JOURNAL OF
EMERGING INVESTIGATORS

Article

Virtual Screening of *Cutibacterium acnes* Antibacterial Agent Using Natural Compounds Database

Chi-Hao Liu[1], Hao-Chun Fan[2]

[1]Taipei Wego Private Senior High School, [2]Graduate Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan

SUMMARY

Acne vulgaris is an immune response leading to the dermatological disease generally known as acne caused by Cutibacterium acnes. It is estimated to affect 9.4% of the global population, and nearly 8 in 10 teens have acne. Acne manifests itself through inflammation of hair follicles roots, skin red-ness, and formation of pimples. Treatments to remove acne scars are often money and time con-suming. Therefore, prevention of acne is beneficial beyond pure cosmetics. Acne symptoms can be alleviated if growth of C. acnes is inhibited; however, more in-depth research is needed for cosmetic care products regarding acne control. We applied reverse pharmacology methods to identify natural small molecule extracts with high affinity to important growth factors in C. acnes, to be employed as anti-bacterial agents in cosmetic or skincare products. With absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction we showed that out of the 2234 natural extracts ana-lyzed, 5 small molecules had low binding energy. These five molecules were further analyzed. Through structural analysis and with reference to prior research, we concluded that these small mol-ecules have the potential to be supplemented in skincare products for acne control.

INTRODUCTION

Acne vulgaris is a type of skin disease commonly caused by the bacterium Cutibacterium acnes, which results in a series of immune reactions that lead to inflammation of the roots of hair follicles, skin redness, and even pimples (1). Acne is one of the most troublesome diagnoses for many adolescent teens, with an estimated 650 million people around the world affected (2). *C. acnes* is an anaerobic, rod-shaped, Gram-positive bacterium that colonizes around the sebaceous glands, an exocrine gland in the skin, and utilizes the lipid secretions of these glands as its primary nutrient source (3). Colonization by the bacterium will eventually cause blockage of the pores which contin-ue to accumulate at skin surface, enlarging the hair follicle and aid the growth of C. acnes (4). The metabolites secreted by *C. acnes* trigger an immune response and activate toll-like receptors, leading to inflammation, redness, and pimple growth (5, 6). People often resort to over-the-counter topical ointments to treat the mild inflammation instead of oral drugs, which is the reason ointments for pimple treatment are one of the best-selling skin care products.

The current treatments for *C. acnes* in skin care products can be separated into two catego-ries, over-the-counter (OTC) drugs and cosmetics. OTC drugs such as tretinoin, benzoyl peroxide, sulfur and salicylic acid are commonly added to skin care ointment (7). Cosmetics often use ethyl lactate, phytosphingosine, nicotinamide or resveratrol to act as the antibacterial reagents (8). Also, plant crude extracts, such as those from cinchona bark or sage, can be added into cosmetics. Plant extracts have proven to be effective antibacterials, but the specific compounds responsible for the effect have not been thoroughly researched to be further utilized as clinical drugs.

The traditional method for pharmaceutical drug development involves testing compounds in animal models or cell cultures for a desired therapeutic effect. However, in reverse pharmacology, a cellular pathway of interest is first identified that could be modified to bring about therapeutic bene-fits (9). In the case of *C. acnes*, targeting an indispensable biosynthesis pathway could provide the desired antibacterial activity. After one of its key enzymes is selected as the specific target, small molecules are docked computationally into the key enzyme's binding pocket to simulate the binding energy. The small molecules with the highest computed affinity (low binding energy and score) are then selected as lead compounds to be further tested for enzyme kinetics and in cell- and animal-based testing. This study describes a virtual screen conducted using a database of plant extracts to identify potential compounds for further development of drugs or skin care products for the treatment of *C. acnes*.

For this study, we chose to target lipid biosynthetic pathways in *C. acnes* because lipid me-tabolism is vital for growth of the bacteria (10). These pathways have often been chosen to be the simulation targets by many published studies for other bacteria (11-13). Bacteria possess two lipid synthesis pathways, Fatty Acid Synthesis I (FAS I) and Fatty Acid Synthesis II (FAS II) (14), and *C. acnes* uses the FAS II pathway. Studies have shown that the interruption of FAS II complex formation is effective for the inhibition of bacterial growth (15, 16). Additionally, beta-ketoacyl-acyl-carrier-protein synthase III (KAS III), part of the FAS II complex, has no homolog in humans, mak-ing it an ideal target for the disruption of bacterial lipid metabolism without affecting human lipid synthesis. We thus selected KAS III as the target

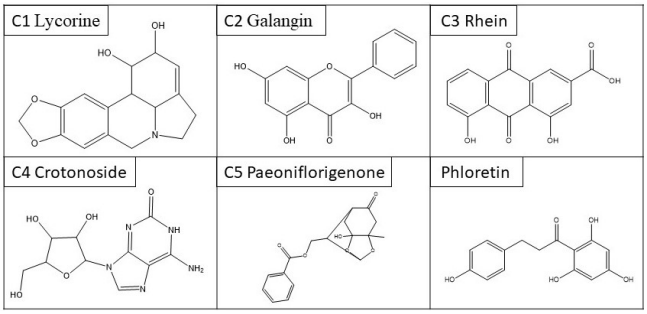

Figure 1: The compound structures of C1-5 and phloretin.

Table 1: Docking Results of compounds with affinity score -9 and higher.

No.	TDEC number	Score	Common names	CAS Number
C1	19CA001722	-10	Lycorine	476-28-8
C2	19CA001765	-9.6	Galangin	548-83-4
C3	19CA001417	-9.5	Rhein	478-43-3
C4	19CA001645	-9.4	Crotonoside	1818-71-9
C5	18CA000840	-9.3	Paeoniflorig-enone	80454-42-8
C6	19CA001486	-9.3	Catalpol	2415-24-9
C7	19CA001491	-9.3	Chrysin	480-40-0
C8	19CA001650	-9.3	Pinocembrin	480-39-7
C9	19CN000683	-9.3	Brazilein	600-76-0
C10	18CN001541	-9.2	Sinularioper-oxide B	NA
C11	19CA001543	-9.2	Wogonin	632-85-9
C12	20CN000243	-9.2	Phaitanthrin A	NA
C13	19CA001418	-9.1	Aloe emodin	481-72-1
C14	19CA001595	-9.1	Ellagic acid	476-66-4
C15	20CN000053	-9.1	Cynandione A	168706-29-4
C16	19CA001017	-9	Emodin	518-82-1
C17	19CA0[1]01508	-9	Alizarin	72-48-0

NA = no CAS Number available.

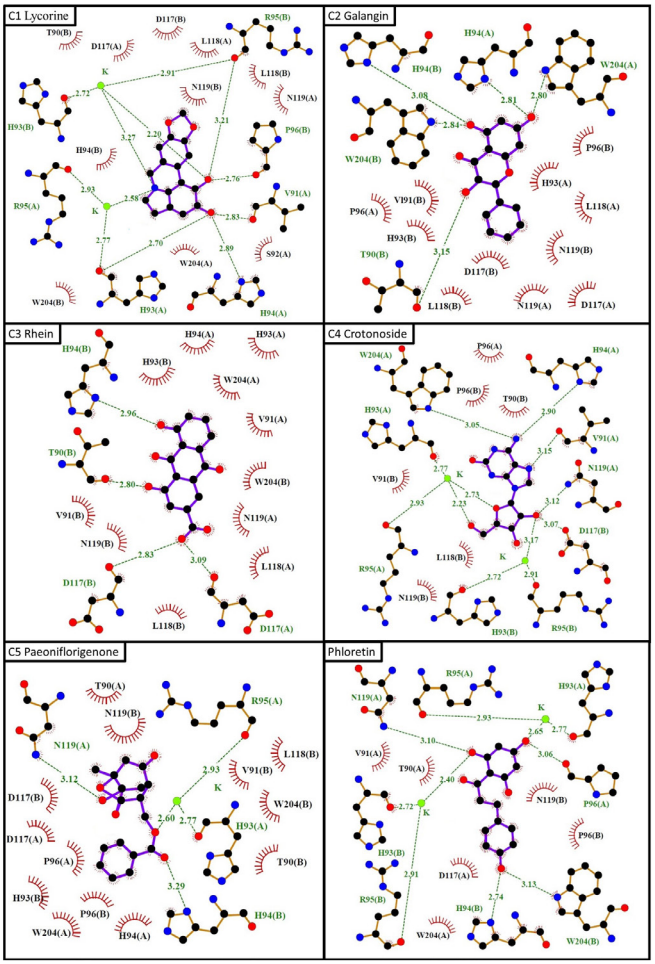

Figure 2. The 2D interaction of C1-5 and phloretin binding to KAS III. Green lines refer to hy-drogen bonds (Å); red dot refers to Oxygen; black dot refers to Carbon; blue dot refers **to** Nitrogen; red eyelashes represent hydrophobic contacts; alphabet in brackets refers to A or B chains of KAS III.

of our screen given that it is unlikely to impact humans, hypothesizing that inhibitory binding by small molecules would lead to FAS II disruption and inhibition of bacterial growth.

For this research, a new pharmaceutical resource integration website was implemented called Taiwan Database of Extracts and Compounds (TDEC). Here, extracts, pure compounds and deriva-tives of Chinese medicines, oceanic organisms and microorganisms were collected and categorized. We used the database of pure compounds from Chinese medicine and their molecular structures to simulate small molecule binding to KAS III in the hopes to find a natural treatment to inhibit the growth of *C. acnes*.

RESULTS

We used a reverse pharmacology approach to look for promising compounds for the reduc-tion of C. acnes bacterial growth. To simulate the docking and binding of our target protein KASIII to different compounds, we downloaded the molecular structure data files of 2234 micromolecular

structures from the TDEC website. We used Autodock vina software to simulate the docking to the active site of KAS III (PDB Accession: 6A9N). We selected compounds with an affinity score under -9, indicating lower binding energy and stronger interaction force with our target protein, for absorption, distribution, metabolism, excretion, and toxicity (ADMET) simulations. Compounds that met Lipinski's rule of five, which is 1) Molecular mass < 500 Daltons, 2) High lipophilicity (expressed as LogP < 5), 3) Less than five hydrogen bond donors, 4) Less than 10 hydrogen bond acceptors, and 5) Molar refractivity between 40-130, and with no toxicity were tabulated (Table 1). We chose the score of -9 as the cutoff because there were too many candidates that shared the same score of -8.9. The top five candidates with the highest affinity scores were, from highest to lowest affinity, lycorine, galangin, rhein, crotonoside, and paeoniflorigenone (Table 1, Figure 1). Their structures and binding with KASIII are also shown (Figure 2, 3).

KAS III functions as a dimer with two identical A and B chains and requires potassium ions for enzyme activity. Upon analysis of the ligand-protein structure (Figures 2, 3), A chain's V91, H93, H94, R95, D117, N119, S191 and W204, and B chain's T90, H93, H94, R95, P96, D117 and W204 (green lines in Figure 2) interact with C1-5. Suggesting that the ligand which interacts with H93(A), H94(A), R95(A), H94(B) scored lower on the binding energy scale. C1, C4, and C5 all in-teracted with the potassium ions; however, not all candidates needed potassium ions to be present to score higher on the docking scale.

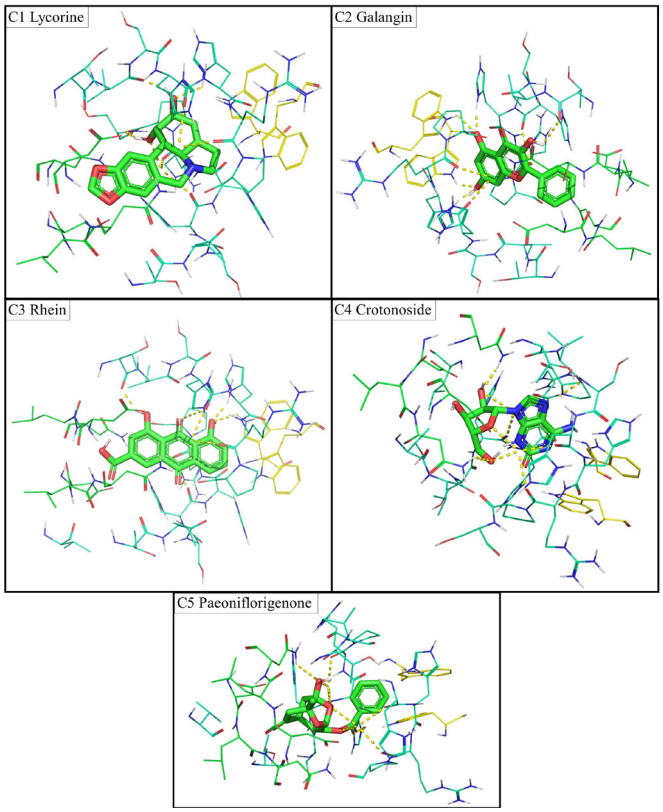

Figure 3. The 3D interaction figures of C1-5 and phloretin binding to KAS III. Red refers to Oxy-gen; Green refers to Carbon; Blue dot refers to Nitrogen.

DISCUSSION

Our study successfully simulated the interaction of different compounds with our target pro-tein KAS III by using the TDEC platform's data on molecular structure. Five candidates were selected for further analysis, which were shown to possess no toxic effects or could be used as drugs by literature review. In reference to previous research on the effects of phloretin, which has been de-scribed to inhibit KAS III function, we found that our candidates' binding scores were much higher than the simulated docking score of phloretin. Therefore, it is reasonable to suppose that the molecules we have selected may perform better in the inhibition of KAS III. Interestingly, lycorine, galangin and rhein have all been reported to have antibacterial effects (17-19). Lycorine (C1) is an alkaloid extract from Lycoris radiata, the Chinese medicinal plant in the Amaryllidaceae family (20). The compound has shown a therapeutic effect in cancer treatment as well as antibacterial activity (21). Galangin (C2) is a polyphenolic compound that can be found in different medicinal herbs, for instance, *Alpinia officinarum* Hance, *Alnus pendula* Matsum, and *Plantago major* (22). Galangin has been studied as a therapy for colitis management (23) and also shows antibacterial activity (24). Rhein (C3) is an anthraquinone group substance obtained from *Rheum*, also known as cassic acid (25). Rhein also has antibacterial activity (26) and other pharma-cological activities, including hepatoprotective, nephroprotective, and anti-inflammatory activities (25). Crotonoside (C4) is a guanosine analog that is isolated from *Croton tiglium* (27). Crotonoside has potentially immunotherapeutic effects for the treatment of arthritic symptoms (27), but no anti-bacterial activity has been previously reported. Paeoniflorigenone (C5) is a monoterpene isolated from the root of *Moutan cortex* (28) that shows strong bioactivity and may induce apoptosis selectively in cancer cell lines (29).

One of the key advantages of molecular simulation is that we can use a database of com-pounds already approved by the FDA or other regulators, making it much easier for the potential drugs to be repurposed after clinical trials (30). This is particularly time saving and prevents the waste of resources. Using natural extracts within the database can yield similar results. The current regulations are more lenient towards plant extracts in skin care and cosmetic products, so if suitable compounds with treatment efficacy are found, plants with high content of said compound can be further tested for toxicity. If there is no observable toxicity, these extracts can be quickly added to skin care products ready for the market.

Phloretin, which has previously been shown to inhibit KAS III (31), only scored a -8.4 on binding energy, comparatively

weaker than our top 5 candidates. This suggests that our candidates might inhibit KAS III more potently than phloretin. However, we would suggest analyzing the in-hibitory effect of the 5 compounds in in vitro studies to show their antibacterial activity in future, which needs to be further explored in a laboratory setting. Since the database we used only includes natural derivatives, we could apply plant-based extracts containing candidate molecules to further expand our antibacterial testing, with the intention for them to be added to skin care products as an acne prevention and treatment additive. In conclusion, this experiment provides a great basis of in-formation that can inform future product development and serve as a reference for antibacterial experiments.

MATERIALS AND METHODS

Files and Software

The SDF files of the ligands were obtained from Taiwan Database of Extracts and Com-pounds (https://tdec.kmu.edu.tw/index.aspx), and the Protein 3D structure files were obtained from PDB (www.rcsb.org). Docking simulations were done in the following platforms: AutoDocksTools 1.5.6 and PyRx 0.9.x. ADMET analysis were done using SwissADME (https://www.swissadme.ch). Finally, the 2D and 3D figures were made using LigPlot+ 2.2 and Pymol 2.4 to conclude the study.

Molecular Docking

PyRx's open label panel was used to convert the SDF files of the ligand into pdbqt files. Au-toDockTools were used to remove water and add hydrogens to the protein and grid them into pdbqt files. Every file was placed in PyRx's autodock vina function to complete docking. Docking was set centralized to (25, 25, 25), dimension=10, and extensive=10. Models that had binding energy less than -9 were selected for further analysis.

ADMET Prediction

SwissADME (http://www.swissadme.ch/) was used to verify whether the candidates met Lipinski's rule of five. Those that did not meet it were eliminated. Lipinski's rule of five is defined as [1] partition coefficient log P in −0.4 to +5.6 range; [2] molar refractivity from 40 to 130; [3] mo-lecular weight from 180 to 480; and [4] number of atoms from 20 to 70 (32).

ACKNOWLEDGMENTS

Special thanks to the TDEC database for providing us the structures of the micromolecules tested, and the free license of LigPlot+ by EMBL-EBI. Also, thanks to NOAH education, Taiwan has provided us a place to finish this project.

Received: April 17, 2021
Accepted: June 21, 2021
Published: March 20, 2022

REFERENCES

1. Platsidaki, Eftychia, *et al.* "Recent advances in understanding Propionibacterium acnes (Cutibac-terium acnes) in acne." F1000Research, vol. 7, no. 1953, 2018, doi: 10.12688/f1000research.15659.1.
2. Blaskovich, Mark A., *et al.* "In Vitro Antimicrobial Activity of Acne Drugs against Skin-Associated Bacteria." Scientific Reports, vol. 9, no. 1, 2019, doi:10.1038/s41598-019-50746-4.
3. Tuchayi, Sara Moradi, *et al.* "Acne vulgaris." Nature Reviews Disease Primers, vol. 1, no. 15029, 2015, doi:10.1038/nrdp.2015.29.
4. Ju, Qiang, *et al.* "Sex hormones and acne." Clinics in Dermatology, vol. 35, no. 2, 2017, pp. 130-137, doi: 10.1016/j.clindermatol.2016.10.004.
5. Kim, Jenny, *et al.* "Activation of toll-like receptor 2 in acne triggers inflammatory cytokine re-sponses" The Journal of Immunology, vol. 169, no. 3, 2002, doi: 10.4049/jimmunol.169.3.1535.
6. Jugeau, S., *et al.* "Induction of toll-like receptors by Propionibacterium acnes" British Journal of Dermatology, vol. 153, no. 6, 2005, pp. 1105 1113, doi:10.1111/j.1365-2133.2005.06933.x.
7. Gollnick, Harald P.M., *et al.* "Topical Treatment in Acne: Current Status and Future Aspects" Dermatology, vol. 206, no. 1, 2003, pp. 29-36, doi: 10.1159/000067820.
8. Dall'oglio F., *et al.* " Cosmetics for acne: indications and recommendations for an evidence-based approach" G Ital Dermatol Venereol, vol. 150, no. 1, 2015, pp. 1-11.
9. Vaidya, Ashok D.B. "Reverse Pharmacology-A Paradigm Shift for Drug Discovery and Devel-opment." Current Research in Drug Discovery, vol. 1, no. 2, 2014, pp. 39-44, doi: 10.3844/crddsp.2014.39.44.
10. Heath, Richard J., *et al.* "Lipid biosynthesis as a target for antibacterial agents." Progress in Lipid Research, vol. 40, no. 6, 2001, pp. 467-497, doi: 10.1016/S0163-7827(01)00012-1.
11. Wang, Xiao-Liang, *et al.* "Design, synthesis and antibacterial activities of vanillic acylhydrazone derivatives as potential β-ketoacyl-acyl carrier protein synthase III (FabH) inhibitors." Europe-an Journal of Medicinal Chemistry, vol. 57, 2012, pp. 373-382, doi: 10.1016/j.ejmech.2012.09.009.
12. Sabbagh, Ghalia, *et al.* "Docking studies of flavonoid compounds as inhibitors of β-ketoacyl acyl carrier protein synthase I (Kas I) of Escherichia coli." Journal of Molecular Graphics and Modelling, vol. 61, 2015, pp. 214-223, doi: 10.1016/j.jmgm.2015.07.005.
13. Wallace, Joselynn, *et al.* "Discovery of Bacterial Fatty Acid Synthase Type II Inhibitors Using a Novel Cellular Bioluminescent Reporter Assay." Antimicrobial Agents and Chemotherapy, vol. 59, no. 9, 2015, pp. 5575-5587, doi: 10.1128/AAC.00686-15.
14. Yao, Jiangwei, *et al.* "Exogenous Fatty Acid Metabolism in Bacteria." Biochimie, vol. 141, 2017, pp. 30-39, doi:

10.1016/j.biochi.2017.06.015.
15. Duan, Xiangke, *et al.* "Crucial components of mycobacterium type II fatty acid biosynthesis (Fas-II) and their inhibitors." FEMS Microbiology Letters, vol. 360, no. 2, 2014, pp. 87-89, doi: 10.1111/1574-6968.12597.
16. Zhang, Yong-Mei, *et al.* "Inhibiting Bacterial Fatty Acid Synthesis." Journal of Biological Chem-istry, vol. 281, no. 26, 2006, pp. 17541-17544, doi: 10.1074/jbc.R600004200.
17. Bendaif, H., *et al.* "Antibacterial activity and virtual screening by molecular docking of lycorine from Pancratium foetidum Pom (Moroccan endemic Amaryllidaceae)." Microbial Pathogenesis, vol. 115, 2018, pp. 138-145, doi: 10.1016/j.micpath.2017.12.037.
18. Cushnie, T. P. T., *et al.* "Assessment of the antibacterial activity of galangin against 4-quinolone resistant strains of Staphylococcus aureus." Phytomedicine, vol. 13, no. 3, 2005, pp. 187-191, doi: 10.1016/j.phymed.2004.07.003.
19. Azelmat, Jabrane, *et al.* "The anthraquinone rhein exhibits synergistic antibacterial activity in as-sociation with metronidazole or natural compounds and attenuates virulence gene expression in Porphyromonas gingivalis." Archives of Oral Biology, vol. 60, no. 2, 2015, pp. 342-346, doi: 10.1016/j.archoralbio.2014.11.006.
20. Roy, Mridul, *et al.* "Lycorine: A prospective natural lead for anticancer drug discovery." Bio-medicine & Pharmacotherapy, vol. 107, 2018, pp. 615-624, doi: 10.1016/j.biopha.2018.07.147.
21. Bendaif, H., *et al.* "Antibacterial activity and virtual screening by molecular docking of lycorine from Pancratium foetidum Pom (Moroccan endemic Amaryllidaceae)." Microbial Pathogenesis, vol. 115, 2018, pp. 138-145, doi: 10.1016/j.micpath.2017.12.037.
22. Fang, Dengyang, *et al.* "Chemopreventive mechanisms of galangin against hepatocellular carci-noma: A review." Biomedicine & Pharmacotherapy, vol. 109, 2019, pp. 2054-2061, doi: 10.1016/j.biopha.2018.09.154.
23. Gerges, Samar H., *et al.* "The natural flavonoid galangin ameliorates dextran sulphate sodium–induced ulcerative colitis in mice: Effect on Toll-like receptor 4, inflammation and oxidative stress." Basic & Clinical Pharmacology & Toxicology, vol. 127, no.1, pp. 10-20, doi: 10.1111/bcpt.13388.
24. Cushnie, T.P.T., *et al.* "Assessment of the antibacterial activity of galangin against 4-quinolone resistant strains of Staphylococcus aureus." Phytomedicine, vol. 13, no. 3, pp. 187-191, doi: 10.1016/j.phymed.2004.07.003.
25. Zhou, Yan-Xi, *et al.* "Rhein: A Review of Pharmacological Activities." Evidence-Based Comple-mentary and Alternative Medicine, vol. 2015, pp. 578107, 2015, doi: 10.1155/2015/578107.
26. Yu, Lu, *et al.* "Global transcriptional response of Staphylococcus aureus to Rhein, a Natural Plant Product." Journal of Biotechnology, vol. 135, no. 3, pp. 304-308, 2008, doi: 10.1016/j.jbiotec.2008.04.010.
27. Lin, Shih-Chao, *et al.* "Alleviation of Collagen-Induced Arthritis by Crotonoside through Modu-lation of Dendritic Cell Differentiation and Activation." Plants-Basel, vol. 9, no. 11, pp. 1535, 2020, doi: 10.3390/plants9111535.
28. Huang, Ying, *et al.* "Apoptosis-inducing activity and antiproliferative effect of Paeoniflorigenone from moutan cortex." Biochemistry & Molecular Biology, vol. 81, no. 6, pp. 1106-1113, 2017, doi: 10.1080/09168451.
29. Huang, Ying, *et al.* "Apoptosis-inducing activity and antiproliferative effect of Paeoniflorigenone from moutan cortex." Bioscience, Biotechnology, and Biochemistry, vol. 81, no. 6, pp. 1106-1113, doi: 10.1080/09168451.
30. Pushpakom, Sudeep, *et al.* "Drug repurposing: progress, challenges and recommendations." Na-ture Reviews Drug Discovery, vol. 18, 2019, pp. 41-58, doi: 10.1038/nrd.2018.168.
31. Cheon, Dasom, *et al.* "Target Proteins of Phloretin for Its Anti-Inflammatory and Antibacterial Activities Against Propionibacterium acnes-Induced Skin Infection." Molecules, vol. 24, no. 7, 2019, pp. 1319, doi: 10.3390/molecules24071319.
32. Ghose, Arup K., *et al.* "A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery. 1. A Qualitative and Quantitative Characterization of Known Drug Databases." Journal of Combinatorial Chemistry, vol. 1, no. 1, pp. 55-68, 1999, doi: 10.1021/cc9800071.