3**. Compound **2** was selectively hydrolysed by replacing ethanol with tetrahydrofuran to afford compound **3**, containing a carboxylic acid side chain.

Using anhydrous potassium carbonate, **s3** and *N*-methyl-4-chloropyridine-2-carboxamide (**s4**) was reacted in dimethylsulfoxide to form compound **4** (Fig. 7). Again, owing to the difference in steric hindrance, only the hydroxyl adjacent to the methoxy group in **s3** reacted with **s4**.

Cinnamic acid compounds and alkyl-substituted phenols were reacted in Eaton's reagent to obtain compounds **5**–**7** (Fig. 8). The cinnamic acid compounds were ferulic, 3,4,5-trimethoxyphenylacrylic, and erucic acids, whereas the phenol compounds were *p*-ethylphenol and *p*-isopropylphenol. An

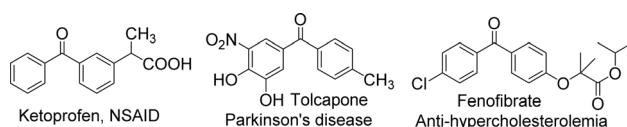


Fig. 3 Several representative benzophenone drugs.

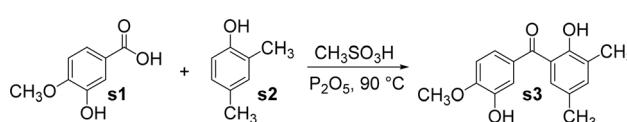


Fig. 4 The synthetic route of compound **s3**.

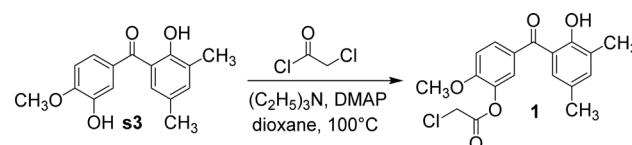


Fig. 5 The synthetic route of compound **1**.



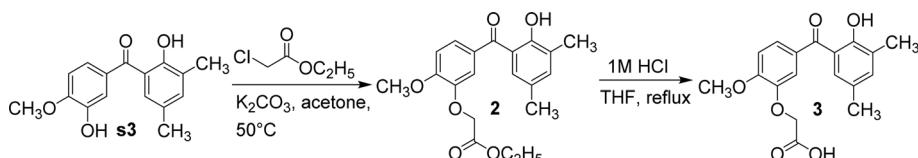


Fig. 6 The synthetic route of compounds 2–3.

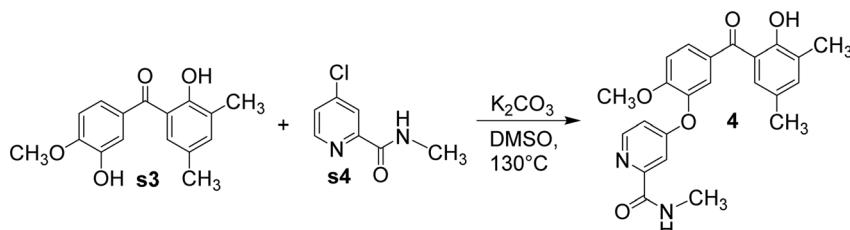


Fig. 7 The synthetic route of compound 4.

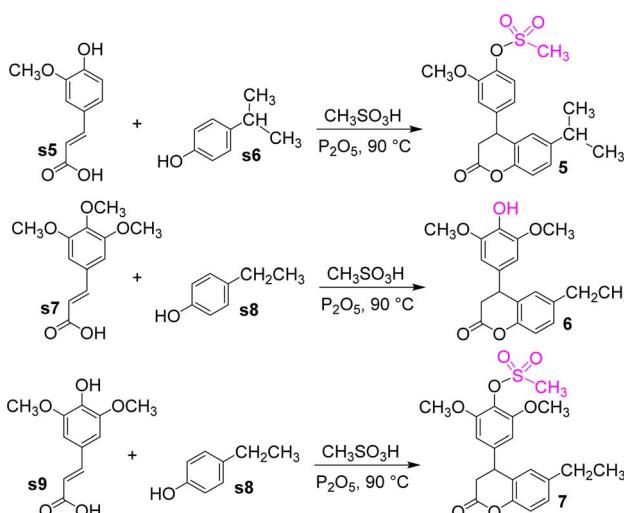


Fig. 8 The synthetic route of compounds 5–7.

unexpected mechanism was found for these reactions, in which the methoxy group at C4 position of the phenyl ring of the dihydrocoumarin intermediate was converted to the hydroxyl group to give compound 6. However, compounds 5 and 7 were esterification products of dihydrocoumarin intermediates and methanesulfonic acid. This finding has a good reference value for the synthesis of related compounds.

Compound s3 reacted with sodium acetate and acetic anhydride to yield compound 8. In this reaction, the phenolic hydroxyl group adjacent to the carbonyl group was involved in the formation of coumarin, while the other phenolic hydroxyl

group was acetylated to ester. Under the catalysis of 9 equivalents of 1 M hydrochloric acid, compound 8 was hydrolysed in ethanol to afford compound 9 (Fig. 9). In this reaction, compound 8 was deacetylated and acidified, exposing the phenol hydroxyl group.

2.2 Biological activity

2.2.1 Antitumor activity. Tables 1 and 2 present the results of the antitumor activity assays. All compounds were evaluated to determine their *in vitro* cytotoxic activity against five human cancer cell lines, specifically the promyelocytic leukemia cell line HL-60, lung cancer cell line A-549, hepatocarcinoma cell line SMMC-7721, breast cancer cell line MDA-MB-231, and colon cancer cell line SW480. The inhibitory percentage of some compounds was greater than 50% and their IC₅₀ values were subsequently determined relative to the corresponding tumor cell lines. Two anticancer drugs, cisplatin and paclitaxel (Taxol), were co-assayed as positive controls.

For the promyelocytic leukemia cell line HL-60, compound 4 exhibited weak inhibitory activity with an IC₅₀ value of 14.30 μM, and compounds 2 and 3 exhibited strong inhibitory activity with IC₅₀ values of 1.28 and 3.65 μM, respectively. Compounds 1, 8, and 9 exhibited very strong inhibitory activity against HL-60 cells with IC₅₀ values of 0.48, 0.15, and 0.16 μM, respectively. Among these, compounds 1, 2, 8, and 9 exhibited stronger inhibitory activity than cisplatin against HL-60 cells.

For the A-549 lung cancer cell line, compounds 2, 3, and 4 exhibited weak inhibitory activity with IC₅₀ values ranging from 10.64 to 27.86 μM, and compounds 8 and 9 exhibited strong

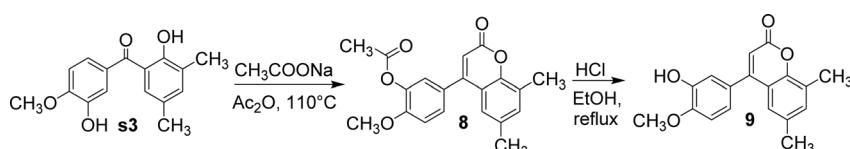


Fig. 9 The synthetic route of compounds 8 and 9.

Table 1 The inhibitory percentage of compounds 1–9 at 40 μ M against five cancer cell lines (%)

Compd	HL-60	A-549	SMMC-7721	MDA-MB-231	SW480
1	98.83 \pm 0.42	74.56 \pm 0.87	84.65 \pm 0.66	82.63 \pm 1.53	58.91 \pm 0.99
2	68.57 \pm 0.48	65.91 \pm 0.80	69.93 \pm 2.18	45.03 \pm 1.40	61.83 \pm 2.32
3	68.71 \pm 0.89	63.12 \pm 2.92	70.99 \pm 3.61	64.86 \pm 2.71	64.58 \pm 0.88
4	84.07 \pm 0.45	49.55 \pm 1.43	68.10 \pm 2.22	51.78 \pm 1.09	45.08 \pm 1.13
5	8.36 \pm 2.75	6.42 \pm 2.85	31.34 \pm 1.45	10.79 \pm 2.55	8.16 \pm 1.53
6	42.41 \pm 0.34	52.87 \pm 1.96	76.09 \pm 0.71	53.96 \pm 0.08	54.40 \pm 0.69
7	9.95 \pm 1.28	4.65 \pm 2.34	25.30 \pm 3.04	9.88 \pm 2.76	0.79 \pm 1.56
8	91.04 \pm 0.65	63.52 \pm 0.50	75.27 \pm 0.95	67.32 \pm 1.48	57.67 \pm 2.40
9	89.08 \pm 0.22	65.71 \pm 1.01	74.72 \pm 0.47	67.37 \pm 1.41	56.45 \pm 1.89

Table 2 IC_{50} values of compounds 1–4, 6, and 8–9 against corresponding cancer cell lines^a

Compd	HL-60	A-549	SMMC-7721	MDA-MB-231	SW480
1	0.48 \pm 0.068	0.82 \pm 0.038	0.26 \pm 0.15	9.97 \pm 0.23	0.99 \pm 0.045
2	1.28 \pm 0.022	10.64 \pm 0.55	1.55 \pm 0.083	—	13.56 \pm 1.88
3	3.65 \pm 0.14	14.25 \pm 0.91	11.26 \pm 1.10	3.77 \pm 0.25	7.61 \pm 0.22
4	14.30 \pm 0.24	—	21.04 \pm 1.11	19.02 \pm 1.23	—
6	—	27.86 \pm 0.94	11.50 \pm 2.33	11.63 \pm 0.73	29.07 \pm 2.01
8	0.15 \pm 0.004	3.92 \pm 0.31	1.02 \pm 0.034	6.13 \pm 0.10	0.51 \pm 0.054
9	0.16 \pm 0.015	4.61 \pm 0.29	0.80 \pm 0.059	5.65 \pm 0.34	0.93 \pm 0.20
Cisplatin	2.50 \pm 0.044	10.10 \pm 0.34	8.68 \pm 0.36	20.27 \pm 1.43	17.70 \pm 0.47
Taxol	<0.008	<0.008	0.11 \pm 0.002	<0.008	<0.008

^a “—” means not being determined.

inhibitory activity with IC_{50} values of 3.92 and 4.61 μ M, respectively. Compound 1 exhibited very strong inhibitory activity against A-549 cells with an IC_{50} value of 0.82 μ M. Among these, compounds 1, 8, and 9 exhibited stronger inhibitory activity than cisplatin against A-549 cells.

For the SMMC-7721 hepatocarcinoma cell line, compounds 3, 4, and 6 exhibited weak inhibitory activity with IC_{50} values in the range of 11.26–21.04 μ M. Furthermore, compounds 2 and 8 exhibited strong inhibitory activity against SMMC-7721 cells with IC_{50} values of 1.55 and 1.02 μ M, respectively. In particular, compounds 1 and 9 exhibited very strong inhibitory activity with IC_{50} values of 0.26 and 0.80 μ M, respectively.

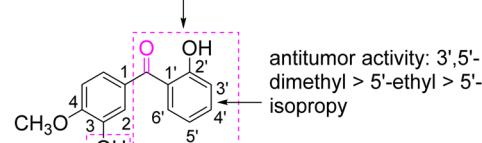
For the MDA-MB-231 breast cancer cell line, compounds 4 and 6 exhibited weak inhibitory activity with IC_{50} values of 19.02 and 11.63 μ M, respectively, and compounds 1, 8, and 9 exhibited moderate inhibitory activity with IC_{50} values of 9.97, 6.13 and

5.65 μ M, respectively. Furthermore, compound 3 exhibited strong inhibitory activity with an IC_{50} value of 3.77 μ M.

For the SW480 colon cancer cell line, compounds 2 and 6 exhibited weak inhibitory activity with IC_{50} values of 13.56 and 29.07 μ M, respectively, and compound 3 exhibited moderate inhibitory activity with an IC_{50} value of 7.61 μ M. In particular, compounds 1, 8, and 9 exhibited very strong inhibitory activity with IC_{50} values of 0.99, 0.51 and 0.93 μ M, respectively.

In summary, 1, 8, and 9 exhibited excellent antitumour activity, whereas 2 and 3 exhibited moderate antitumour effects, and the other compounds exhibited weak antitumour activity. In other words, the above nine compounds, designed and synthesised based on s3, exhibited varying antitumour activities. We previously reported on the synthesis and antitumour activities of s3 and its six benzophenone analogues, which also exhibited varying antitumour activities.⁷ Combining the results

After the structure is converted to coumarin, the antitumor activity is retained or slightly weakened. When it is converted to dihydrocoumarin, the antitumor activity is significantly reduced.



This hydroxyl group is very important. After it forms ester bond with carboxylic acid or acyl chloride, the antitumor activity is retained or slightly weakened.

After the hydroxyl group forms ether bond with alkyl chloride or pyridine chloride, the antitumor activity is significantly weakened.

Fig. 10 Summary of the SAR of the prepared compounds.



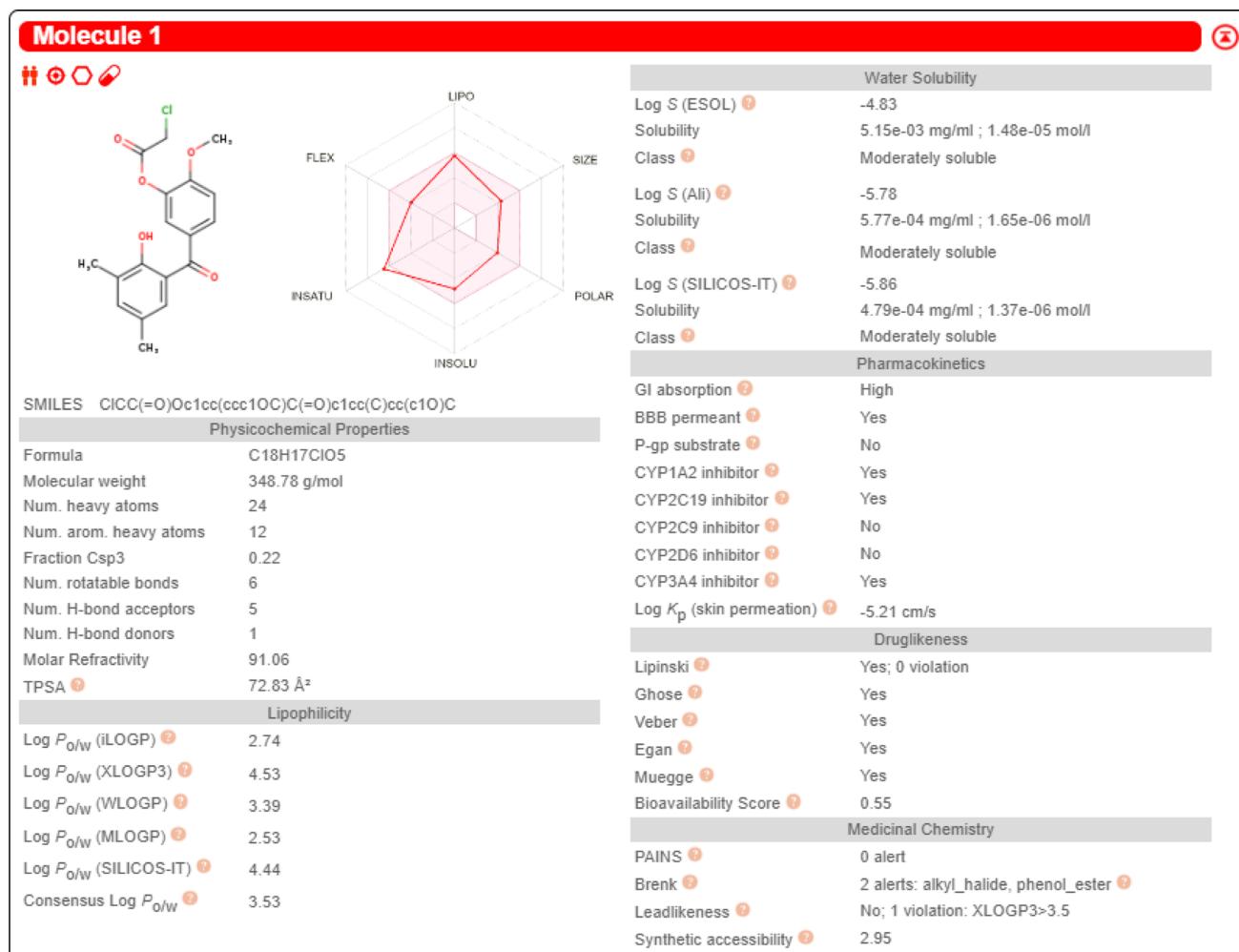


Fig. 11 The prediction data for compound 1.

of this study and ref. 7, the structure–activity relationships (SAR) of benzophenone and its derivatives are analysed in Fig. 10.

2.2.2 Network pharmacology

2.2.2.1 Selection of compounds for network pharmacology. Of these compounds, compound 1 exhibited the best antitumor activity, with IC_{50} values of less than 1.0 μ M for four tumor cell lines, so compound 1 was selected for network pharmacology study.

2.2.2.2 SwissADME prediction of compound 1. The properties of compound 1, including lipophilicity, water solubility, pharmacokinetics, and druglikeness, were predicted by the SwissADME database. The consensus $\log P_{o/w}$ value for compound 1 was 3.53. The solubility class of compound 1 was moderate. The gastrointestinal absorption of compound 1 was “High”. For the BBB permeant, compound 1 was shown as “Yes”. So compound 1 has the potential to treat brain diseases. For druglikeness, compound 1 showed five “Yes”. This indicates that compound 1 has good pharmacokinetic properties and bioavailability. The predicted data for compound 1 are shown in Fig. 11.

2.2.2.3 Target prediction of compound 1. Using the SwissTargetPrediction, PharmMapper, and TargetNet databases to

predict target genes for compound 1, 366 target genes were identified after removing duplicates.

2.2.2.4 Tumor-related genes. The words “carcinoma,” “cancer,” and “tumor” were searched in the GeneCards database respectively, and 39 549 oncogenes were obtained after removing duplicates.

2.2.2.5 Oncogenes predicted by compound 1. The genes predicted by compound 1 and tumor-related genes were intersected on the Venny 2.1 website, and 351 oncogenes of compound 1 were obtained (Fig. 12).

2.2.2.6 Construction of the protein–protein interaction network. A total of 351 target genes were input into the String

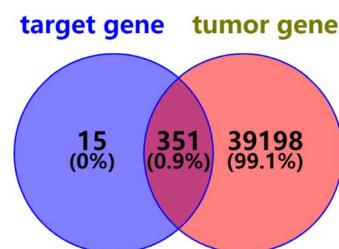


Fig. 12 Intersection diagram of target genes and oncogenes.

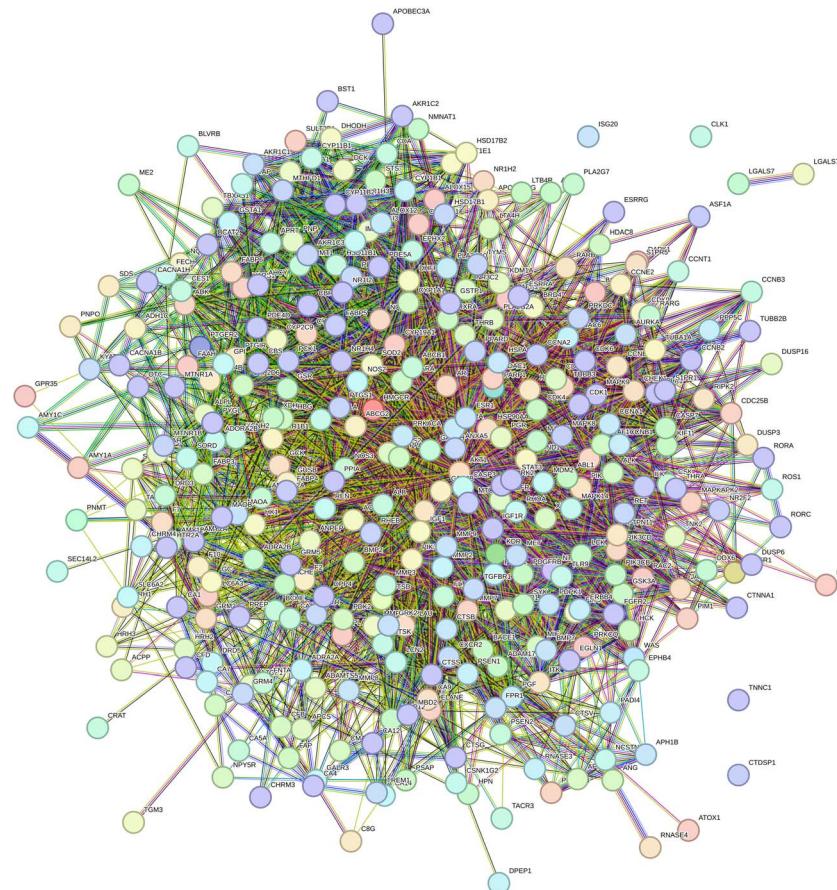


Fig. 13 PPI network of potential targets of compound 1.

website, and protein–protein interaction (PPI) network of 349 nodes and 4531 sides were obtained after analysis (Fig. 13).

2.2.2.7 Calculation of the topological parameters of proteins. The table in tsv format obtained from the String analysis was imported into Cytoscape 3.7.2 software. A table containing different parameters such as degree, betweenness centrality, closeness centrality, and clustering coefficient was built. Target genes whose degree value was ≥ 5 were selected to obtain 315 target genes.

2.2.2.8 The target major genes was obtained by molecular docking simulations. Using AutoDock Vina final v 1.0 (Beikwx, China), 315 target proteins and compound 1 were docked, respectively. A protein with a docking score ≤ -5 was considered the target major protein, and its corresponding gene was considered the target major gene. Thus, 294 genes were considered the target major genes.

2.2.2.9 Identification of important target genes. The above table (in tsv format), obtained from the String analysis tool, was imported into the Cytoscape 3.7.2 software; then, 21 target genes with a docking score greater than -5 were removed. The remaining 294 genes were analysed using the CytoHubba plugin, employing three algorithms (maximal clique centrality (MCC), maximum neighbourhood component (MNC), and degree) to identify the top 10 hub genes based on their scores. Then, the results of the three algorithms were intersected to

obtain seven hub genes, namely, AKT1, ALB, CASP3, ESR1, GAPDH, HSP90AA1, and STAT3 (Fig. 14), which were considered as potential target hub genes for compound 1.

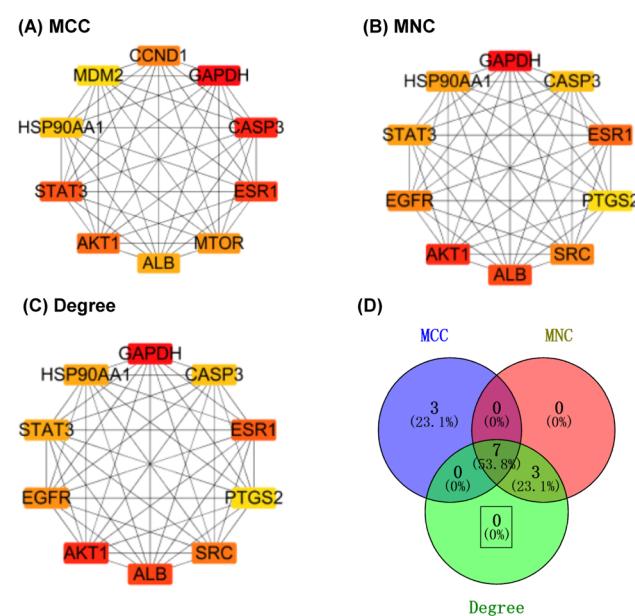


Fig. 14 The network of the first 10 key genes of compound 1.

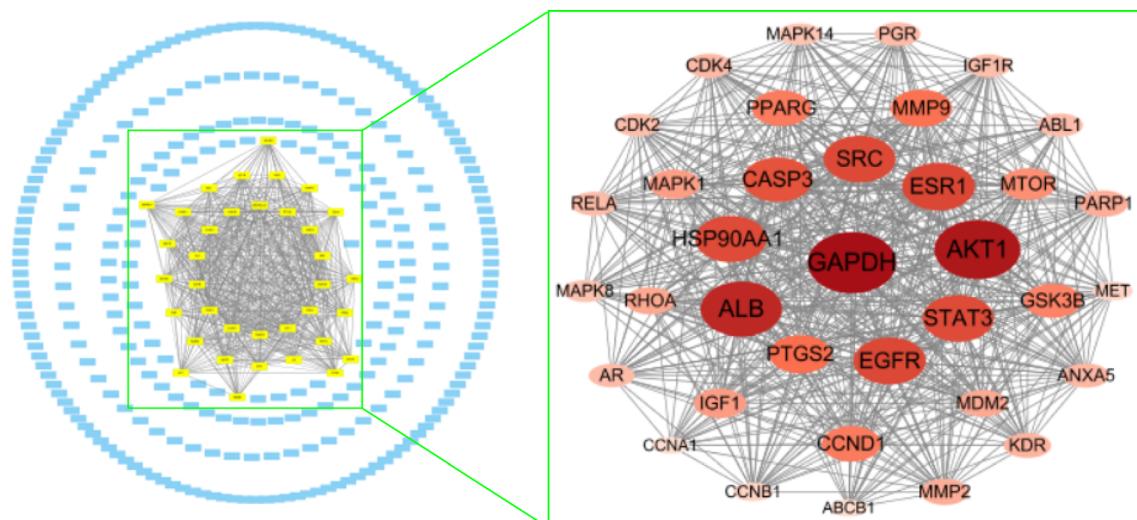


Fig. 15 A total of 294 target major genes and 36 important target genes.

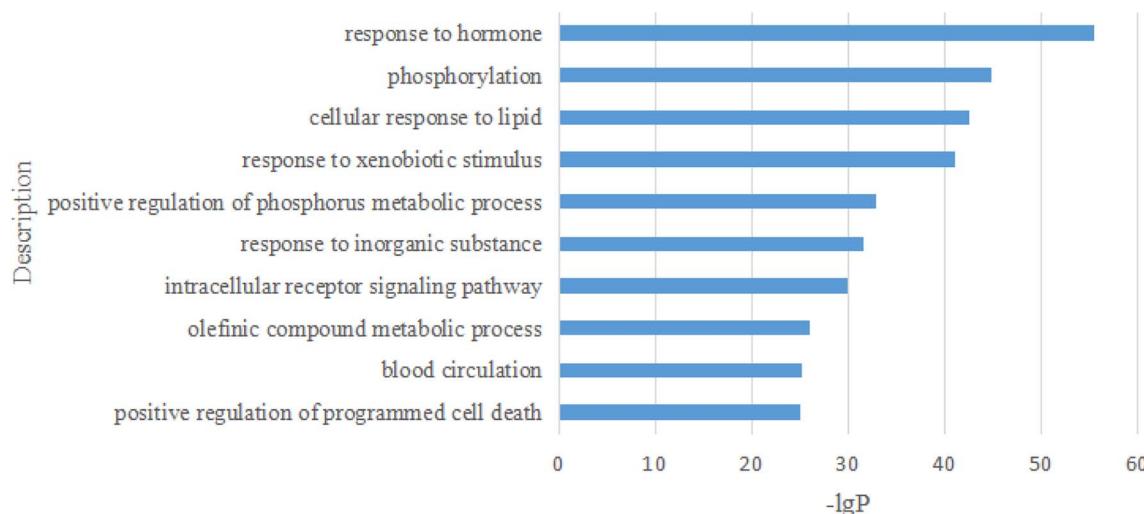


Fig. 16 The analysis of GO BP.

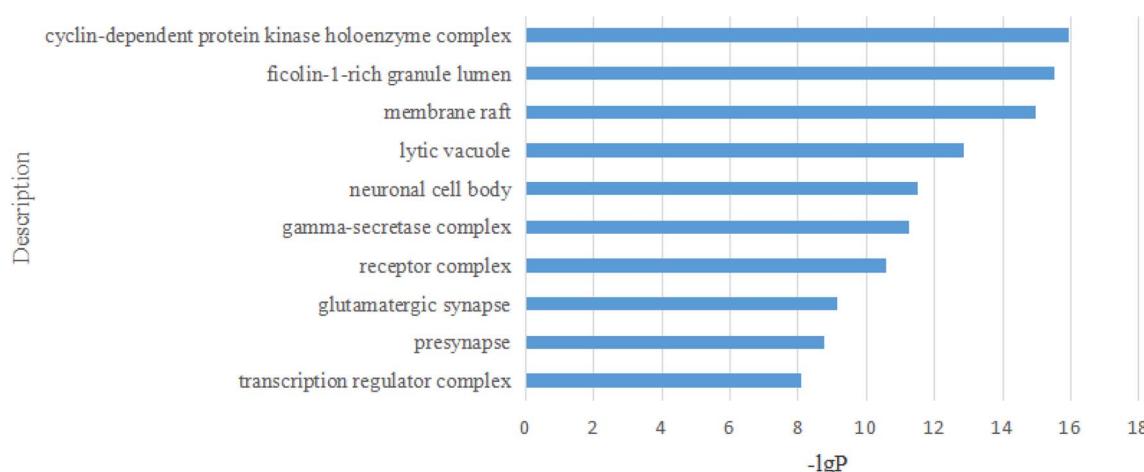


Fig. 17 The analysis of GO CC.



Moreover, the remaining 294 genes were analysed using the Molecular Complex Detection (MCODE) plug-in, enabling the selection of the module with the highest score, comprising 36 nodes (Fig. 15). This module had a score of 32.1, while the average of the degree values was 26.6; it was the only module with a score greater than the average of the degree values. This

suggests that these 36 nodes are important target genes, containing the seven potential target hub genes mentioned above.

2.2.2.10 Enrichment analysis. These 294 main genes were imported into the Metascape website (<https://metascape.org/gp/index.html#/main/step1>), and then their Gene Ontology (GO) in terms of biological processes (BP), cellular components (CC), molecular functions (MF), and Kyoto Encyclopedia of Genes

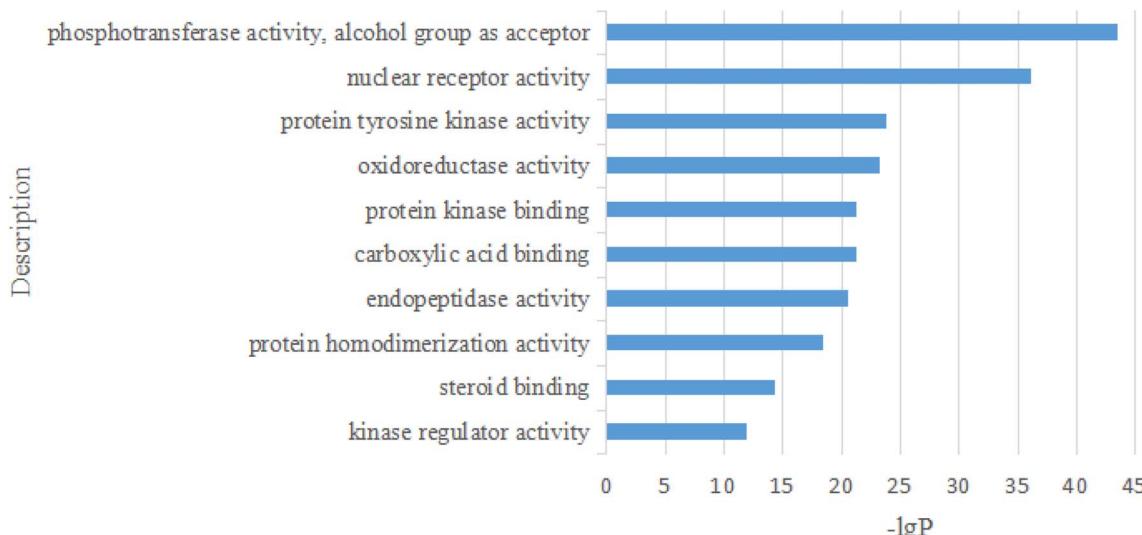


Fig. 18 The analysis of GO MF.

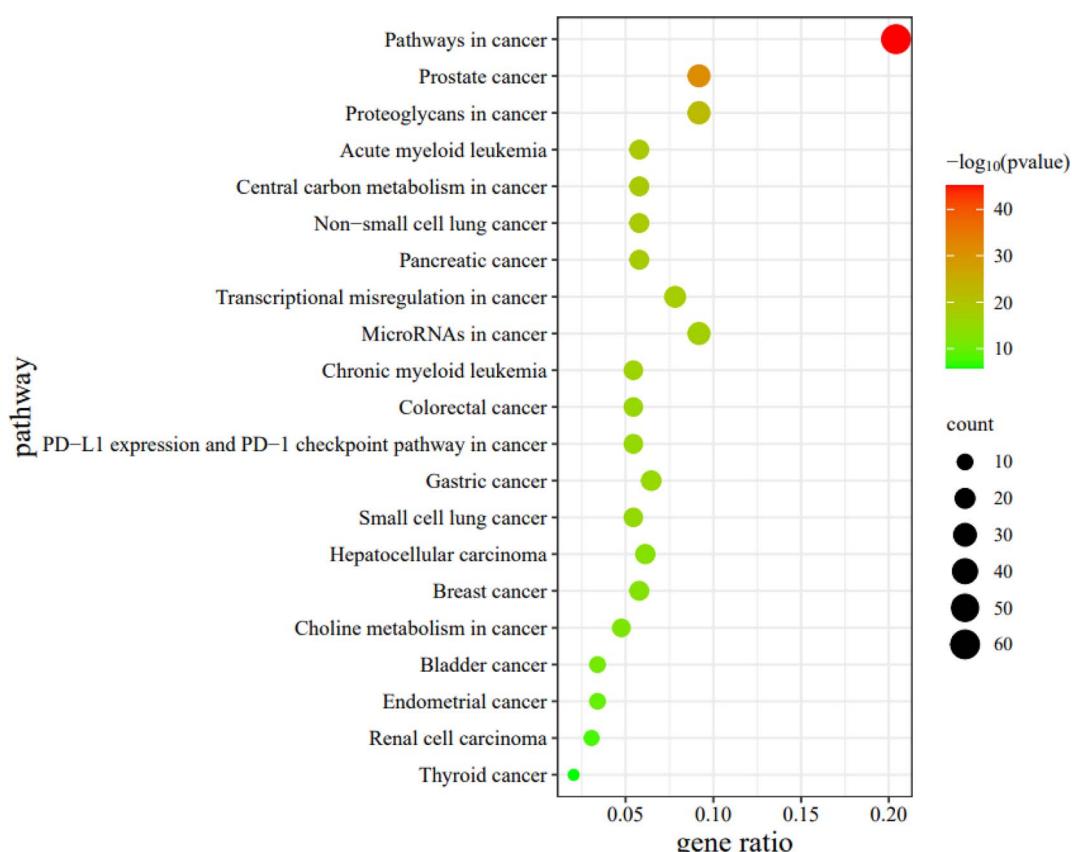


Fig. 19 The analysis of KEGG pathway.

and Genomes (KEGG) pathway enrichment were analysed (Fig. 16–19).

On the bar graph, the longer the bar, the smaller the *P*-value, indicating that the difference between the experimental and control groups was more statistically significant. For the GO BP analysis, the top 10 representative processes are illustrated in Fig. 16.

For the GO CC analysis, the top 10 representative components are illustrated in Fig. 17.

For the GO MF analysis, the top 10 representative functions are illustrated in Fig. 18.

For the bubble map, the redder the color of the bubble, the smaller the *P*-value, indicating that the difference between the experimental and control pathways was more statistically

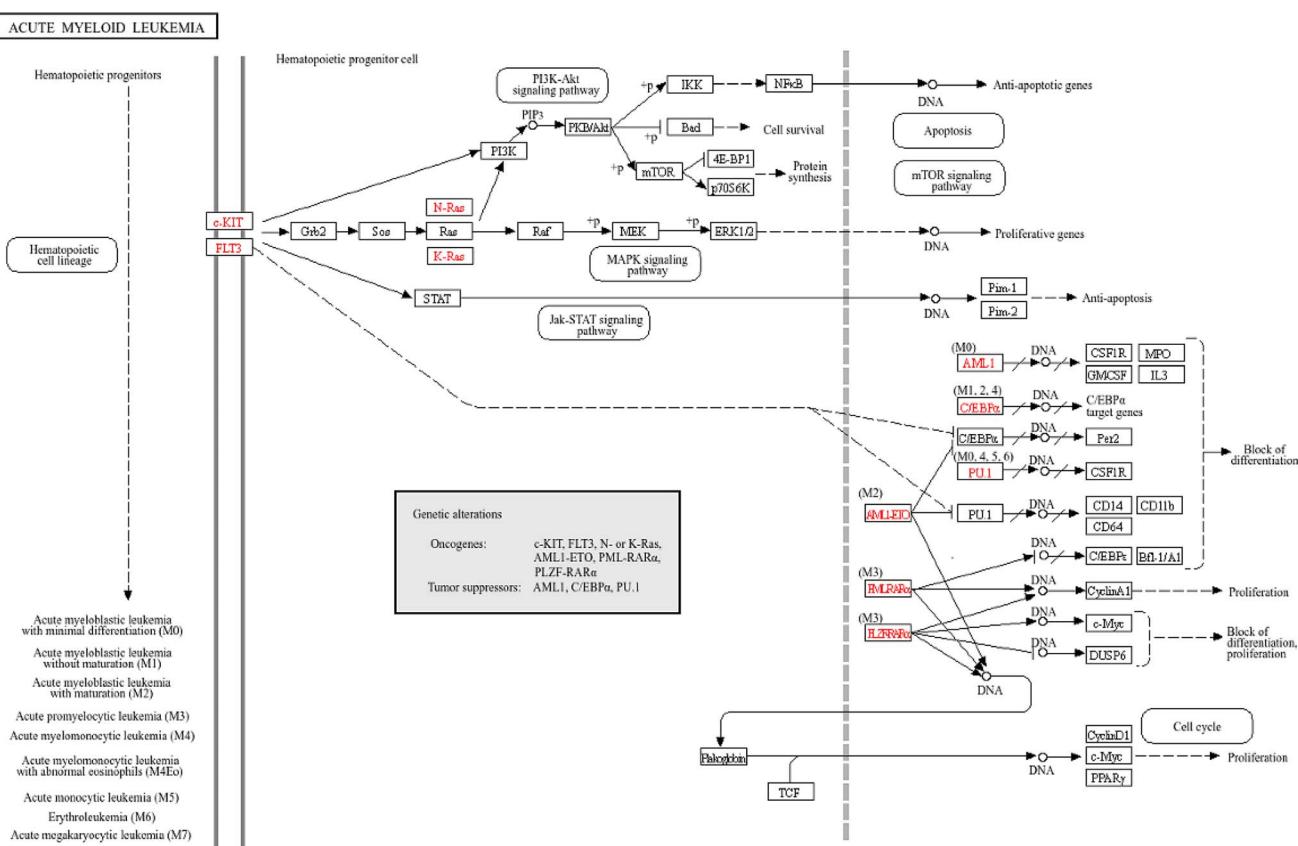


Fig. 20 Potential targets and mechanism of compound 1 against AML. The red codes represent targeted genes involved in the AML pathway.

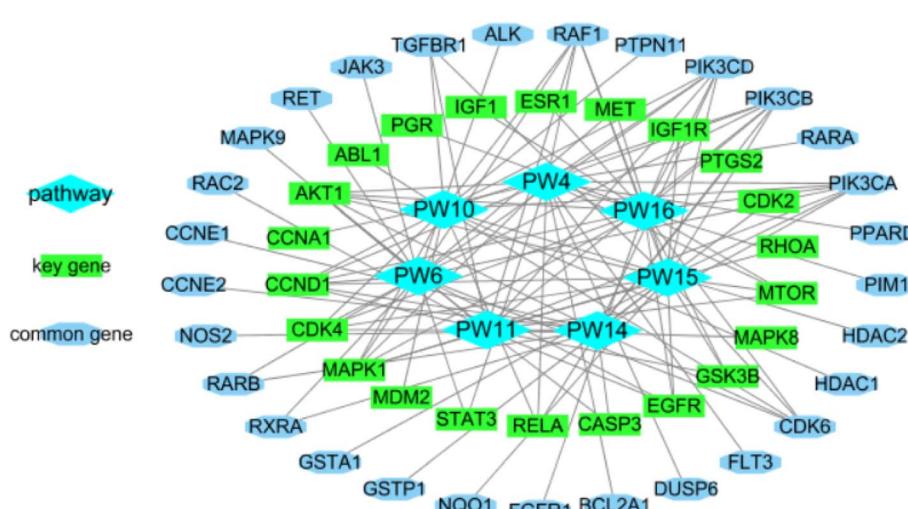


Fig. 21 The seven cancer pathways and their 51 genes.



significant. Furthermore, the larger the bubble coverage area, the more genes in the experimental pathway. In this study, the top 21 cancer pathways were selected in order from the smallest to the largest P -value. For the analysis of the KEGG pathway, the top 21 cancer pathways are illustrated in Fig. 19. For simplicity, these 21 pathways were abbreviated PW1-21 in turn.

Compound 1 exhibited strong inhibitory activity against HL-60 cells. Therefore, the potential targets and mechanism of

compound **1** against acute myeloid leukemia (AML) are shown in Fig. 20.

2.2.2.11 Identification of the potential mechanism of action.

The analysis of the KEGG pathway included tumor pathways such as AML, non-small cell lung cancer, chronic myeloid leukemia, colorectal cancer, small cell lung cancer, hepatocellular carcinoma, and breast cancer, which matched the bioactivity test. When the related genes from the PW4, 6, 10, 11, 14,

Table 3 Result of the molecular docking simulation

Protein	Binding affinity (kcal mol ⁻¹)	Ligand interaction	Interacting amino acids			
			Hydrophobic interaction	Hydrogen bond	π-Stacking (perpendicular)	Salt bridge
AKT1 (PDB ID: 2JDO)	-7.80		LEU-158, VAL-166, LYS-181, THR-213, MET-229, PHE-439	LYS-160, THR-292	—	ARG-6
CASP3 (PDB ID: 3GJR)	-7.1		HIS-121, GLU-123, PHE-128, TYR-204, TRP-206, PHE-256	GLY-122, ARG-207	HIS-121	HIS-121
CDK2 (PDB ID: 3PXY)	-9.6		ILE-10, VAL-18, ALA-31, PHE-80, LEU-134, ALA-144	ASP-86, ASP-145	—	LYS-33
ESR1 (PDB ID: 2OCF)	-8.1		LEU-346, LEU-349, LEU-384, LEU-387, PHE-404, LEU-525	HIS-524, LEU-525	PHE-404	—
MAPK1 (PDB ID: 3W55)	-7.8		ALA-52, TYR-113, LYS-114, LEU-156	ILE-31, LYS-114, ASN-154	—	—
STAT3 (PDB ID: 6NJS)	-5.7		ASP-661, ALA-662, VAL-667	LYS-626	—	—

15, and 16 were aggregated, 51 genes were obtained. They intersected with 36 important genes, resulting in 22 key genes. The 22 key genes were ABL1, AKT1, CASP3, CCNA1, CCND1, CDK2, CDK4, EGFR, ESR1, GSK3B, IGF1, IGF1R, MAPK1, MAPK8, MDM2, MET, MTOR, PGR, PTGS2, RELA, RHOA, and STAT3, respectively. Among them, AKT1, CASP3, and ESR1 are potential target hub genes. The identified pathways and their related genes are shown in Fig. 21.

2.2.2.12 Molecular docking simulation of compound 1 with some representative target proteins. Molecular docking simulations were carried out with six proteins and the docking results of binding affinities for compound 1 were computed in kcal mol⁻¹ as -7.9, -7.1, -9.6, -8.1, -7.8, and -5.7 with target proteins AKT1, CASP3, CDK2, ESR1, MAPK1, and STAT3, respectively. Compound 1 showed significant binding affinities with the six proteins. Table 3 described the significant binding characteristics of compound 1 with the six proteins in detail.

3 Conclusion

Four benzophenones, three dihydrocoumarins, and two coumarins were synthesised. They are analogues or derivatives of the lead compound s3. Compounds 1, 8, and 9 exhibited excellent antitumor activity through evaluation of antitumor activity *in vitro*, especially compound 1. Therefore, the anti-tumor mechanism of compound 1 was investigated through network pharmacology and molecular docking analyses. These results provide an useful reference for the development of benzophenone and its derivatives as potential anticancer agents.

4 Experimental

4.1 Chemistry

The chemical reagents purchased (Shanghai Aladdin Biochemical Technology Co., Ltd, Shanghai, China) were of the highest commercially available purity and were used as received. Melting points were determined on a micro melting point apparatus and the data were uncorrected (Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China). ¹H and ¹³C NMR spectra were determined in acetone-*d*₆ or pyridine-*d*₅ at 500 MHz for ¹H and 125 MHz for ¹³C (Bruker, Fällanden, Switzerland). IR spectra were recorded with KBr pellets (Bruker, Bremen, Germany). High-resolution mass (HRMS) data were recorded *via* a negative ion electron impact mass spectrometry using a time-of-flight analyzer (Bruker Daltonics Inc., Boston, USA).

4.2 Synthesis

Due space constraints of the manuscript, general procedures and spectral data of compounds 1–9 have been placed in the ESI.†

4.3 Cytotoxicity assay

Experimental methods were in accordance with ref. 11. The human cell lines A-549, HL-60, SMMC-7721, MCF-7, MDA-MB-

231 and SW480 were used in the cytotoxic assay. These cell lines were obtained from ATCC (Manassas, VA, USA). Cells were cultured in RMPI-1640 or DMEM medium (Biological industries, Kibbutz Beit-Haemek, Israel), supplemented with 10% fetal bovine serum (Biological Industries) at 37 °C in a humidified atmosphere with 5% CO₂. The cytotoxicity assay was evaluated by MTS (Promega, Madison, WI, USA) assay. Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 µM) was added. After incubation for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8, and 40 µM in triplicate. Cisplatin and Taxol (Sigma, St. Louis, MO, USA) served as positive controls. The IC₅₀ value of each compound was calculated using the method of Reed and Muench. The results were expressed as average ± standard deviation.

4.4 Network pharmacology

4.4.1 SwissADME prediction of compounds. The compound in sdf format was imported into the SwissADME data database (<http://www.swissadme.ch/>), then the SMILES number of the compound was obtained, and finally a “run” was pressed to obtain the data.^{12–15}

4.4.2 Target prediction of compounds. Compounds in sdf format were imported into the SwissTargetPrediction (<http://swisstargetprediction.ch/>),^{16,17} PharmMapper (<http://www.lilab-ecust.cn/pharmmapper/>),^{18–20} and TargetNet (<http://targetnet.scbdd.com/>) databases,²¹ respectively, then “*Homo sapiens*” was select as the target species. Following the instructions, the targets of the compounds were obtained.

4.4.3 Tumor-related genes. The GeneCards database was searched using tumor-related terms to obtain tumor-related genes (<https://www.genecards.org/>).

4.4.4 Oncogenes predicted by compounds. The genes predicted by the compounds and cancer-related genes were intersected on the Venny 2.1 website to obtain the cancer target genes of the compounds (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>).

4.4.5 Construction of the PPI network. The target genes were input into the String website multiple proteins module (<https://string-db.org/>), “*Homo sapiens*” was selected as the target organisms, and “evidence” of the meaning of the network edges was selected. Parameters such as experiments and co-expression in the active interaction sources were selected. The medium confidence was set at 0.400. The PPI network was obtained after analysis.

4.4.6 Calculation of the topological parameters of proteins. The table in tsv format obtained by String analysis was imported into Cytoscape 3.7.2 software. Then, the respective tools, network analyzer, network analysis, and analysis of network settings were selected, respectively. A table in csv format with parameters such as betweenness centrality, closeness centrality, clustering coefficient, and degree was constructed. Degree values ≥ 5 are considered highly connected proteins, which are more important for maintaining the connectivity of complex biological networks.²² Thus, target genes with degree values ≥ 5 were selected.

4.4.7 The main target was obtained by molecular docking. The protein names of potential target genes were obtained from the UniProt website (<https://www.uniprot.org/>), and verified human proteins were obtained from the PDB website (<https://www.rcsb.org/>). Most of these proteins contained ligands with aromatic structures and resolutions in the range of 1–4 Å. These proteins were modified using PyMOL software, hydrogenated,²³ and then separately docked with promising compounds using AutoDock Vina final v 1.0 (Beikwx, China).

4.4.8 Identification of important target genes. A table (in tsv format) listing target genes with good docking scores to compound **1** was imported into the Cytoscape 3.7.2 software, followed by analyses using the CytoHubba²⁴ and MCODE²⁵ plugins to obtain the important target genes.

4.4.9 Enrichment analysis. The genes were input into the Metascape website and then their GO BP, GO CC, GO MF, and KEGG pathways were analysed. The pathway map was downloaded from the KEGG Mapper – Color website (https://www.genome.jp/kegg/tool/map_pathway2.html).

4.4.10 Molecular docking simulation. The protein names of potential target genes were obtained from the UniProt website (<https://www.uniprot.org/>), and verified human proteins were obtained from the PDB website (<https://www.rcsb.org/>). Most of these proteins contained ligands with aromatic structures and resolutions in the range of 1–4 Å. These proteins were modified using PyMOL software, hydrogenated,²³ and then separately docked with promising compounds using AutoDock Vina final v 1.0 (Beikwx, China).¹²

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors gratefully thank the Natural Science Foundation of Fujian Province (Grant No. 2022J011158), Putian City Science and Technology Plan Project (Grant No. 2021S2001-9), Project Approved by Fujian Key Laboratory of New Drug Targets Research (Grant No. FJ-YW-2021KF01) and Project Approved by Fujian Shanhe Pharmaceutical Co., Ltd (Grant No. 2022AHX211(L)) for the support of this program.

References

- 1 B. D. Zhou, C. Lei, X. M. Liao, H. Zhu, Z. P. Ruan, Y. Y. Fang, G. F. Xu and Y. L. Chen, *J. Chin. Pharm. Sci.*, 2022, **31**, 738–745.
- 2 C. Álvarez, R. Álvarez, P. Corchete, J. L. López, C. Pérez-Melero, R. Peláez and M. Medarde, *Bioorg. Med. Chem.*, 2008, **16**, 5952–5961.
- 3 S. A. Khanum, S. Shashikanth, S. Umesha and R. K. Europ, *J. Med. Chem.*, 2005, **40**, 1156–1162.
- 4 H. T. Zeng, Y. H. Yu, X. Zeng, M. M. Li, X. Li, S. S. Xu, Z. C. Tu and T. Yuan, *J. Nat. Prod.*, 2022, **85**, 162–168.
- 5 Glaxo Group Ltd, *Expert Opin. Ther. Pat.*, 2001, **11**, 1637–1640.
- 6 K. Surana, B. Chaudhary, M. Divaker and S. Sharma, *MedChemComm*, 2018, **9**, 1803–1817.
- 7 B. D. Zhou, R. R. Wei, J. L. Li, Z. H. Yu, Z. M. Weng, Z. P. Ruan, J. Lin, Y. Y. Fang, G. F. Xu and D. B. Hu, *J. Asian Nat. Prod. Res.*, 2022, **24**, 170–178.
- 8 J. H. Jeon, D. M. Yang and J. G. Jun, *Bull. Korean Chem. Soc.*, 2011, **32**, 65–70.
- 9 A. R. Jagdale and A. Sudalai, *Tetrahedron Lett.*, 2008, **49**, 3790–3793.
- 10 M. M. Garazd, Y. L. Garazd and V. P. Khilya, *Chem. Nat. Compd.*, 2005, **41**, 245–271.
- 11 B. D. Zhou, Z. M. Weng, Y. G. Tong, Z. T. Ma, R. R. Wei, J. L. Li, Z. H. Yu, G. F. Xu, Y. Y. Fang and Z. P. Ruan, *J. Asian Nat. Prod. Res.*, 2020, **23**, 271–283.
- 12 B. D. Zhou, X. M. Liao, S. Y. Liu, G. Q. Gao, Y. T. Gao, W. T. Gan, J. L. Ke, Y. X. Wu, F. F. Wang, B. C. Huang, W. J. Yang, R. P. Ye, Y. H. Liu and Y. C. Lin, *J. Mol. Struct.*, 2024, **1312**, 138467.
- 13 A. Daina, O. Michielin and V. Zoete, *Sci. Rep.*, 2017, **7**, 42717.
- 14 A. Daina, O. Michielin and V. Zoete, *J. Chem. Inf. Model.*, 2014, **54**, 3284–3301.
- 15 A. Daina and V. Zoete, *ChemMedChem*, 2016, **11**, 1117–1121.
- 16 A. Daina, O. Michielin and V. Zoete, *Nucleic Acids Res.*, 2019, **47**, W357–W364.
- 17 D. Gfeller, O. Michielin and V. Zoete, *Bioinformatics*, 2013, **29**, 3073–3079.
- 18 X. F. Liu, S. S. Ouyang, B. A. Yu, Y. B. Liu, K. Huang, J. Y. Gong, S. Y. Zheng, Z. H. Li, H. L. Li and H. L. Jiang, *Nucleic Acids Res.*, 2010, **38**, W609–W614.
- 19 X. Wang, C. X. Pan, J. Y. Gong, X. F. Liu and H. L. Li, *J. Chem. Inf. Model.*, 2016, **56**, 1175–1183.
- 20 X. Wang, Y. H. Shen, S. W. Wang, S. L. Li, W. L. Zhang, X. F. Liu, L. H. Lai, J. F. Pei and H. L. Li, *Nucleic Acids Res.*, 2017, **45**, W356–W360.
- 21 Z. J. Yao, J. Dong, Y. J. Che, M. F. Zhu, M. Wen, N. N. Wang, S. Wang, A. P. Lu and D. S. Cao, *J. Comput.-Aided Mol. Des.*, 2016, **30**, 413–424.
- 22 R. Y. Lin, L. J. Yang, X. L. Yu and L. Y. Huang, *J. Fujian Med. Univ.*, 2021, **55**, 217–223.
- 23 J. M. Word, S. C. Lovell, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, 1999, **285**, 1735–1747.
- 24 A. N. S. S. Azman, J. J. Tan, M. N. H. Abdullah, H. Bahari, V. Lim and Y. K. Yong, *Foods*, 2003, **12**, 1779.
- 25 J. M. Zhuang, J. H. Mo, Z. G. Huang, Y. L. Yan, Z. Y. Wang, X. Q. Cao, C. K. Yang, B. Shen and F. Zhang, *Chin. Med.*, 2023, **18**, 104.

