Article

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Inhibitory Effects and Mechanisms of the Novel Shikonin Derivative DMAKO-20 on Melanoma Metastasis and Invasion

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Background: Cutaneous melanoma is a malignant tumor with an increasing incidence, prone to recurrence and metastasis. This study aims to explore the effects and mechanisms of the novel shikonin derivative 5,8-dimethyl alkannin oxime derivative (DMAKO-20) on the metastasis and invasion of melanoma cells.
Methods: The inhibitory effects of DMAKO-20 on the melanoma cell line A375 were investigated through Cell Counting Kit-8 (CCK-8), Transwell and angiogenesis experiments. Network pharmacology and Gene Ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were employed to explore potential sites and pathways involved in this process. Additionally, quantitative polymerase chain reaction (qPCR) and Western blot experiments were conducted before and after drug treatment to verify the expression trends of related pathways and proteins.
Results: DMAKO-20 demonstrated selective inhibition of proliferation, invasion and migration of melanoma cells at low concentrations. The WNT pathway appears to be implicated in this process, as DMAKO-20 effectively attenuates its activation, consequently reducing matrix metalloproteinase 9 (MMP9) and Cellular Communication Network Factor 1 (CCN1)/cysteine-rich angiogenic inducer 61 (CYR61) levels. Such modulation inhibits melanoma dissemination and invasion into other tissues.
Conclusion: DMAKO-20 exhibits the capability to suppress metastasis and invasion of melanoma cells, suggesting its potential for clinical application as an adjuvant therapy against melanoma.

Keywords: shikonin; melanoma; WNT pathway; metastasis; CYR61

Introduction

Melanoma is a malignant skin tumor originating from the ectodermal nerve edges. Annually, approximately 99,780 individuals in the US are diagnosed with this cancer, accounting for around 0.29 percent of the population. Among them, approximately 7650 individuals will succumb to the disease, representing about 0.03% of the population, with the rate of distant metastasis reaching as high as 5% [1]. The incidence rate in Europe and Australia ranges from 10 to 30 cases per 100,000 people [2]. Furthermore, the recurrence rate of melanoma is notably high, with approximately 20–30% of patients in the early stage experiencing recurrence within five years and a staggering 95% of patients with stage III and IV melanoma experiencing recurrence within three years [3]. Therefore, melanoma constitutes a significant disease that profoundly affects the quality of human life.

Melanoma can manifest in various tissues and organs, such as the skin, eye choroid, genital, oral, and nasal sinus mucosa. Additionally, some melanomas may originate from internal organs. Among these, skin melanoma is the most prevalent type, distinguished by characteristics like asymmetry, irregular margins, changes in color and an increase in diameter [4]. Owing to its typical early manifestations, melanoma is often overlooked and misdiagnosed. A significant challenge in treating melanoma lies in its propensity for early-stage metastasis; once metastasized, the prognosis is typically bleak [5].

Melanoma can be effectively treated through various approaches. For instance, comprehensive surgical excision can effectively manage the recurrence rate of early (*in situ*) melanoma to 5.7% [6]. Additionally, for cases where surgical intervention is not feasible, treatment options such as radiotherapy, chemotherapy, molecular targeted therapy, and immune checkpoint inhibitors are available. Notably, biological agents like the molecularly targeted drugs Dabrafenib and Trametinib as well as the anti-programmed death-1 (PD1) drug Nivolumab have significantly enhanced

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therapeutic outcomes. These advancements have led to an increased three-year relapse-free survival (RFS) rate of approximately 60% for patients with stage III melanoma [7–9]. Nevertheless, a considerable subset of patients still exhibit resistance to treatment and are susceptible to developing drug resistance post-treatment. Hence, there is a crucial need to delve into the pathogenesis and metastatic mechanisms of melanoma to devise tailored treatment strategies.

In recent years, our team has conducted research on the biological effects of shikonin and its derivatives on melanoma. Our findings indicate that shikonin exhibits promising efficacy and high selectivity in *in vitro* experiments [10,11]. Shikonin, an active compound extracted from the roots of *Lithospermum erythrorhizon* possesses anti-inflammatory properties targeting multiple pathways. Its antitumor mechanisms are multifaceted, involving diverse targets. Various strategies have been proposed to inhibit tumor growth and metastasis. Which include topoisomerase inhibition [12], induction of apoptosis [13,14], programmed cell necrosis [15], autophagy in tumor cells [16], enhancement of radiation and chemotherapy sensitivity in tumor cells [17], anti-angiogenic effects and more [11]. Consequently, these mechanisms influence the metabolism, proliferation, differentiation, signaling pathways, gene expression and other processes of tumor cells, ultimately impeding their growth.

Due to the potent cytotoxicity of shikonin, there are currently no medications available for direct clinical use. Hence, the authors of the present study undertook efforts to enhance the structure of shikonin, resulting in the synthesis of 5,8-dimethyl alkannin oxime derivative (DMAKO-20). This novel compound is a 5,8-dimethyl shikonin oxime derivative characterized by high efficacy and low toxicity. Furthermore, earlier work by the authors has confirmed that it can directly stop cancer cells from growing through the cytochrome P450 family 1 subfamily B member 1 (CYP1B1) enzyme [11].

Through molecular biology experiments, the current investigation delves into the mechanism and effects of DMAKO on melanoma metastasis, clarifying its therapeutic potential and the involvement of other pertinent proteins in this process. Bioinformatics technology was employed to analyze potential pathways and mechanisms, laying the foundation for the preclinical application of this drug.

Materials and Methods

Cell Culture and Drugs

Malignant melanoma cells A375 (SCSP-533), A431 (TCHu188) and YUMM (CRL-3367) and the human keratinocyte cell line HaCaT (SCSP-5091) were acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). We conducted mycoplasma contamination testing to confirm the absence of mycoplasma contamination. The identities of the A375, A431 and the HaCaT cell lines were verified through short tandem repeat (STR) validation analysis. The YUMM cell line was derived from mice and identified through morphological observation by using an optical microscope. Almost all the cells were adherent growth and had strong adherent ability. Most of them were spindle-shaped or irregular triangular. We cultured melanoma cells and HaCaT in Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA, USA, 12800017), supplemented with 10% fetal bovine serum (Lonsera, Shanghai, China, S711-001) and 1:100 streptomycin and penicillin antibiotics (Biosharp, Hefei, China, BL505A). The synthesis of DMAKO-20 was carried out following the reported procedures [18].

Cell Proliferation

Cell viability was assessed using a Cell Counting Kit-8 (CCK-8) kit following the manufacturer's instructions (CCK-8, CK04-1000t, Dojindo, Kumamoto, Japan). Melanoma cell lines A375, A431, YUMM and the control group HaCaT cell suspension were incubated in 96-well plates and cultured in an incubator (37 °C, 5% CO2). Various concentrations of DMAKO-20 were applied to treat the cells with each concentration repeated three times. After 24 hours, 10 µL of CCK-8 solution was added to each well and the 96-well plates were further incubated in the incubator for 2 hours. Absorbance at 450 nm was measured using a microplate reader (Model 680 Microplate Reader, BIO-RAD, Hercules, CA, USA) and relative viability was calculated.

Cell Invasion and Migration

Cell migration and invasion were evaluated using Transwell assays. Melanoma cells were seeded in the upper chamber of Transwell plates (Transwell 3401, Costar, Cambridge, MA, USA), while a culture medium containing 10% FBS was placed in the lower chambers. For Transwell migration assays, 5×10^4 cells were seeded in the upper chamber. For invasion assays, Transwell inserts were precoated with Matrigel solution and polymerized in the upper chamber. After 12 hours of incubation at 37 °C, cells that had migrated or invaded the lower surface of the membrane were fixed for 20 minutes with 4% (v/v) paraformaldehyde, stained with Giemsa solution and counted in five randomly selected microscopic fields at ×200 magnification to determine migration or invasion. Apart from the membrane filters being pre-coated with Matrigel, the cell invasion assays were virtually identical to the cell migration assays.

Tube Formation Assay

The supernatant was collected subsequent to culturing A375 cells in a serum-free medium with or without DMAKO-20. BD Matrigel (356234, BD Biosciences, San Jose, CA, USA) matrix was then plated in 96-well plates. Following a 30-minute incubation period with or without DMAKO treatment, ten thousand HUVEC cells per well

and 50 µL of A375 supernatant were seeded onto the Matrigel. The plate was subsequently incubated at 37 °C for 20 hours. Following the 20-hour incubation period at 37 °C, the wells were photographed and analyzed. The authors chose 1µmol as the concentration of DMAKO-20 for the tube-forming experiment.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Assay

Total RNA was extracted from A375 cells using TRIzol Reagent (10296010, Invitrogen, Carlsbad, CA, USA) and subsequently reverse-transcribed into first-strand cDNA. qRT-PCR was performed using the SYBR® Premix Ex TaqTM II kit from Takara (RR820B, Tokyo, Japan) on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Human β-actin (Forward Primer: CATGTACGTTGCTATCCAGGC, Reverse Primer: CTCCTTAATGTCACGCACGAT) was employed as the internal reference primer. Normalization to β-actin was conducted using the $2^{-\Delta\Delta CT}$ method for result calculation. The sequences of the qRT-PCR primers are as follows: cysteine-rich angiogenic inducer 61 (*CYR61*, 5′-CTCGCCTTAGTCGTCACCC-3′ (forward) and 5′-CGCCGAAGTTGCATTCCAG-3′ (reverse); matrix metalloproteinase 9 (*MMP9*), 5′-GCTGGGCTTAGATCATTCCTC-3′ (forward) and 5′-GCCATTCACGTCGTCCTTAT-3′ (reverse).

Transfection

Short hairpin RNA (shRNA) sequences were utilized in the construction of sh-*CYR61* transfection plasmids. The sequence of sh-*CYR61* is CCTGTGAATATAACTCCAGAA. Sh-*CYR61* transfection plasmids were individually constructed using their specific shRNAs (Gene Pharma, Shanghai, China). Non-specific shRNAs were employed as controls (shCtrl). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was used for the transfection of A375 cells. Following a 48-hour incubation period, qRT-PCR analysis was conducted on transfected cells. All samples were assayed in triplicate.

Western Blotting

Western blotting was conducted using standard procedures. Following treatment with 1 µM DMAKO or DMSO for 60 minutes, whole-cell protein extracts were prepared using lysis buffer (RIPA buffer, 9806S, Cell Signaling Technology, Danvers, MA, USA) containing a protease and phosphatase inhibitor cocktail (P8340, Sigma Aldrich, St. Louis, MO, USA). Total proteins were extracted in this experiment and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by protein transfer to Polyvinylidene fluoride (PVDF) membranes (ZY101123, ZEYE, Shanghai, China). After blocking, primary antibodies were applied overnight, followed by secondary antibodies (1:5000, ab205718) for 2 hours in the dark. Primary antibodies against β-catenin (ab224803), MMP9 (1:500, ab76003), GSK3 (1:500, ab185141), CYR61 (1:500, ab228592) and β-actin (1:5000, ab8226) were all obtained from Abcam (Cambridge, MA, USA). Antibody dilutions and product numbers are indicated in parentheses.

Discovery of Drug and Disease Targets

The targets of DMAKO-20 were identified from the Pharm Mapper database, Gene Cards (https://www.genecards.org/), and the comparative toxic genomics database (CTD, http://ctdbase.org/). Utilizing the available 3D structures of DMAKO-20, the authors employed the web server Pharm Mapper to predict DMAKO-20 target genes. Additionally, by inputting DMAKO-20 into the CTD, the target genes were obtained. Simultaneously, the desired targets of DMAKO-20 and melanoma were acquired using the Gene Cards database.

Melanoma and DMAKO-20 Target Intersection

Using the R programming language, a Venn diagram was generated by intersecting the disease target with the drug target. These overlapping targets are regarded as potential targets of DMAKO-20 action on melanoma.

Protein-Protein Interaction Enrichment Analysis

The protein-protein interactions (PPI) network of co-targets associated with melanoma and DMAKO-20 was established using String (http://string-db.org/) based on the obtained PPI relationships. PPI pairs with an interaction score exceeding 0.4 were utilized, as per standard practice [19]. The targets were ranked by degree and the top 50 were highlighted. Furthermore, the subnet was analyzed using the Molecular Complex Detection (MCODE) toolkit, accessible through the METASCAPE website (http://metascape.org/gp/index.html). For each MCODE component, a separate pathway and process enrichment analysis were conducted. We selected terms with the highest p-value to describe the functionality of each component.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

After identifying the intersection between the PPI network nodes and the core targets of DMAKO-20 and melanoma, the key targets were subjected to functional enrichment analysis, including GO and KEGG. The GO enrichment analysis encompassed three categories: cellular components (CC), molecular functions (MF) and biological processes (BP). This analysis was conducted using the R package cluster profile. Additionally, KEGG pathway enrichment analysis was carried out using the R packages limma, cluster profile and path view to visualize the targeted pathways of core genes within the KEGG pathways.

The analyses were conducted with a significance threshold of p-value < 0.05 and an adjusted p-value < 0.05.

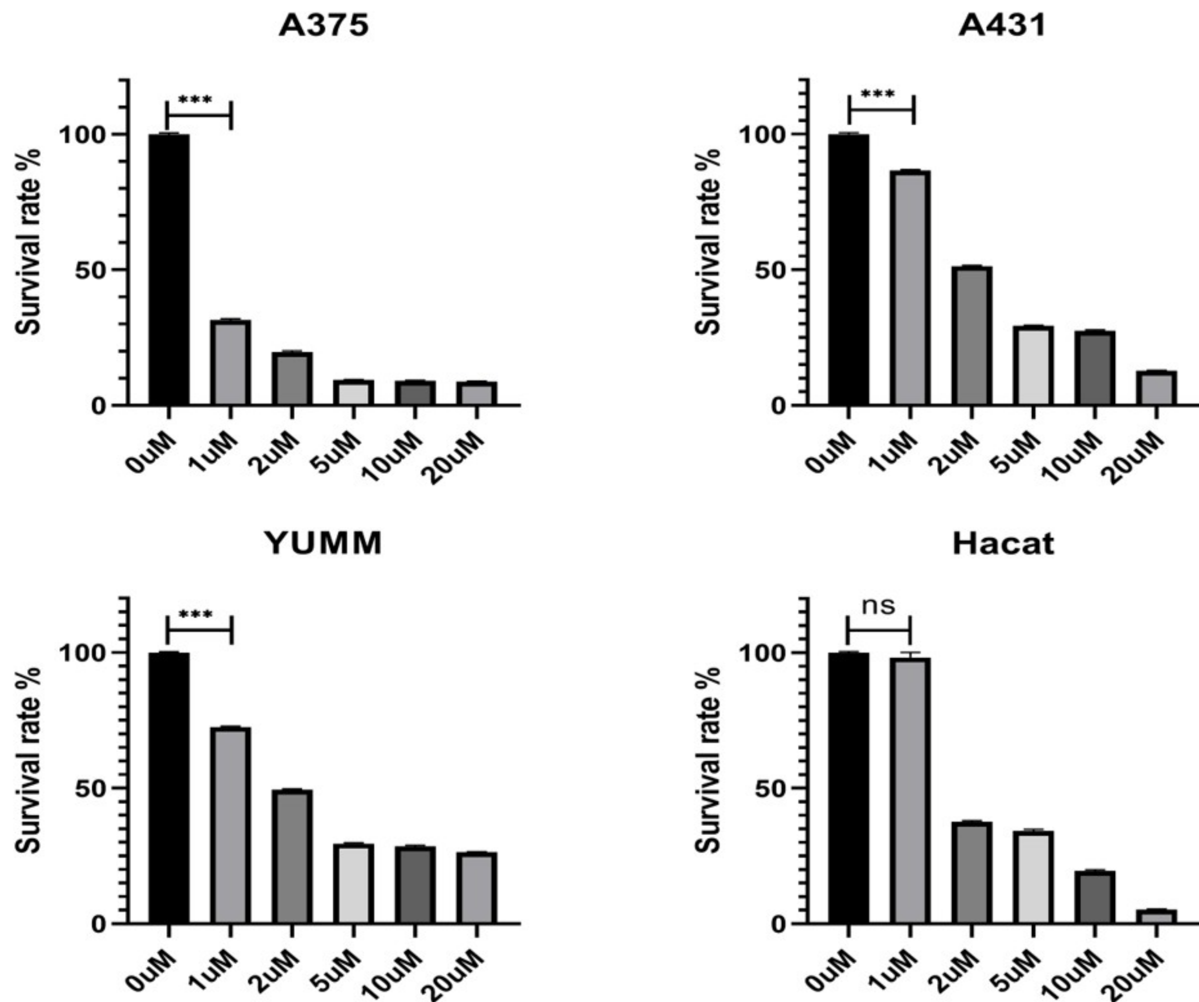

Fig. 1. Selective killing effect of 5,8-dimethyl alkannin oxime derivative (DMAKO-20) on different melanoma cell lines. Through the Cell Counting Kit-8 (CCK-8) assay, it was found that DMAKO-20 could effectively inhibit the proliferation of melanoma cells at low concentrations. While at the same dosage, it did not appear to exert any cytotoxic effects on keratinocytes Quantification data are shown as averages of three biological replicates. ***$p < 0.001$, ns, no significance.

The top 15 terms for each GO category and the top 30 enriched KEGG pathways are depicted in the bubble diagram.

Results

DMAKO-20 Specifically Inhibits Melanoma Cell Proliferation

Fig. 1 illustrates that following a 24-hour treatment with 1 µM DMAKO-20. The survival rate of melanoma cells decreased by 70% compared to the control group ($p < 0.001$). However, at the same dosage, the medication did not appear to exert any cytotoxic effects on keratinocytes. Nevertheless, with a further increase in concentration, the drug demonstrated significant cytotoxicity in both cell types. In melanoma cells, the cytotoxic effect did not escalate beyond a 2 µM concentration. Conversely, a dose-dependent cytotoxic effect was observed in HACAT cells.

DMAKO-20 Inhibits the Formation of Vascular Endothelial Cells and Invasion or Migration of Melanoma

To investigate the impact of DMAKO on angiogenesis, the authors conducted a vascular endothelial cell tubulogenesis experiment. The results depicted in Fig. 2 revealed a significant reduction in tubulogenesis in the DMAKO administration group. The Transwell experiment also showed that after 48 hours of cell growth, the DMAKO-20 treatment group had much lower movement and invasion skills than the control group. Additionally, cell morphology appeared relatively shrunk and the cell volume was observed to be smaller as depicted in Fig. 3.

DMAKO-20 Blocks the WNT Pathway that Helps Prevent Melanoma Metastasis

The structure of DMAKO-20 is shown in Fig. 4A. Through network pharmacology analysis, a total of 79 common targets were identified between melanoma target genes

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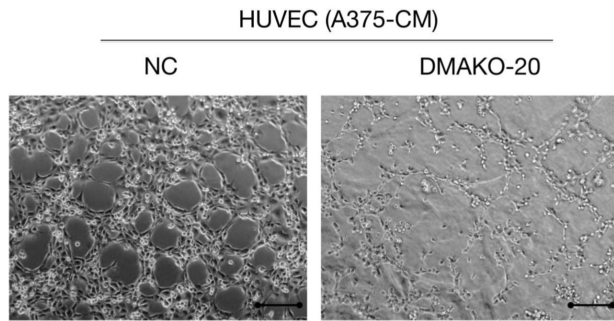


Fig. 2. DMAKO-20 inhibits angiogenesis of melanoma. Tube formation assay showed that the melanoma supernatant treated by DMAKO-20 could effectively inhibit the tabulation of human vascular endothelial cells (HUVEC) (Scale bar = 100 µm).

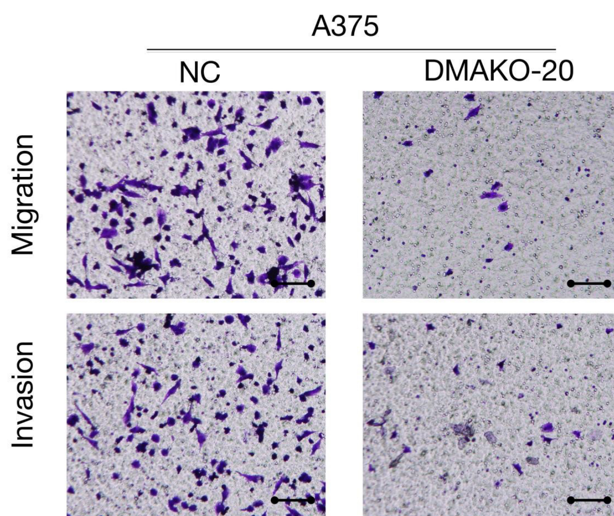


Fig. 3. DMAKO-20 inhibits the invasion and migration of melanoma. The Transwell assay showed that 1 µM DMAKO-20 could effectively inhibit the invasion and migration of melanoma cells (Scale bar = 50 µm).

and drug molecular structures. This analysis aimed to comprehend the possible mechanisms underlying the phenomena depicted in Fig. 4B,C. Subsequently, after conducting a PPI analysis of the related targets, it was observed that *AKT1, EGFR, CASP3, MMP9* and *WNT3A* were among the top-weighted proteins, as shown in Fig. 5A,B.

Further analysis involved exploring the pathways and mechanisms associated with these targets. KEGG and GO analyses were performed, revealing their involvement in cancer-related pathways, particularly highlighting the significance of the IL17 and WNT pathways, as illustrated in Fig. 6A,B.

We further elucidated the role of key genes within the WNT pathway in DMAKO's anti-melanoma invasion, building upon these findings. Additionally, Western blot experiments confirmed that DMAKO effectively inhibited the activation of the WNT pathway, as depicted in Fig. 7.

Inhibition of CYR61 Gene Expression in Melanoma can Reduce the Invasiveness of Melanoma

Quantitative polymerase chain reaction (qPCR) tests were employed to investigate whether DMAKO-20 alters the expression of genes involved in angiogenesis. Fig. 8 depicts the downregulation of both *CYR61* and *MMP9* following DMAKO-20 treatment with a decrease of over 45% and 60% respectively.

Furthermore, to validate the role of *CYR61*, a Transwell assay was conducted after inhibiting its expression using shRNA. The results showed a significant reduction in the migration and invasion of *CYR61*-inhibited cells, as shown in Fig. 9. Thus, it is possible that DMAKO further attenuated the aggressiveness of melanoma by suppressing the expression of *CYR61*.

Discussion

This study conducted cell biology experiments to validate the inhibitory effect of the shikonin derivative DMAKO-20 on the proliferation, invasion and migration of melanoma cells. Despite the efficacy demonstrated by cisplatin, carboplatin, vinblastine, vincristine and paclitaxel in systemic chemotherapy for melanoma, current clinical studies suggest that they fail to enhance the overall survival rate and may even induce additional chemotherapy-related side effects [20]. Moreover, newly developed biological or targeted agents still encounter challenges such as drug resistance and high costs [8,9].

Therefore, this study focuses on identifying plant-based medicines that demonstrate efficacy without causing harm. Herbal medicines have been extensively studied for their anti-tumor properties. For instance, ginsenoside Rg3 has been shown to induce apoptosis in melanoma cells by inhibiting nuclear factor kappa-B (NF-κB), Vascular endothelial growth factor (VEGF) and PI3K/Akt signal transduction pathways, thereby suppressing tumor cell proliferation and metastasis [21]. Researchers have found that resveratrol inhibits melanoma cell proliferation and induces apoptosis by up-regulating p53 and negatively regulating the pyruvate kinase M2 (PKM2)/Bcl-2 axis in a concentration-dependent manner [22]. Oridonin, by activating p53 and extracellular signal-regulated kinase (ERK) pathways, increases the expression ratio of Bax/Bcl-xL protein, leading to the release of cytochrome C into the cytoplasm and induction of apoptosis in A375-S2 cells [23].

However, these natural drugs do not surpass traditional chemotherapeutic drugs in terms of anti-tumor activity. This study selected shikonin as a more potent anticancer drug candidate, citing its strong inhibition of A375 cell growth at concentrations less than two micromolar in previous studies [24]. Nevertheless, naturally occurring shikonin exhibits high reactive oxygen species (ROS)-generation activity and displays broad cytotoxic effects. Therefore, in modifying the chemical structure of shikonin, the naphtho-

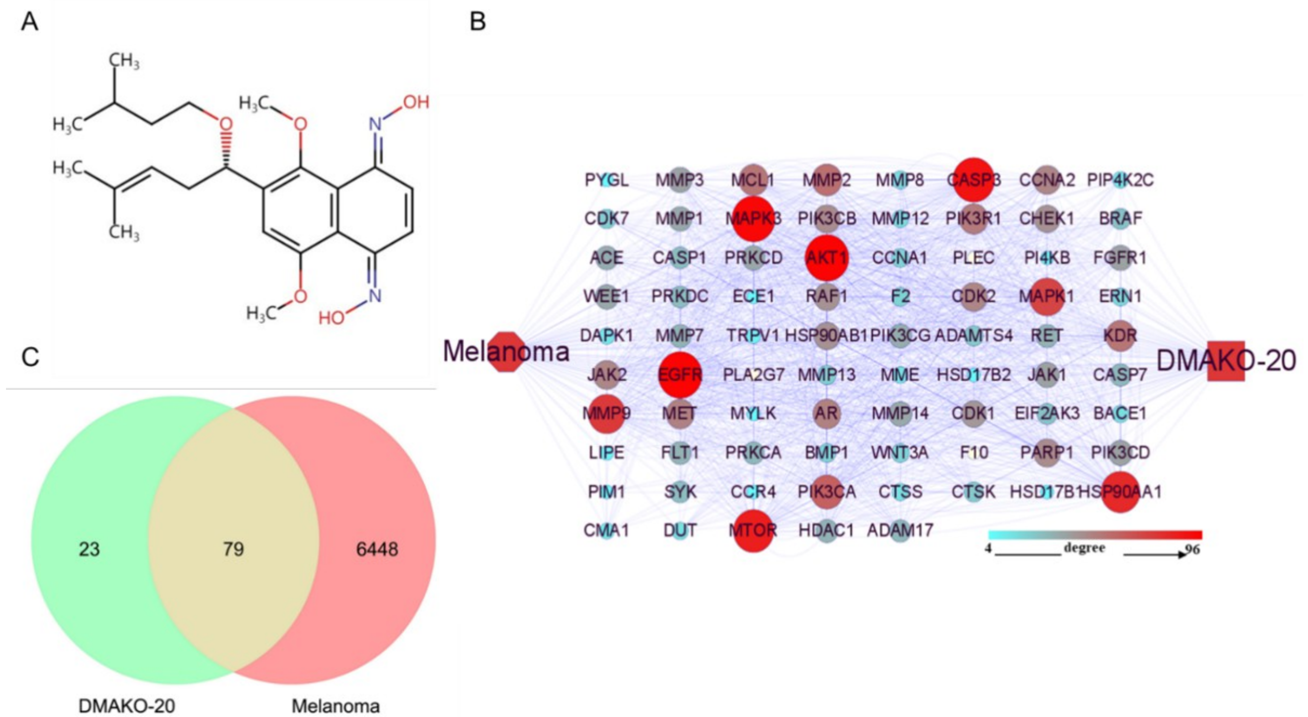


Fig. 4. Network pharmacological analysis. (A) Molecular structure of DMAKO-20. (B,C) Venn diagram and Common target of DMAKO-20 and melanoma derived from Pharm Mapper database, Gene Cards and comparative toxic genomics database (CTD).

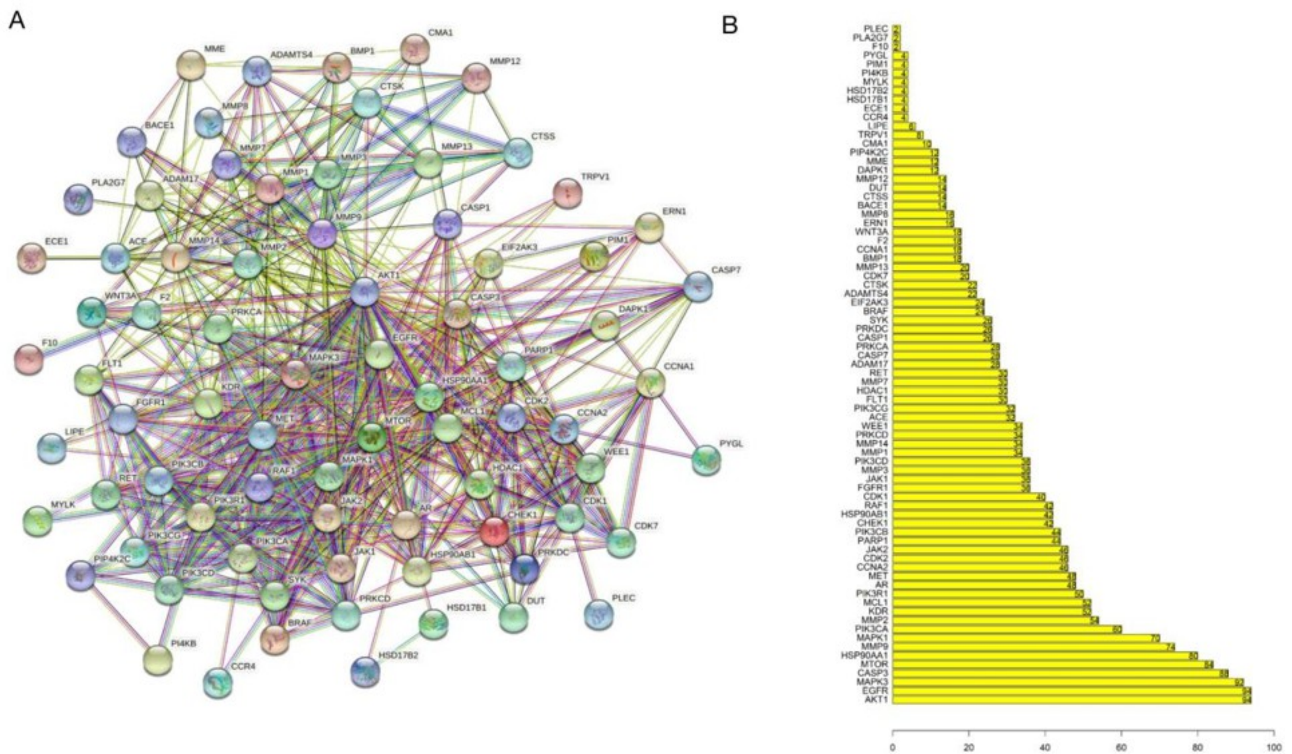
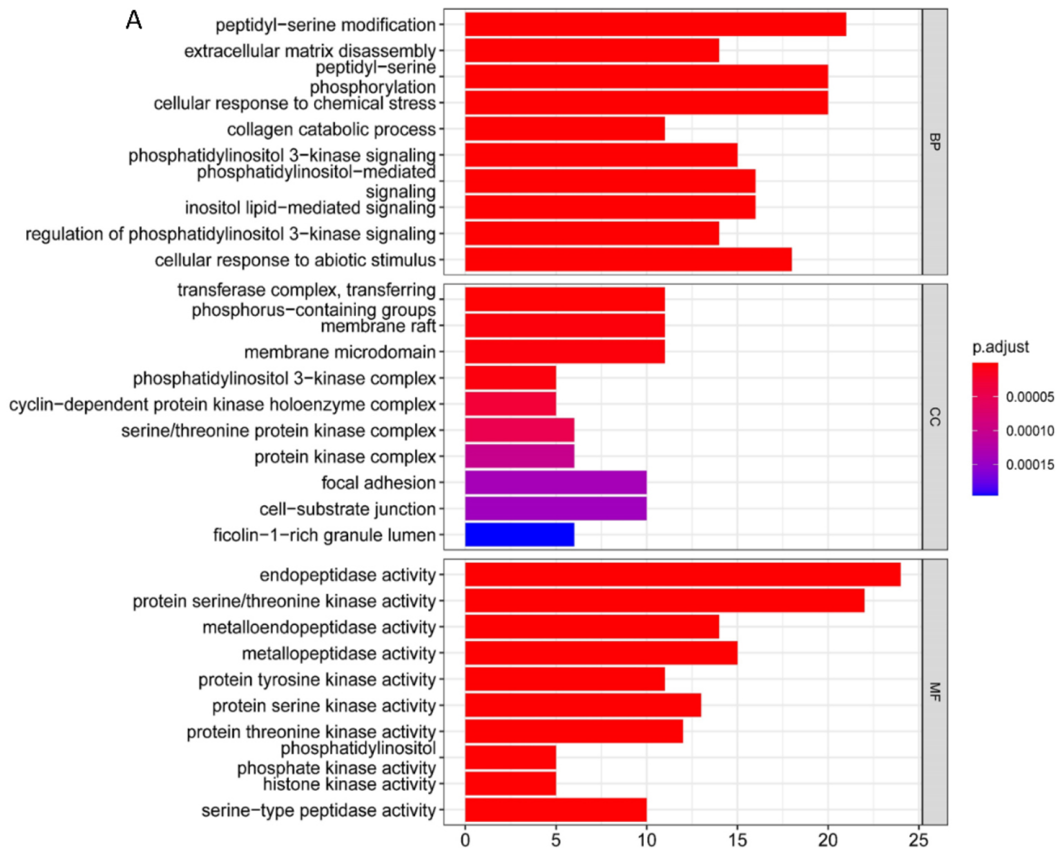


Fig. 5. The protein-protein interactions. (A) The protein-protein interactions network co-targets of DMAKO-20 and melanoma were constructed by the String database. (B) The top 50 proteins in the network.

quinone structure is protected to avoid non-selective cytotoxicity. The resulting shikonin oxime acts as a prodrug against tumor cells, where the active form with cytotoxicity and anti-invasiveness is generated only after metabolism



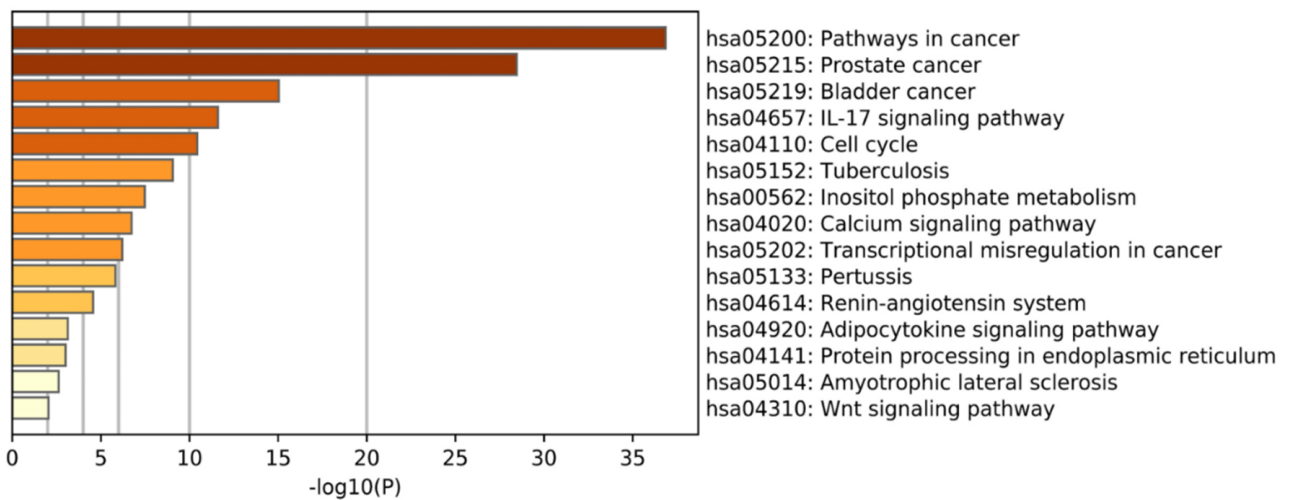


Fig. 6. GO & KEGG analysis. (A) GO analysis of the core targets, including the top 10 terms in BP, MF and CC. (B) KEGG pathway enrichment analysis of the core targets and the top 15. BP, biological process; MF, Molecular Function; GO, Gene Ontology; CC, cellular components; KEGG, Kyoto Encyclopedia of Genes and Genomes.

by tumor cells. This mode of action is considered safer and more effective than the parent compound shikonin [11].

The mechanism of action of shikonin against tumor migration and invasion has long been a focal point of research. This study employed bioinformatics technology to identify the WNT pathway as a potential common pathway between melanoma and DMAKO targets. The WNT pathway is a signaling pathway strongly associated

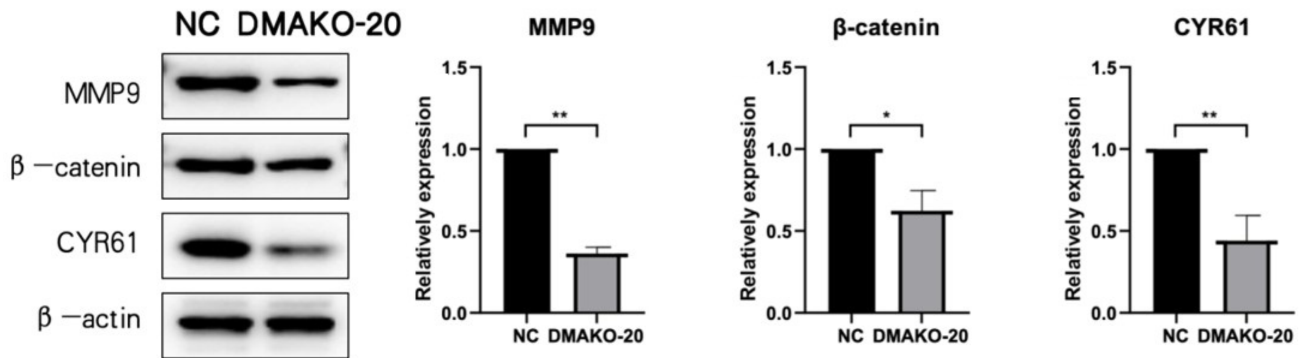


Fig. 7. Effect of DMAKO-20 on proteins related to WNT pathway. Western blot assay inhibited the expression of proteins related to the WNT pathway after DMAKO-20 treatment. Quantification data are shown as averages of three biological replicates. *$p < 0.05$, **$p < 0.01$.

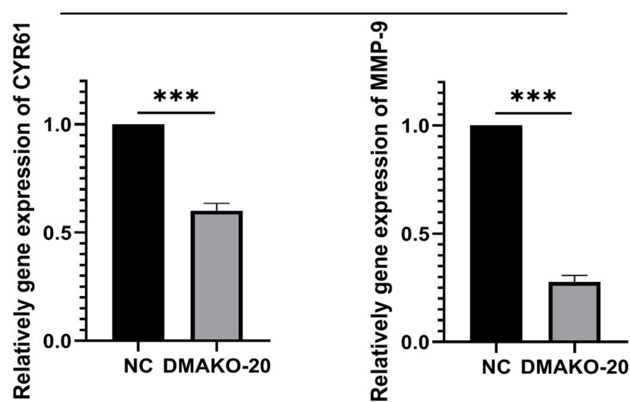


Fig. 8. Effect of DMAKO-20 on the expression of CYR61 and MMP9 in A375 cells qPCR verified that DMAKO-20 could inhibit the expression of CYR61 and MMP9 in melanoma through the WNT pathway. Quantification data are shown as averages of three biological replicates. ***$p < 0.001$. CYR61, cysteine-rich angiogenic inducer 61; MMP9, matrix metalloproteinase 9; qPCR, quantitative polymerase chain reaction.

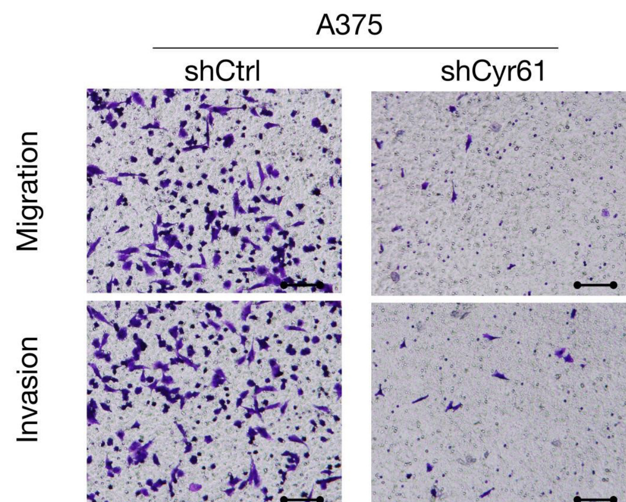


Fig. 9. Inhibition of CYR61 expression by shRNA and its effect on invasion and migration of melanoma cells. Transwell assay showed that the invasion and migration ability of melanoma cells were decreased after shRNA interfered with CYR61 (Scale bar = 50 µm). shRNA, Short hairpin RNA.

with tumor proliferation, epithelial-mesenchymal transition (EMT), migration, and invasion [25]. The prediction results also included other pathways like PI3K/Akt, MAPK and JAK-STAT but only under experimental conditions. In this research, the authors initially focused on the WNT pathway, traditionally linked to tumor invasion and metastasis for subsequent verification. The WNT pathway plays a pivotal role in tumor cell self-renewal and drug resistance. Traditional shikonin has been reported to inhibit tumor proliferation and invasion effects through this pathway [26]. This study utilized qPCR and WB techniques to investigate the changing trends of related genes and proteins after drug treatment. The findings revealed that WNT pathway-related proteins were inhibited following shikonin treatment.

Particularly, an important target gene within the WNT pathway is matrix metalloproteinase 9 (MMP9). Increased expression of MMP9 can establish favorable conditions for tumor invasion [27]. For the first time, this study used computer experiments to predict that this pathway would be involved. The results showed that DMAKO could stop MMP9 from activating in melanoma to stop tumor invasion and migration. This inhibition may be linked to the suppression of the WNT pathway.

Due to the intimate association between tumor metastasis and angiogenesis, cysteine-rich angiogenic inducer 61 (CYR61, also known as Cellular Communication Network Factor 1 (CCN1)), a protein present in the extracellular matrix, assumes a pivotal role in vascularization [28]. Hence, this study was selected to investigate the significance of this protein in melanoma. Currently, CYR61 has been identified as a protein closely correlated with tumor metastasis, playing a crucial role in tumor initiation, progression and

epithelial-mesenchymal transition (EMT) among other processes [28,29]. While a study has revealed that CYR61 promotes tumor metastasis in gastric and breast cancer [30]. The mechanistic role appears inconsistent across various cancers. For instance, it acts as a tumor suppressor in non-small cell lung cancer [31]. Inhibition of CYR61 expression in tumors has been demonstrated to suppress tumor metastasis and recurrence by attenuating the function of tumor stem cells [32].

However, the precise function of CYR61 in the metastasis and invasion of melanoma remains unclear. In this study, qPCR experiments demonstrated that DMAKO not only effectively reduced the expression of MMP9 in melanoma but also inhibited the vascular proliferation ability of tumor cells by downregulating CYR61 expression, consequently diminishing their migratory potential. Researchers used shRNA to lower CYR61's transcript to increase their confidence that it was involved in this process. They observed a significant reduction in melanoma invasion and migration following CYR61 inhibition. Hence, it was conclusively demonstrated for the first time that DMAKO could impede the invasion and migration of melanoma by suppressing the expression of MMP9 and CYR61.

The main difference between this study and others is that the drug structure of traditional shikonin has been improved. This is done to reduce its widespread cytotoxicity and increase its clinical potential. However, the modified shikonin may exert different mechanisms of action on tumors due to structural alterations. Therefore, this study employed bioinformatics techniques such as network pharmacology to analyze and predict the common action sites of DMAKO and melanoma. It was confirmed that DMAKO could diminish the expression of MMP9, thereby reducing melanoma metastasis. Additionally, this study delved into the role of the vascular-associated protein CYR61 for the first time, demonstrating that DMAKO may curb tumor angiogenesis through inhibition of CYR61 expression. Consequently, DMAKO exhibits the potential to mitigate melanoma metastasis through dual mechanisms.

Conclusion

The findings of this study suggest that the shikonin derivative DMAKO-20 has the potential to inhibit the activation of MMP9 in melanoma, consequently impeding the metastasis of this cancer. Moreover, DMAKO-20 may enhance this effect by suppressing the expression of *CYR61*, thus offering promising prospects for its adjunctive application in clinical anti-melanoma therapy.

Availability of Data and Materials

Data and materials will be available upon request from the corresponding authors.

Author Contributions

XBZ contributed to writing-original draft, investigation and data curation. JH contributed to writing-review & editing and formal analysis, investigation and data curation. SGL contributed to writing-review & editing, validation and methodology. JHC contributed to conceptualization, project administration and resources, and revising critically for important intellectual content. JC contributed to writing-review, conceptualization and funding acquisition. All authors gave their final approval and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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