A Practical Strategy for Exploring the Pharmacological Mechanism of Chrysophanol Against Obesity Asthma/Childhood Asthma Comorbidity

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Abstract

Background and purpose Obesity may be more likely to lead to asthma, that is, obesity asthma. Children are the age stage of high incidence of asthma. Obesity asthma may be more refractory in children with asthma, and is more likely to produce glucocorticoid resistance, which greatly leads to the risk of severe disease in children with asthma. However, approaches to combating Obesity Asthma/Childhood Asthma complications are limited by conditions. Existing evidence shows that chrysophanol has antibacterial and anti-inflammatory, fat reduction, anticough, promoting gastrointestinal functional homeostasis and immune regulation. Experimental methods Through systematic pharmacological and bioinformatics analysis, we evaluated the physical and chemical properties and biological activities of chrysophanol, and further analyzed its binding activities, targets, biological functions and mechanisms. Key results It was found that chrysophanol can play the ideal physical and chemical properties and biological activities. The PPI network screened 144 common targets of drugs and diseases, and 15 hub targets were obtained. Then, the top 10 hub targets were identified, namely EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, PIK3CA, and verified in the protein-ligand blind docking. Enrichment analysis showed that chrysophanol may be involved in inflammation regulation, EGFR tyrosine kinase inhibitor resistance, HIF-1 signaling pathway, Neutrophil extracellular trap formation,and so on. Conclusions and implications Our fingdings indicate that chrysophanol can reduce airway inflammation and remodeling through multi-pathway and multi-target, and provide evidence for the application of chrysophanol in Obesity Asthma/Childhood Asthma comorbidities. The predicted results will be strictly verified by experiments.

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Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.*AbstractBackground and purpose*

Obesity may be more likely to lead to asthma, that is, obesity asthma. Children are the age stage of high incidence of asthma. Obesity asthma may be more refractory in children with asthma, and is more likely to produce glucocorticoid resistance, which greatly leads to the risk of severe disease in children with asthma. However, approaches to combating Obesity Asthma/Childhood Asthma complications are limited by conditions. Existing evidence shows that *chrysophanol* has antibacterial and anti-inflammatory, fat reduction, anticough, promoting gastrointestinal functional homeostasis and immune regulation.

Experimental methods

Through systematic pharmacological and bioinformatics analysis, we evaluated the physical and chemical properties and biological activities of *chrysophanol* , and further analyzed its binding activities, targets, biological functions and mechanisms.

Key results

It was found that *chrysophanol* can play the ideal physical and chemical properties and biological activities. The PPI network screened 144 common targets of drugs and diseases, and 15 hub targets were obtained. Then, the top 10 hub targets were identified, namely EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, PIK3CA, and verified in the protein-ligand blind docking. Enrichment analysis showed that *chrysophanol* may be involved in inflammation regulation, EGFR tyrosine kinase inhibitor resistance, HIF-1 signaling pathway, Neutrophil extracellular trap formation,and so on.

Conclusions and implications

Our fingdings indicate that *chrysophanol* can reduce airway inflammation and remodeling through multi-pathway and multi-target, and provide evidence for the application of *chrysophanol* in Obesity Asthma/Childhood Asthma comorbidities. The predicted results will be strictly verified by experiments.

Keywords: *Chrysophanol* ; Obesity Asthma; Childhood Asthma; System Pharmacology; Bioinformatics Analysis.

Introduction

Chrysophanol (Chrysophanic acid, CA) is a natural anthraquinone, which can inhibit EGF-induced EGFR phosphorylation and inhibit the activation of AKT and mTOR/p70S6K. It is mainly found in Ligusticum ligusticum, Rhubarb, bloody-rattan, senna, Polygonum multiflorum, Polygonum polygonum, cassia seed, aloe vera, and achyranthes radix. In addition, chrysophanol is also found in pinellia pinellia and other expectorants. The molecular formula of CA is $C_{15}H_{10}O_4$. It has antibacterial effect on a variety of bacteria, can antibacterial cough, anti-aging, promote defecation, promote nerve excitation and muscle paralysis.In the process of screening and treating the comorbidity of obesity asthma and childhood asthma, it is found that*chrysophanol* has obvious network pharmacological inhibition and molecular targeting docking with the comorbidity factors of obesity asthma and childhood asthma, which may have a good effect on controlling the attack of obesity asthma and childhood asthma. Therefore, through the systematic network pharmacological and bioinformatics analysis, to analyze and predict the comorbidity of obesity asthma and children asthma systematically, in order to provide effective treatment measures and new medication methods for children obesity-asthma comorbidity.

Chrysophanol has been reported to inhibit lipid accumulation 3T3-L1 adipocytes. It can down-regulate adipogenic factors in 3T3-L1 adipocytes and induces thermogenic factors in primary cultured brown adipocytes, it also can inhibit adipogenesis and induces thermogenesis by activating AMPK pathway(Lim and Park et al., 2016). In addition,*chrysophanol* inhibited the production of IL-6, TNF-a and monocyte chemoattractant

protein-1 as well as the reduction of adiponectin production in 3T3-L1 cells(Rim and Moon et al., 2013). Researches have shown that the antioxidant and anti-in- flammatory effects of*chrysophanol* play a role in protecting nerve cell. Chrysophanol (10.0 mg/kg) could alleviate hip-pocampal neuronal injury in lead-exposed neonatal mice by promoting the excretion of lead, while significantly reducing the level of malondialdehyde in the brain, liver and kidney, and increasing the activity of superoxide dis-mutase (SOD) and glutathione peroxidase(Zhang and Yan et al., 2014).*Chrysophanol* (20 lM) effectively attenuated overall clinical scores as well as various pathological markers of colitis. Additionally, chrysophanol inhibited the production of tumor necrosis factor (TNF)-a, interleukin (IL)-6 and the expression of cyclooxygenase (COX)-2 levels induced by LPS through suppression of the activation of NF-jB and caspase-1(Kim and Kim et al., 2010). *Chrysophanol* had a protective effect on ovalbumin-induced asthma in mice,reducing the number of eosinophils and inflammatory cytokines in bronchoalveolar lavage fluid and inhibited the expression of RAW, Th17 percentage and RORct(Zhang and Song et al., 2017).

The possible "dangerous link" between Obesity Asthma/Childhood Asthma remains a potential threat to the prognosis of Childhood Asthma. Clinical and experimental evidence suggests that obese children are at increased risk of asthma compared to children of healthy weight. Obese children with asthma had more severe and poorly controlled asthma symptoms, more frequent asthma exacerbations, and an overall lower asthma-related quality of life than children with healthy weight asthma(Bapat and Whitty et al., 2022; Reyes-Angel and Kaviany et al., 2022). Juan R etl. found the co-occurrence of asthma and obesity with gene inversion 16p11.2.16 through clinical samples(Gonzalez and Caceres et al., 2014; Gonzalez and Ruiz-Arenas et al., 2020). In asthma, raised BMI has recently been shown to affect airway closure independently of asthma control(Kaminsky and Chapman et al., 2019). Adipose "tissue" forms a complex endocrine organ intimately involved in inflammation homeostasis and plays a pro-inflammatory role(Kiefer, 2017; Hildebrand and Stumer et al., 2018). Studies have shown that *chrysophanol*can promote adipose metabolism and exert an anti-allergic inflammatory effect by regulation of Th2 cytokines, inflammatory cytokines, caspase-1, chemokines, and inflammatory cells in ovalbumin-induced allergic rhinitis mouse model(Kim and Jee et al., 2019). However, the therapeutic targets and molecular mechanisms of *chrysophanol* for Obesity Asthma/Childhood Asthma complications have not been explored, the therapeutic targets and molecular mechanisms of *Chrysophanol*against Obesity Asthma/Childhood Asthma Comorbidity patients have not been previously explored. Based on this, we used system pharmacology and bioinformatics analysis to assess the drug likeness and bioactivity of*Chrysophanol* and analyze the targets and signaling pathways of combating Obesity Asthma/Childhood Asthma Comorbidity. The flow diagram of our research is shown in **Figure 1** .

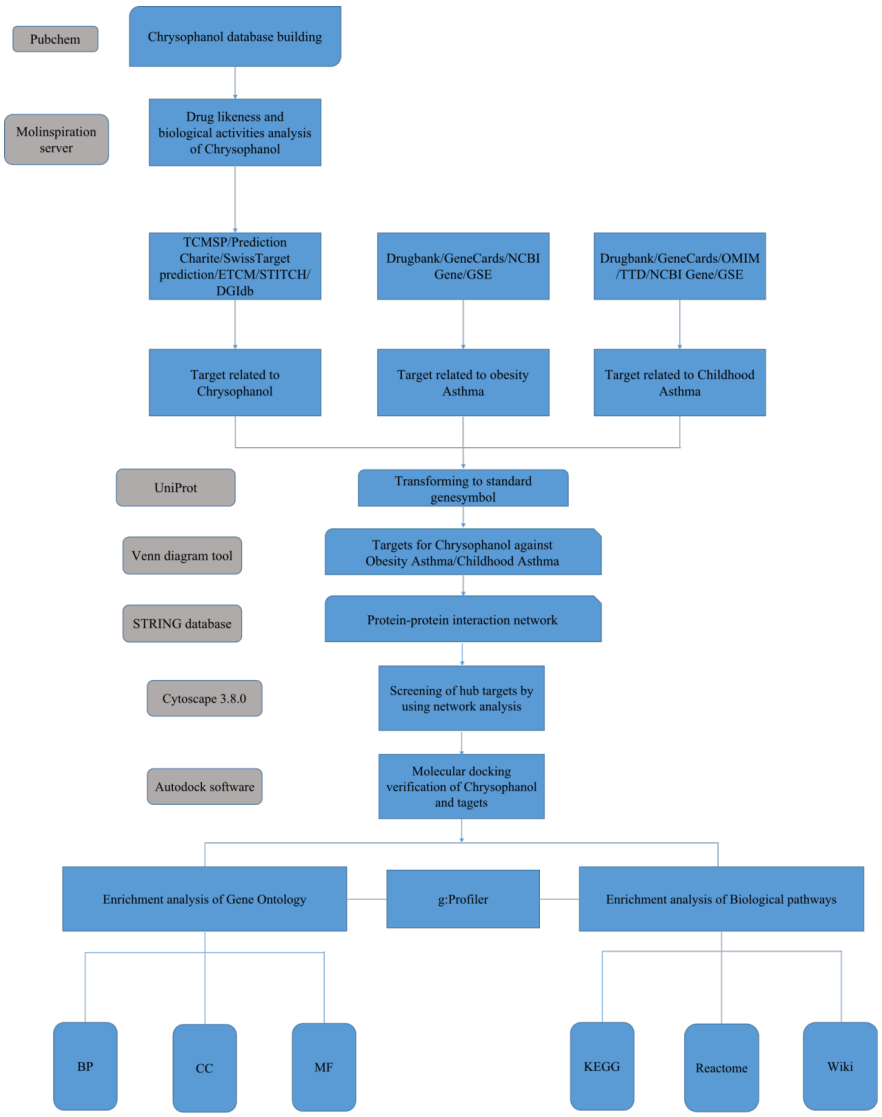


Figure 1

The flow diagram of this research showing a pragmatic strategy for identifying the pharmacological mechanisms of *Chrysophanol*Against Obesity Asthma/Childhood Asthma Comorbidity based on system pharmacology and bioinformatics analysis.

Materials and Methods

Ethics Statement

The data were obtained from open-source databases, and thus, ethics committee approval was not applicable in this study.

Chrysophanol Database Building

The 2D structure, 3D structure, InChI, and canonical SMILES profiles of Chrysophanol were obtained from PubChem(Kim and Chen et al., 2021).

Analyses of Physicochemical Properties and Biological Activities

Molinspiration server (https://www.molinspiration.com/) works based on the Lipinski Rules of Five,can carry out molecular manipulation and processing(Carlsson and Spjuth et al., 2010). The most "drug-like" compound must possess the following properties: LogP [?]5, molecular weight [?]500Da, number of hydrogen bond acceptors(n-ON)[?]10, and number of hydrogen bond donors(n-OHNH) [?]5. Compounds that failed to show these characters are least considered as a drug. Molinspiration server calculates the important molecular properties of compounds based on partition coefficient (LogP), polar surface area, number of hydrogen bond donors and acceptors, and also prediction of bioactivity score for the drug targets such as G-Protein Coupled Receptor (GPCR) ligands, kinase and protease inhibitors (KIs and PIs), ion channel modulators (ICMs), and nuclear receptor ligand (NRL). Topographical polar surface area (TPSA) was used to calculate the percentage of absorption using the following equation: Percentage of absorbance = 109 - 0.345 × TPSA(Carlsson and Spjuth et al., 2010). Thus, the Molinspiration server was used to evaluate the molecular descriptors, drug likeness, and bioactivity of *Chrysophanol* (Rashid, 2020). The standard SMILES profile of *Chrysophanol* was uploaded to "Calculation of Molecular Properties and Bioactivity Score" section, the drug likeness analyzed based on the Lipinski's rule of five comprised the parameters described above.

Fishing of Chrysophanol-Related Targets

Different types of pharmacological targets related to Chrysophanol were collected from the following databases: 1) Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/) (Ru and Li et al., 2014), 2)Prediction.charite (https://prediction.charite.de/), 3) Swiss Target Prediction (http://www.swisstargetprediction.ch/)(Daina and Michielin et al., 2019), 4) Chemical Association Networks (STITCH, http://stitch.embl.de/)(Szklarczyk and Santos et al., 2016), 5) Drug Gene Interaction Database (DGIdb, https://www.dgidb.org/), and 6) Encyclopedia of Traditional Chinese Medicine (ECTM, http://www.tcmip.cn/ETCM/)(Xu and Zhang et al., 2019),The target proteins were transformed to standard gene symbols by using the UniProt database (https://www.uniprot.org/) with the limitation of "Human species".

Collection of Obesity Asthma/Childhood Asthma-Related Targets

The Obesity Asthma-related targets were identified from differentially expressed genes (DEGs) by analyzing available transcriptomic RNA-seq data of Obesity Asthma (GSE110551) from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo)(Barrett and Wilhite et al., 2013). The "limma" in R (version 3.6.2, https://www.r-project.org/) was applied to access the profile of DEGs, which must fit the screening criteria of adjusted P-value <0.05 and |log2FC| >1(Gu and Xue et al., 2020). DEGs were visualized by volcano plots, which were drawn by "ggpubr" and "ggthemes" of R-language package. Moreover, the Obesity Asthma-related targets were also gathered from seven open-source databases listed as follows:1) DrugBank (https://go.drugbank.com/)(Wishart and Feunang et al., 2018), 2) GeneCards (https://www.genecards.org/)(Stelzer and Rosen et al., 2016) , and 3) NCBI Gene (https://www.ncbi.nlm.nih.gov/).

As for Childhood Asthma-related targets, we first analyzed the DEGs from the GSE1743254 and GSE188424 datasets from the GEO database, which were also assessed by the "limma" package of R-language Bioconductor with the criteria of adjusted P-value <0.05 and |log2FC| >1(Gu and Xue et al., 2020). Additionally, targets related to Childhood Asthma were also acquired by exploring the following six databases: 1) GeneCards, 2) Online Mendelian Inheritance in Man (OMIM, https://omim.org/)(Amberger and Bocchini et al., 2015), 3) Therapeutic Target Database (TTD, http://db.idrblab.net/)(Wang and Zhang et al., 2020), and 4) NCBI Gene.

Targets of Chrysophanol Against Obesity Asthma/Childhood Asthma Comorbidity Acquisition

The overlapping targets between Chrysophanol, Obesity Asthma and Childhood Asthma were further obtained by using the Venn diagram tool (http://jvenn.toulouse.inra.fr/app/index.html)(Bardou and Mariette et al., 2014). The intersection between Chrysophanol-related targets and Obesity Asthma and Childhood Asthma-related targets was the final targets of Chrysophanol against Obesity Asthma and Childhood Asthma comorbidity.

Analyses of the Protein–Protein Interaction Network and Hub Targets

The protein–protein interaction (PPI) network helps to better understand the biological mechanisms involved in target-related pathogenesis at the protein level. Thus, the STRING 11.5 database (https://string-db.org/) was used to construct the PPI network and receive hub targets. The organism was set to "Homo sapiens" and the minimum required interaction score was 0.4 (Szklarczyk and Gable et al., 2019). Subsequently, the PPI network was visualized and analyzed by Cytoscape 3.8.0 software (https://cytoscape.org/). The degree values in the PPI network were calculated by using the NetworkAnalyzer plugin of Cytoscape 3.8.0 software. Then, targets with degree values higher than the median were filtered as hub targets(Xia and Xun et al., 2020).

Enrichment Analyses for Hub Targets

Enrichment analyses of Gene Ontology (GO) (including molecular function, cellular component, and biological process) and biological pathways (including KEGG pathways, Reactome pathways, and Wiki pathways) of hub targets were carried out through g:Profiler (https://biit.cs.ut.ee/gprofiler/gost)(Raudvere and Kolberg et al., 2019). The organism was set to "Homo sapiens" and a term with adjusted P-value <0.05 was considered significantly enriched. The GO terms or pathway terms with smaller adjusted P-values were believed to have more potent effects on fighting Obesity Asthma/Childhood Asthma comorbidity, and the top 15 GO terms and pathway terms were illustrated in the results ranked by adjusted P-value from low to high.

Molecular Docking Verification of Chrysophanol and Targets

The protein structures of targets were downloaded from the PDB database (https://www.rcsb.org/)(Rose and Prlic et al., 2017). The 3D structure of Chrysophanol was provided by the PubChem database. Cavity-detection guided blind Docking was applied to extract the ligand from the target protein, then the original ligand to the active site of the complex was redocked, and the conformation of the original ligand was compared with the conformation of the ligand after docking(Liu and Yang et al., 2022; Yang and Liu et al., 2022). Furthermore, the water molecules were removed, hydrogen atoms were added, and the spatially active sites of ligand molecules docking in the target protein compound were determined for docking preparation.

Chrysophanol was docked with targets, including EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, PIK3CA, the top 10 targets predicted by the PPI network through the Cavity-detection guided blind Docking.

Results

Analysis of the Physicochemical Properties of Chrysophanol

The evaluation of drug likeness is of vital importance in the production and upgrading of drug entities, and we first predicted the physicochemical properties of *chrysophanol* according to the Lipinski's rule of five. The criteria of the Lipinski's rule of five are as follows: logP [?]5, MW [?]500 Da, n-ON [?]10, and n-OHNH [?]5 . In addition, the topological polar surface area (TPSA) value is a key indicator for evaluating and predicting the oral bioavailability of molecular compounds, and the Topological Polar Surface Area (TPSA) value of [?]140 A represents good oral bioavailability(Ertl and Rohde et al., 2000; Kunick and Lauenroth et al., 2004; Jamuna and Rathinavel et al., 2018; Rashid, 2020). %ABS value calculated according to TPSA value between 67% and 83% means an ideal oral bioavailability. Surprisingly, the results showed that *Chrysophanol* met the criteria of miLogP = 3.54 < 5, MW = 254.24 < 500 Da, n-ON, number of hydrogen bond acceptors: n-ON = 4 < 10, and number of hydrogen bond donors:n-OHNH = 2 <5, and the value of TPSA at 74.6 2< 140 and %ABS at 83, the number of Lipinski's rule-of-five violation and number of rotatable bonds are 0, which are all in agreement with Lipinski's rules, indicating that Chrysophanol has a good permeability in the cellular membrane(Lipinski and Lombardo et al., 2001; Veber and Johnson et al., 2002; Da and Comin et al., 2015). The topological polar surface area (TPSA) is recognized as a good indicator of drug absorption in the intestine (TPSA less than 140 Angstroms squared [A^2]) and blood-brain barrier penetration (TPSA less than 60 A^2). The TPSA value of Chrysophanol is 74.6 and it has a good intestinal absorption(Da and

Comin et al., 2015). All of these mean that chrysophanol is at the range of ideal oral bioavailability as shown in Table 1 .

Table 1. Physicochemical properties of Chrysophanol evaluated by Molinspiration

Compound	%ABS	miLogP	TPSA (Å)	n-atoms	MW	n-ON	n-OHNH	n-violations	n-rotb	MV
Standard criteria		<5			<500	<10	<5	[?]1	[?]10	
Chrysophanol	83	3.54	74.6 [2]	19	254.24	4	2	0	0	161.55

%ABS, percentage of absorption; miLogP, logarithm of partition coefficient between n-octanol and water; TPSA, topological polar surface area; n-atoms, number of atoms; MW, molecular weight; n-ON, number of hydrogen bond acceptors; n-OHNH, number of hydrogen bond donors; n violations, number of Lipinski's rule-of-five violation; n-rotb, number of rotatable bonds; MV, molecular volume.

Bioactivity Prediction of Chrysophanol

As summarized in ***Table 2,*** the physiological role of Chrysophanol may be associated with a variety of mechanisms, including possible interactions with GPCR ligands, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor. Moreover, Chrysophanol exhibited promising Enzyme inhibitor, Nuclear receptor ligand affinities with bioactivity scores greater than $0.02 > 0$, while it showed moderate Kinase inhibitor, Ion channel modulator, GPCR ligand, Protease inhibitor affinities with bioactivity values between -0.50 and 0.00. The results indicated that Chrysophanol had better nuclear receptor ligand affinity (Enzyme inhibitor > Nuclear receptor ligand > Kinase inhibitor > Ion channel modulator > GPCR ligand > Protease inhibitor).

Table 2. Bioactivity scores of Chrysophanol based on Molinspiration cheminformatics.

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor
Chrysophanol	-0.23	-0.17	-0.06	0.02	-0.26

Bioactivity score of >0 represented promising activity, bioactivity score between -5.00 and 0.00 represented moderate activity, and bioactivity score of [?]5.0 represented no activity.

Target Identification of Obesity Asthma/Childhood Asthma Comorbidity and Chrysophanol

Subsequently, we obtained the DEGs of Obesity Asthma and Childhood asthma from GEO via analyzing GSE110551(4754). Volcano plots of DEGs for Obesity Asthma and Childhood asthma infected patients are shown in**Figures 3E˜H** . Then, we continued to collect target genes related to Obesity Asthma from seven open-source databases, namely, DrugBank (8), GeneCards (3209), and NCBI Gene (596). After checking duplications, 8567 target genes were obtained **(Figure 2A)** .

As for Childhood asthma-related genes, we obtained the targets from six open-source databases as follows: 1) GeneCards(4822), 2) OMIM(22), 3) TTD(168),4) Drugbank (4), 5) NCBI Gene (396), and 6) GSE1743254(20) and GSE188424(36). A total of 5468 target genes were achieved after the removal of duplications **(Figure 2B)** .

Eight open-source databases were used to obtain the targets related to Chrysophanol, namely, TCMSP(12), Swiss Target Prediction(100), prediction.charite.de(105, Probability>50%), STITCH(2), DGIdb(5), and ECTM(18). We established a Chrysophanol-related target set by syndicating a union of the predicted results and 144 targets related to Chrysophanol were gathered after the removal of duplications and transferring gene symbols **(Figure 2C)** .

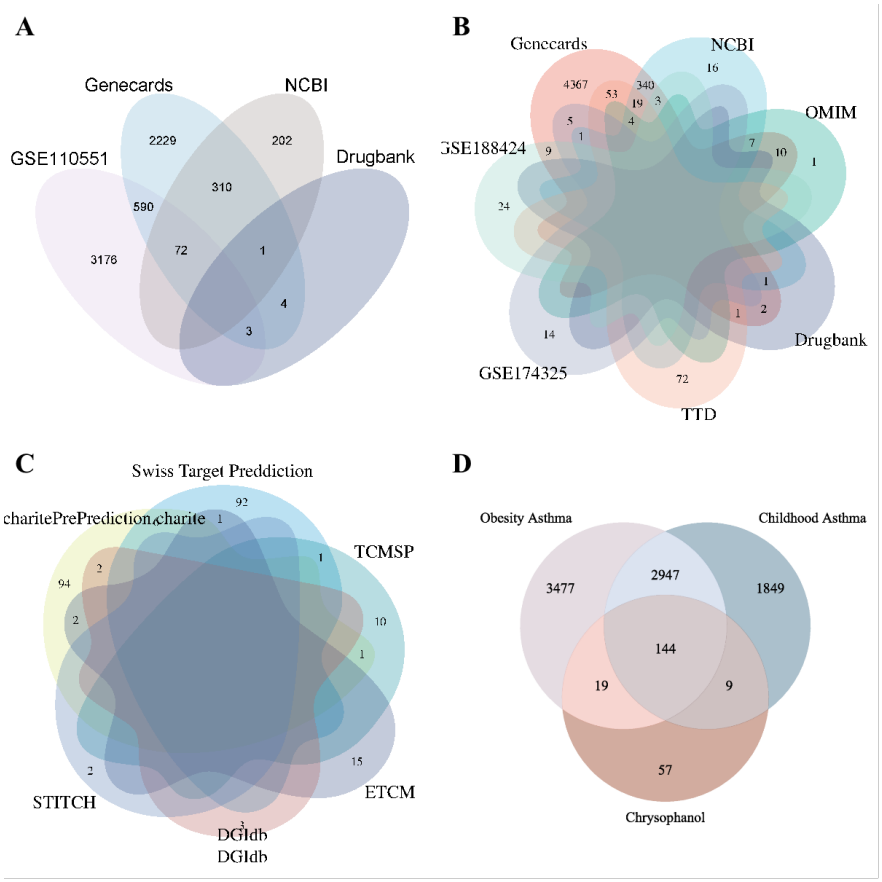
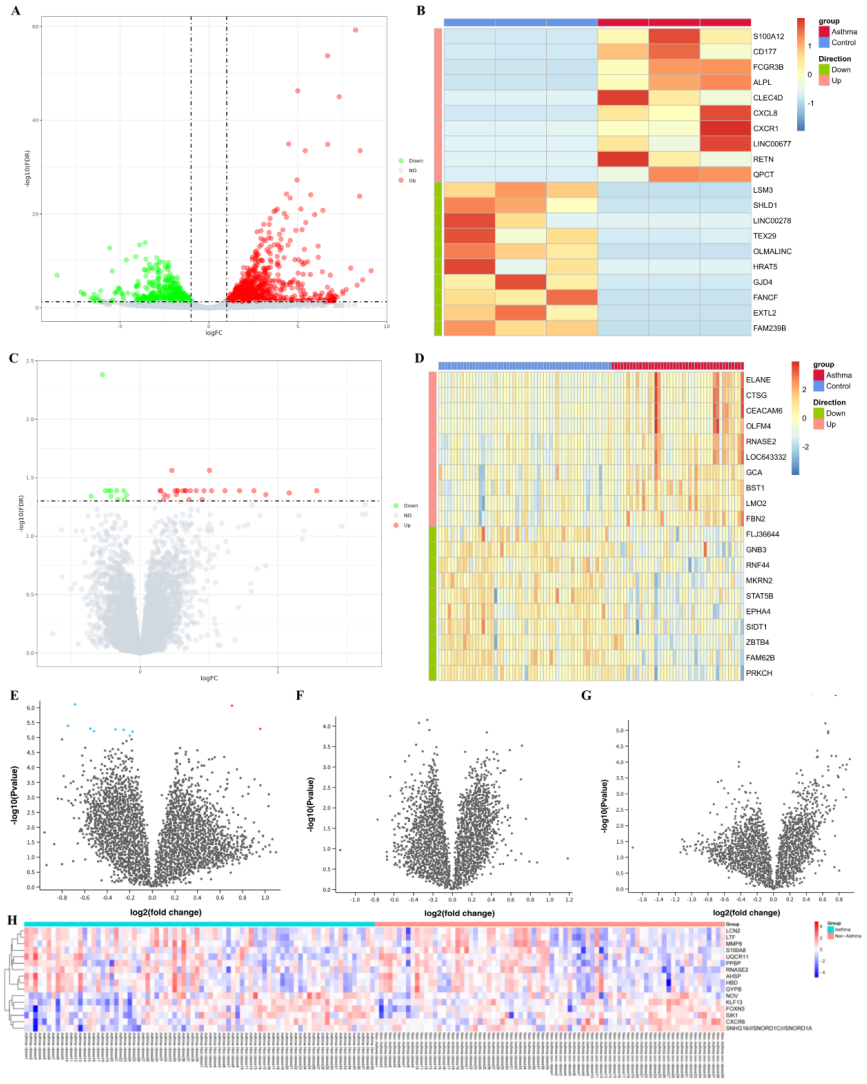


Figure 2

The number of target genes related to Obesity Asthma from four open-source databases. (B) The numsber of target genes related to Childhood asthma from seven open-source databases. (C) The number of target genes related to Chrysophanol from six open-source databases. (D) Venn diagram depicting common target genes between Obesity Asthma, Childhood Asthma and Chrysophanol.

Subsequently, we obtained the DEGs of Childhood Asthma from GEO via analyzing GSE174325(20) and GSE188424(36) recruited 6 asthma patients and 3 healthy controls to isolate from peripheral blood mononuclear cells (PBMCs), and the profile of GSE188424 collected from whole blood comprised 43 participants with asthma and 56 healthy donors. Volcano plots and Heatmaps of DEGs for asthma patients are shown in**Figures 3A˜D** . GSE110551(4754) information originated from the whole blood of 156 donors with 39 Obese Asthma, 39 Non-Obese Asthma, 39 Non-Obese Non-Asthma and 39 Obese Non-Asthma. Volcano plots and Heatmaps of DEGs for Obesity Asthma-infected patients are shown in **Figures 3E˜H** .



Figures 3

Volcano plots and Heatmaps of differentially expressed genes (DEGs) for Childhood Asthma and Obese Asthma patients. The abscissa represented log2FC and the ordinate indicated the -log10 (adjusted P-value) of the genes. The red and blue points, respectively, represented the upregulated and downregulated genes with the criteria of adjusted P-value <0.05 and |log2FC| >1. (A) and (B) DEGs from GSE174325. (C) and (D) DEGs from GSE188424. (E) ˜(H) DEGs from GSE110551.

Finally, we received 144 common target genes between Obesity Asthma/Childhood Asthma and Chrysophanol, which were analyzed by the JVenn tool(**Figure 2D**) . The 144 target genes were used to further screen the hub target genes to construct the PPI network for Chrysophanol against Obesity Asthma/Childhood Asthma comorbidity.

PPI Network Analysis

The interaction network between 144 common target genes was analyzed to screen the hub targets for Chrysophanol against Obesity Asthma/Childhood Asthma comorbidity. The targets with degree values

greater than the median were chosen as the hub targets, and the results showed that the median degree value was 30 and the targets with degree values greater than 30 were regarded as hub targets. Thus, a total of 15 hub targets were identified and the PPI network was constructed by the STRING 11.0b database and visualized by Cytoscape 3.8.0 software as shown in**Figure 4A** . The nodes and edges, respectively, represented targets and interactions between targets, and there were 14 nodes and 81edges in the PPI network of hub targets. In particular, the sizes and color shades of nodes presented positive correlation with degree values, indicating that a node with a darker color and larger shape plays a more important role in fighting Obesity Asthma/Childhood Asthma comorbidity. As seen in **Figure 4B, C and D** , the top 10 targets with the highest degree values were EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, and PIK3CA (Degree shown in Table 3). Consequently, EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, and PIK3CA as the crucial players for *Chrysophanol* to treat Obesity Asthma/Childhood Asthma comorbidity were further used to perform molecular docking with *Chrysophanol* .

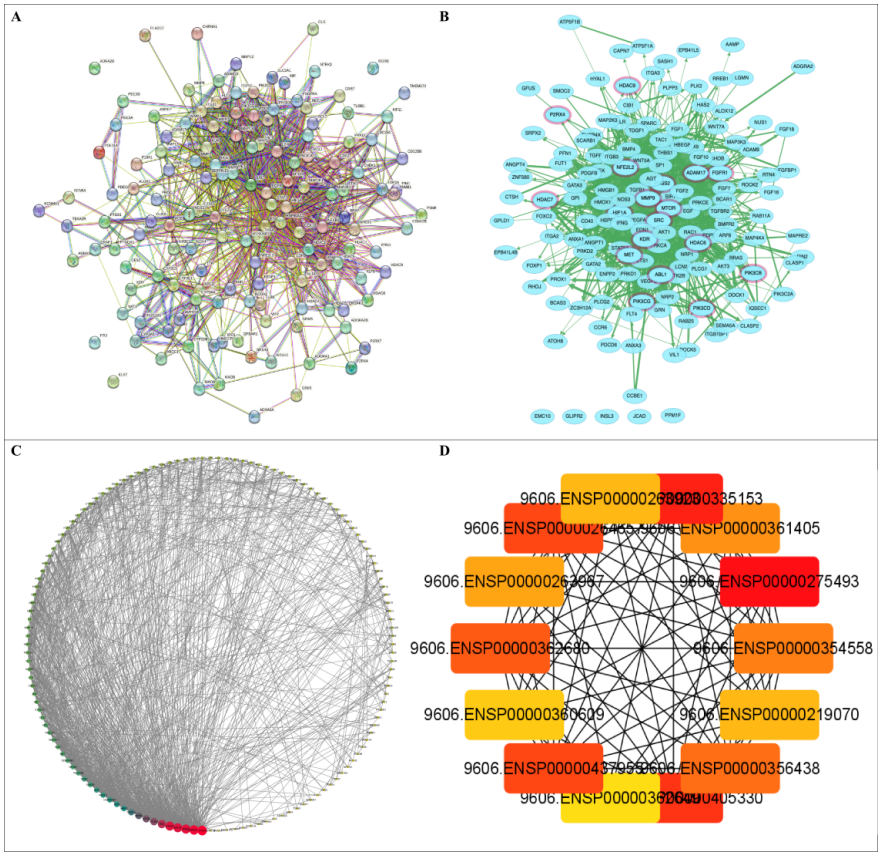


Figure 4

PPI network for hub targets of *Chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity. The nodes and edges, respectively, represented hub targets and interactions between targets. The sizes and color shades of nodes presented a positive correlation with degree values, and a node with a brighter color and larger shape played a more important role in fighting Obesity Asthma/Childhood Asthma comorbidity.

Table 3. Top 15 in network STRING network ranked by Degree method

Rank	Name	Targets	Score

1	9606.ENSP00000275493	EGFR	66
2	9606.ENSP00000335153	HSP90AA1	64
3	9606.ENSP00000405330	ESR1	62
4	9606.ENSP00000437955	HIF1A	61
4	9606.ENSP00000264657	STAT3	61
6	9606.ENSP00000362680	SRC	55
7	9606.ENSP00000356438	PTGS2	48
8	9606.ENSP00000354558	MTOR	46
9	9606.ENSP00000361405	MMP9	43
10	9606.ENSP00000263967	PIK3CA	38
11	9606.ENSP00000263923	KDR	32
11	9606.ENSP00000219070	MMP2	32
13	9606.ENSP00000360609	HSP90AB1	31
14	9606.ENSP00000362649	HDAC1	30
14	9606.ENSP00000290866	ACE	30

GO Enrichment Analysis

To further explore the biological functions of *Chrysophanol*against Obesity Asthma/Childhood Asthma comorbidity, hub targets were submitted to g:Profiler for GO enrichment analysis. A total of 374 GO terms were obtained consisting of 240 BP terms, 77 CC terms, and 57 MF terms. The top 20 terms of BP, CC, and MF were ranked by adjusted P-value and the enrichment condition of LYN, RET, ABCC1, HDAC2, ACE, HSP90AB1, MAOB, SRC, MMP2, AHR, PTGS2, ADRA1A, P2RX7, ADAM17, TBXA2R, LCK, APEX1, PDE3A, BCL2, CDK1, and ABL1 are shown in **Figures 5A˜D** . BP enrichment analysis mainly contained cellular response to chemical stimulus; response to xenobiotic stimulus, peptidyl-tyrosine phosphorylation, extracellular matrix disassembly, protein phosphorylation, inflammatory response, and so on. CC enrichment analysis mainly contained cytoplasm, receptor complex, cytosol, histone deacetylase complex, nucleoplasm, plasma membrane, and so on. MF enrichment analysis mainly contained endopeptidase activity, transmembrane receptor protein tyrosine kinase activity, protein tyrosine kinase activity, NAD-dependent histone deacetylase activity (H3-K14 specific), transcription factor binding, ATP binding, and so on. The results of GO terms suggested that *Chrysophanol* may regulate the xenobiotic stimulus, peptidyl-tyrosine phosphorylation, extracellular matrix disassembly, inflammatory response through the cytoplasm, receptor complex, cytosol, histone deacetylase complex, nucleoplasm, plasma membrane based on endopeptidase activity, transmembrane receptor protein tyrosine kinase activity, ATP binding etc. to perform its therapeutic effects against Obesity Asthma/Childhood Asthma comorbidity.

Figure 5

Gene Ontology enrichment analysis results of *Chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity. (A) The results of biological process (BP), cellular component (CC), and molecular function (MF) enrichment analyses of top 20. The abscissa indicated the kind of GO enrichment analyses, while the ordinate represented the Enrichment score of the terms. The red, green, and blue points, respectively,

represented the BP, CC, and MF enrichment analyses terms. (B) Identification result of BP terms according to adjusted P-value. (C) Identification result of CC terms according to adjusted P-value. (D) Identification result of MF terms according to adjusted P-value. (B) ˜ (D) The abscissa indicated the kind of GO enrichment analyses, while the ordinate represented the -log10 (adjusted P-value) of the terms.

Pathway Enrichment Analysis

A total of 647 pathway terms were recognized consisting of 142 KEGG pathways, 280 Reactome pathways, and 225 Wiki pathways. The top 20 pathways were ranked by adjusted P-value and the enrichment condition of EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, and PIK3CA are presented in **Figures 6A˜D** . KEGG pathways were mainly involved in the Prostate cancer, Pathways in cancer, Central carbon metabolism in cancer, EGFR tyrosine kinase inhibitor resistance, MicroRNAs in cancer, Endocrine resistance, Chemical carcinogenesis - receptor activation, HIF-1 signaling pathway, and so on. Reactome pathways were significantly enriched in Diseases of signal transduction by growth factor receptors and second messengers, Signal Transduction, Signaling by Receptor Tyrosine Kinases, Interleukin-4 and Interleukin-13 signaling, Collagen degradation, PIP3 activates AKT signaling, Signaling by NOTCH1, and so on. Wiki pathways predominantly included the Relationship between inflammation, COX-2 and EGFR, EGFR tyrosine kinase inhibitor resistance, Aryl hydrocarbon receptor pathway, Malignant pleural mesothelioma, Glioblastoma signaling pathways, Chronic hyperglycemia impairment of neuron function, and so on. The enriched pathways of *Chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity were strongly associated with inflammation, immune response, endocrine regulation, nervous regulation, oxidative stress, collagen degradation and signal transduction.

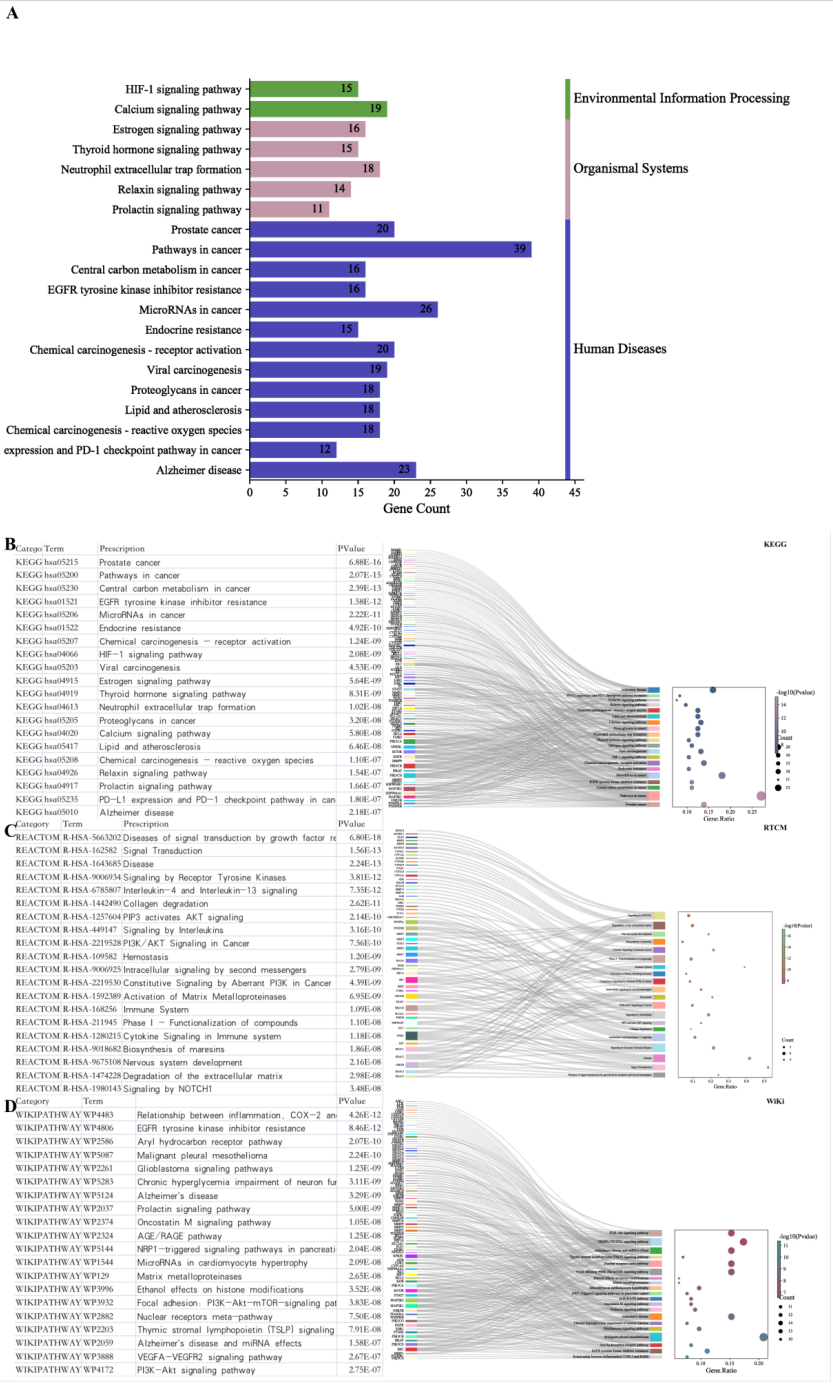


Figure 6

The top 20 biological pathway enrichment analysis results of*Chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity. (A) KEGG pathway enrichment analysis results. The abscissa indicated the kind of KEGG enrichment analyses, while the bar chart represented the number of gene enrichment of the terms. (B) KEGG pathway enrichment analyses identification result according to

adjusted P-value. (C) Wiki pathway enrichment analyses identification result according to adjusted P-value. (D) Reactome pathway enrichment analysis identification result according to adjusted P-value. (B) ˜ (D) The abscissa indicated the kind of biological pathway enrichment analysis, while the ordinate represented the -log10 (adjusted P-value) of the terms.

Molecular docking of Chrysophanol, Obesity Asthma/Childhood Asthma-Related Targets

Galaxy Visualizer is a web tools that allows easy creation of 3D molecular structures from SMILES by using Molinspiration Galaxy 3D generator. Created molecules may be interactively examined in various display modes, including visualization of various surface properties, such as molecular lipophilicity potential (MLP) and polar surface area (PSA). MDL Molfiles of generated structures may be downloaded for use by in-house programs. We learned from Molinspiration about chrysophanol Wireframe models, tube models, dotted molecular surfaces and space-filling CPK models of visualized Molecules to *Chrysophanol*(**Figures 7A˜C)** , and found the three-dimensional hydrophobicity distribution of chrysophanol (logP of several common drugs is the same (about 2.5), but the three-dimensional hydrophobicity distribution is very different)(**Figures 7D˜G)** .

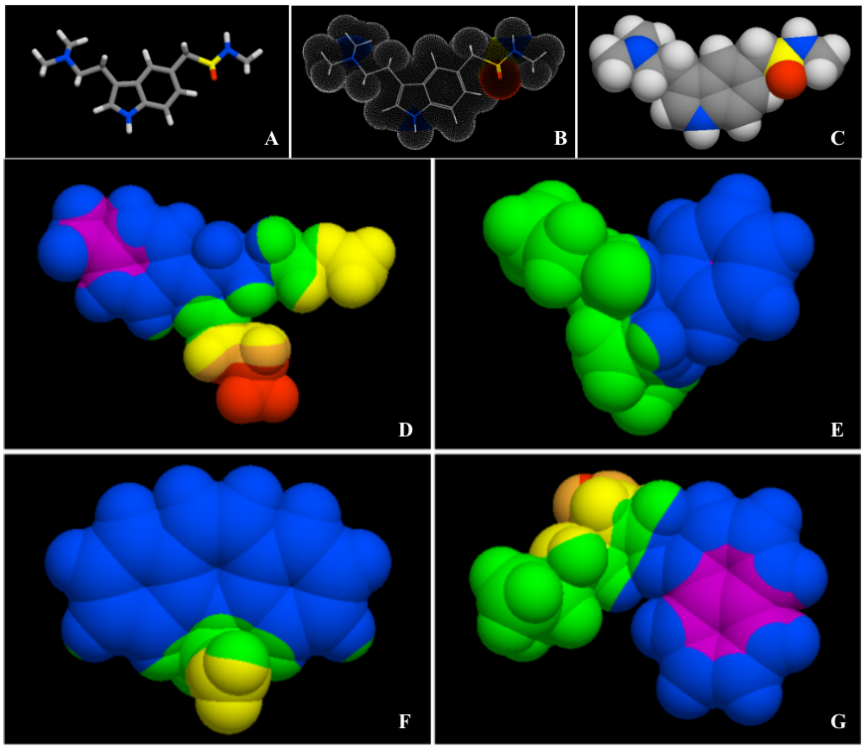


Figure 7

(A)˜(C).Wire-frame models, tube models, dotted molecular surfaces and space-filling CPK models of visualized Molecules to*Chrysophanol.* (D)˜(G). 3D hydrophobicity distribution of *Chrysophanol* (Several common drugs having the same logP (about 2.5) but exhibiting very different 3D hydrophobicity distribution).

After that, 3D structure of *chrysophanol* was determined based on pubchem and Molinspiration. The binding of 3D small molecule structure of *chrysophanol* with 10 target proteins was established through Auto blind docking, and therapeutic effect and binding site of*chrysophanol* were determined. The results showed that*chrysophanol* had good binding activities with the top 10 hub targets and as shown in **Figures 8A˜J** . Among the top 10 hub targets of *chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity

predicted by the PPI network, the results showed that binding activity of *chrysophanol* with MMP9, STAT3, and PTGS2 was the best, and EGFR, HSP90AA1, ESR1, HIF1A, SRC, MTOR, and PIK3CA performed compact binding patterns with*chrysophanol* , but secondary to MMP9, STAT3, and PTGS2 (Table 4 and Table 5) .

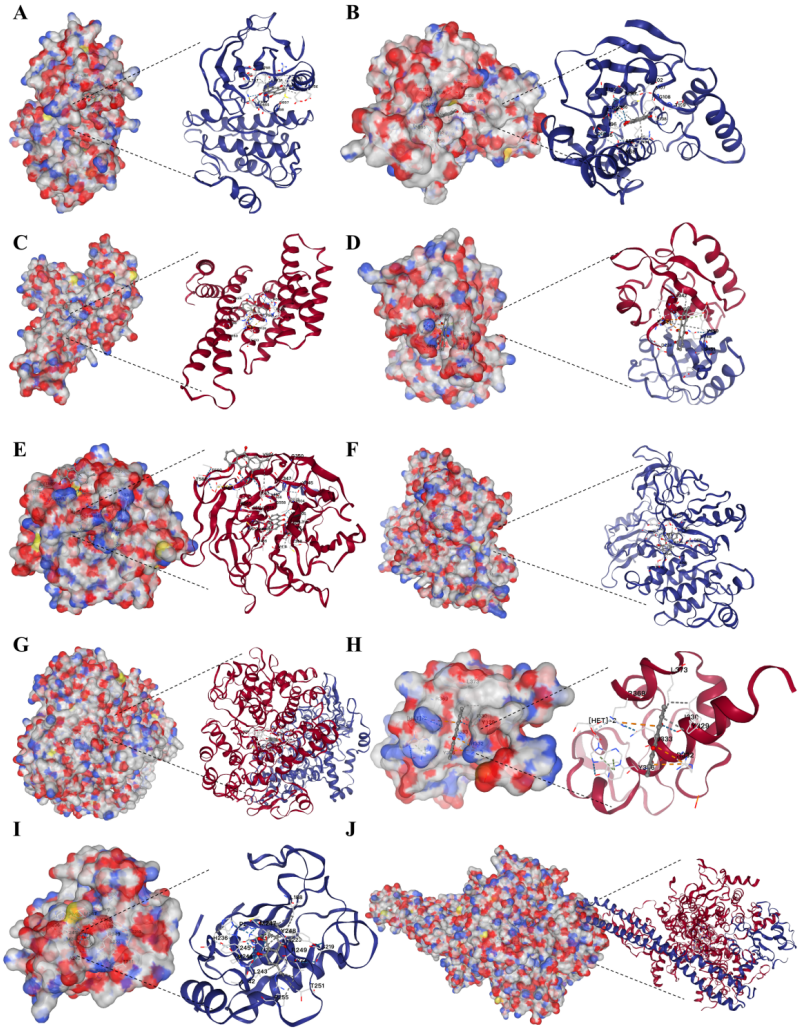


Figure 8

Docking results of small molecules of *chrysophanol* with 10 hub targets. (A) *chrysophanol* /EGFR. Combine pockets mainly in Chain A: LYS745 LEU747 GLU758 ILE759 GLU762 ALA763 MET766 ARG776 LEU777 LEU788 THR790 THR854 ASP855 GLY857 LEU858. (B) *chrysophanol* /HSP90AA1. Combine pockets mainly in Chain A: LEU48 ASN51 SER52 ALA55 LYS58 ASP93 GLY95 ILE96 GLY97 MET98 ASP102 ALA107 GLY108 THR109 PHE138 THR184 VAL186. (C) *chrysophanol* /ESR1. Combine pockets mainly in Chain A: LYS49 GLY53 ARG56 ALA57 ARG60 ARG129 TYR130 GLU133 ASN175 VAL178, Chain B: PHE591 PRO592 ALA593 VAL595. (D) *chrysophanol* /HIF1A. Combine pockets mainly in Chain A: ASP238 LYS240 THR241 TYR340 VAL341 VAL342, Chain B: ASN448 TYR450 SER451 GLU453. (E) *chrysophanol* /STAT3. Combine pockets mainly in Chain X: GLY364 LEU365 ALA366 GLY367 CYS368 ILE416 VAL418 VAL463 GLY464 VAL465 GLY509 ALA510 GLY511 VAL512 CYS513 ALA556 LEU557 GLY558 ILE559

GLY603 VAL604 GLY605 VAL606 ALA607. (F)*chrysophanol* /SRC. Combine pockets mainly in Chain A: LEU273 GLY274 VAL281 ALA293 THR338 GLU339 TYR340 MET341 SER342 GLY344 SER345 ASP348 ALA390 LEU393 ALA403 ASP404. (G) *chrysophanol* /PTGS2. Combine pockets mainly in Chain A: VAL349 LEU352 SER353 TYR355 PHE381 TYR385 TRP387 PHE518 MET522 VAL523 GLY526 ALA527 PHE529 LEU531 LEU534. (H) *chrysophanol* /MTOR. Combine pockets mainly in Chain A: TYR329 ILE330 ARG332 GLN333 TYR336 ARG369 LEU373. (I) *chrysophanol* /MMP9. Combine pockets mainly in Chain A: LEU188 SER219 LEU222 VAL223 HIS226 GLN227 HIS236 ALA242 LEU243 MET244 TYR245 PRO246 MET247 TYR248 ARG249 THR251 PRO255.(J) *chrysophanol* /PIK3CA. Combine pockets mainly in-Chain A: TRP424 PRO449 HIS450 ASN457 ILE459 GLY460 VAL461 THR679 LEU1006 GLY1007 SER1008 GLY1009 MET1010 PRO1011 GLU1012 LEU1013 GLN1014.

Table 4. The PDB ids corresponding to the top 10 target genes and the corresponding compounds of Chrysophanol

Target Gene	**PDB ID**	**R-Value Observed**	**Method and Score**	**Species**	**Corresponding c**
EGFR	8a27	0.126	X-RAY DIFFRACTION 1.07 Å	Homo sapiens	Chrysophanol
HSP90AA1	5J80	0.180	X-RAY DIFFRACTION 1.17 Å	Homo sapiens	
ESR1	7BAA	0.185	X-RAY DIFFRACTION 1.1 Å	Homo sapiens	
HIF1A	4H6J	0.216	X-RAY DIFFRACTION 1.52 Å	Homo sapiens	
STAT3	2FLU	0.180	X-RAY DIFFRACTION 1.5 Å	Homo sapiens	
SRC	1fmk	0.210	X-RAY DIFFRACTION 1.50 Å	Homo sapiens	
PTGS2	5f19	0.172	X-RAY DIFFRACTION 2.04 Å	Homo sapiens	
MTOR	7SOQ	0.176	X-RAY DIFFRACTION 1.15 Å	Homo sapiens	
MMP9	6ESM	0.157	X-RAY DIFFRACTION 1.104 Å	Homo sapiens	
PIK3CA	7PG5	0.199	X-RAY DIFFRACTION 2.2 Å	Homo sapiens	

Table 5. Auto blind docking score of PDB of 10 Hubba gene with Chrysophanol

Gene/Small molecule	CurPocket ID	Detected CurPockets	Vina score	Cavity volume (Å3)	Center (x, y, z)	Docking size (x, y, z)
EGFR	C1	5	-8.6	3691	18, -10, -13	30, 28, 31
HSP90AA1	C1	5	-7.9	561	0, 9, -24	19, 19, 19
ESR1	C1	5	-7.8	887	20, 15, 9	19, 19, 26
HIF1A	C1	5	-7.2	444	24, -5, -15	19, 19, 19
STAT3	C1	5	-9.5	875	18, 17, 8	19, 19, 19
SRC	C2	5	-8	1362	5, -6, 5	29, 19, 19
PTGS2	C2	5	-9.5	4431	14, 49, 65	26, 29, 29
MTOR	C4	5	-6.3	43	12, -5, 10	19, 19, 19
MMP9	C2	5	-9.9	350	1, 48, 23	19, 19, 19
PIK3CA	C1	5	-8.3	40963	-22, -19, 38	35, 35, 35

Discussion

We obtained 144 hub targets for *chrysophanol* against Obesity Asthma/Childhood Asthma comorbidity. GO analysis predicted that chrysophanol may regulate the xenobiotic stimulus, peptidyl-tyrosine phosphorylation, Chrysophanol may regulate the xenobiotic stimulus, Peptidyl-Tyrosine phosphorylation, extracellular matrix disassembly, inflammatory response through the cytoplasm, receptor complex, cytosol, histone deacetylase complex, nucleoplasm, plasma membrane based on endopeptidase activity, transmembrane receptor protein tyrosine kinase activity, ATP binding etc. to perform its therapeutic effects against Obesity Asthma/Childhood Asthma comorbidity. Pathway analysis suggested that Diseases of signal transduction

by growth factor receptors and second messengers, Signal Transduction, Diseases of signal transduction by growth factor receptors and second messengers, signal transduction, Signaling by Receptor Tyrosine Kinases, Interleukin-4 and Interleukin-13 signaling, Collagen degradation, PIP3 activates AKT signaling, Signaling by NOTCH1 and other approaches in *chrysophanol* to combat obesity asthma/childhood asthma comorbidity. Molecular docking demonstrated that chrysophanol had good binding activity to the top 10 genes and proteins related to obesity asthma/childhood asthma comorbidity. Therefore, these findings suggest that *chrysophanol* has good drug similarity and biological activity, and has great potential to be an effective treatment for Obese Asthma/Childhood Asthma complications.

The Ideal Physicochemical Properties and Bioactivity of chrysophanol

It is well accepted that a compound that complies with the Lipinski's rule of five tends to have favorable pharmacokinetic properties and further improves the possibility of evolving into a drug candidate, such as milogP [?]5, MW [?]500 Da, n-ON [?]10, n-OHNH [?]5, and TPSA value of [?]140 Å and n-rotb [?]10, which means the drug has a bigger potential to show good oral bioavailability. To sum up, drug likeness and ADME (including absorption, distribution, metabolism, and excretion) are evaluated according to the following aspects: Lipinski's rule of five, TPSA, and %ABS. The results showed that *chrysophanol* fitted with miLogP = 3.54 < 5, MW = 254.24 < 500 Da, n-ON, number of hydrogen bond acceptors: n-ON = 4 < 10, and number of hydrogen bond donors: n-OHNH = 2 <5, and the value of TPSA at 74.6 2< 140 and %ABS at 83 were at the range of ideal oral bioavailability. These results reveal that *chrysophanol*theoretically would not have caveats about drug likeness and ADME. The above results illustrate that *chrysophanol* is expected to perform pharmacological effects via the five mechanisms listed above with relatively good biological activity scores.

Chrysophanol Might Trigger Hub Targets to Fight Against Obesity Asthma/Childhood Asthma comorbidity

We first obtained 144 common targets of the *chrysophanol* and the Obesity Asthma/Childhood Asthma comorbidity, and 15 targets with degree values greater than 30 in the PPI network were selected as the hub targets. Moreover, we chose the top 10 targets in the PPI network and critical proteins related to Obesity Asthma/Childhood Asthma comorbidity to perform molecular docking with *chrysophanol* . The top 10 target genes included EGFR > HSP90AA1 > ESR1 > HIF1A > STAT3 > SRC > PTGS2 > MTOR > MMP9 > PIK3CA, and the other relatively hub targets comprised KDR, MMP2, HSP90AB1, HDAC1, ACE, and so on.

It has been reported that increasing rates of obesity among young people in the UK are a major cause of high rates of asthma, as well as in Italy and China(Manninen and Latvala et al., 2019).

In obesity-asthma comorbidities, EGFR derived from Adipose tissue macrophages (ATMs) not only plays a pre-inflammatory response, but also promotes the secretion of mucus from the airway mucosa of asthma, leading to airway hyperresponsiveness and wheezing(Jia and Bao et al., 2021; Cao and Pan et al., 2022). Wang et al.(Wang and Chang et al., 2020) performed bisulfite pyrosequencing on three genes associated with selected DMRs (BCL6, HSPG2, and HSP90AA1) by including 13 children with allergic asthma who received allergen specific immunotherapy (SIT), 12 controls with allergic asthma who did not receive SIT, and 12 healthy controls. Compared with allergic asthmatic group, HSPG2 and HSP90AA1 in SIT group were hypermethylated. ESR1, one of the candidate asthma genes, can lead to decreased lung function or airway remodeling(Hur and Broide, 2019). STAT3 is essential for ILC2 effector function and promotes ILC2-driven allergic inflammation in the lung. STAT3 deficiency, inhibition of STAT3 mitochondrial translocation, or blockade of methionine metabolism markedly dampened the ILC2 allergic response and ameliorated allergic lung inflammation(Fu and Zhao et al., 2022).NF-ϰB/HIF-1α and MAPK/STAT1 pathways are highly expressed in OVA-induced asthma and contribute to lung injury mediated by inflammatory cell infiltration(Mo and Deng et al., 2023) mainly focuses on HIF1A signaling pathway, which consistent with the enrichment of signal pathway.Activation of the Src/EGFR pathway in HCDM induced mouse asthma leads to increased IL-4, IL-5, and Mucin5AC(Deng and Zhang et al., 2022). PTGS2, also known as COX-2, is associated with inflammation and is a coding gene related to oxidative phosphorylation in mitochondria. It is involved in iron

death and is the main marker of airway inflammation in asthma(Lv and Xu et al., 2020; Bao and Liu et al., 2023).The imbalance of Th1/Th2 and Th17/Treg is a critical factor in asthma pathogenesis,manipulation of these with signaling molecules such as mTOR, PI3K, Akt, and MyD88 can control asthma(Ma and Athari et al., 2021).The main function of MMp9 is to degrade and restore the dynamic balance of extracellular matris. As one of the gelatin enzymes, MMP-9 binds to CD44 to release stored TGF-β1, which may participate in the remodeling of respiratory tract and lung, such as asthmatic airway remodeling. In addition, OVA-sensitized MMP-2, MMP-8, MMP-9 and MMP-12 in the lung tissue of obese mice were imbalanced, accompanied by high degradation, resulting in excessive deposition of type I and Type III collagen in the lung stroma of obese animals(Vieira and de Oliveira et al., 2020; Huo and Tian et al., 2021).

We found that *Chrysophanol* can efficiently bind to EGFR, HSP90AA1, ESR1, HIF1A, STAT3, SRC, PTGS2, MTOR, MMP9, and PIK3CA, suggesting that *Chrysophanol* may directly target the asthma and Obesity Asthma to perform Anti-inflammation and anti-collagen deposition as well as anti-oxidation function. To summarize, we believe that*Chrysophanol* has the great potential to help increase the treatment effect of present clinical approaches and immunotherapy to treat the lethal Obesity Asthma/Childhood Asthma comorbidity.

The Critical Mechanisms for Chrysophanol to Combat Obesity Asthma/Childhood Asthma comorbidity

The GO results showed that BP was enriched in response to xenobiotic stimulus, peptidyl-tyrosine phosphorylation, extracellular matrix disassembly, protein, phosphorylation, positive regulation of MAP kinase activity, positive regulation of protein kinase B signaling, negative regulation of apoptotic process, positive regulation of cell migration, inflammatory response, collagen catabolic process. It is suggested that*chrysophanol* may interfere with the biological process of Obesity Asthma/Childhood Asthma through these mechanisms. The MF showed that*chrysophanol* mainly through endopeptidase activity, transmembrane receptor protein tyrosine kinase activity, protein tyrosine kinase activity, NAD-dependent histone deacetylase activity (H3-K14 specific), transcription factor binding, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, metalloendopeptidase activity, ATP binding, protein deacetylase activity, histone deacetylase activity etc. to play a therapeutic role for Obesity Asthma/Childhood Asthma comorbidity.

The enrichment of signaling pathway was found to be closely related to Obesity Asthma/Childhood Asthma comorbidity. EGFR tyrosine kinase inhibitor resistance is mainly associated with asthma-related airway inflammation that is steroid insensitive. One of the characteristics of obese asthma is steroid insensitive, leading to wheezing that is difficult to control(Pan and Hsiao et al., 2019). Endocrine regulation of the airway associated preganglionic nerve may contribute to airway hyperreactivity(AHR)in obese states(Leiria and Martins et al., 2015). Obesity asthma is characterized by low immunoinflammatory Th2 tendency, and is mainly characterized by neutrophil infiltration(Telenga and Tideman et al., 2012; Hammad and Lambrecht, 2021; Yamasaki and Okazaki et al., 2022). Neutrophil extracellular traps (NETs) mediate the activation of inflammasome and IL-1β secretion by monocytes, causing damage to airway epithelial cells and activation of neutrophils leading to severe asthma(Lachowicz-Scroggins and Dunican et al., 2019), NETs and HIFA signaling pathways work together to induce neutrophil infiltration(Burczyk and Cichon et al., 2022), which has been shown to be a major inflammatory state in obesity-related asthma(Hammad and Lambrecht, 2021). In addition, the Interleukin-4 and Interleukin-13 signalin and Notch pathways are also key signaling pathways for Childhood asthma attacks, mediating airway inflammation and airway hyperresponsiveness(Jiang and Xiao et al., 2017; Conde and Bertrand et al., 2021; Iosifidis and Sutanto et al., 2021). The results of systematic pharmacological studies show that *chrysophanol* can control airway inflammation and airway hyperresponsiveness and airway remodeling of asthma and obese asthma by intervening the above signaling pathways, which is expected to be a new drug for the treatment of Obesity Asthma/Childhood Asthma comorbidities.

Calcium signaling pathway

Chrysophanol can reduce inflammatory response, interfere with cell migration and apoptosis, and help to

alleviate the transformation of airway epithelium and produce airway hyperresponsiveness in asthma. It can promote collagen catabolism, extracellular matrix decomposition and reduce airway remodeling. In additionIn addition, it can reduce the production of neutrophil trapping net, improve oxidative stress response and endocrine and metabolic disorders. In Obesity Asthma, the immune inflammatory response is mainly based on neutrophil infiltration. In conclusion, *chrysophanol* has the effects of anti-airway hyperresponsiveness, anti-airway remodeling and anti-allergen stimulation in the treatment of Obese Asthma/Childhood Asthma, which are the pathological basis of recurrent attacks of Obese Asthma/Childhood Asthma.

Conclusions

In conclusion, the results of systematic pharmacological and bioinformatic analyses highlight that antiviral, anti-inflammatory, antioxidant, immunomodulatory, and anti-collagen generation are key targets/pathways of rhubarb against Obesity Asthma and Childhood Asthma comorbidities. In addition, *chrysophanol* has good physical and chemical properties and biological activities, and can be used in the clinical treatment of Obesity Asthma and Childhood Asthma comorbidities according to the predicted biological process and pharmacological mechanism. In addition, the potential and key pharmacological targets of*chrysophanol* against Obesity Asthma /Childhood Asthma comorbidities provide directions for further research, but the predicted results need to be rigorously verified. In subsequent studies, we intend to use adipose cells in conjunction with airway and bronchial epithelial cells to simulate a state of Obesity Asthma and Childhood Asthma comorbidities. In addition, we will further analyze the mechanism of*chrysophanol* against Obesity Asthma/Childhood Asthma comorbidities through proteomics, genomics, metabolomics and proteomics.

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Conflict of interest

We declare that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

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