UNIVERSITI PUTRA MALAYSIA

REPURPOSING OF FDA-APPROVED DRUGS AS NEW TREATMENT FOR SKIN INFLAMMATORY DISEASES TARGETING TNF-α AND IL-1α

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ABSTRACT

REPURPOSING OF FDA-APPROVED DRUGS AS NEW TREATMENT FOR SKIN INFLAMMATORY DISEASES TARGETING TNF-α AND IL-1α

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Introduction: Frequent use of topical corticosteroid as the primary treatment in skin inflammatory diseases remained insufficient due to relapse and regression in most of the treatment as well as the adverse effect on systemic level. TNF-α and IL-1α are examples of inflammatory cytokines that play important role as therapeutic targets for skin inflammatory conditions. **Objective:** *In silico* technique approaches were implemented to identify alternative treatment for skin inflammatory diseases via drug repurposing of existing FDA-approved drugs by targeting the TNF-α and IL-1α. **Methodology:** A total of 29 drugs that are frequently prescribed for treatment of skin diseases was chosen and classified into two different groups which are biologic and non-biologic drugs. A pharmacophore model for both drug groups was constructed via LigandScout 4.4.7. Next, a database of FDA-approved drugs obtained from DrugBank was subjected to virtual screening employing the previously constructed pharmacophore model and LigandScout 4.4.7 to yield possible hit compounds as drug candidates. Subsequently, molecular docking analysis using AutoDock Vina tool of the hit compounds against TNF-α and IL-1α was performed to determine the binding affinity of each hit compound with the proteins**. Results:** Docking results showed that the top binding affinity scores were exhibited by sulfasalazine and meclocycline to both TNF-α (at -8.3 kcal/mol and -7.6 kcal/mol respectively) and IL-1α (at -8.1 kcal/mol and -7.1 kcal/mol respectively). A number of strong, moderate and weak hydrogen bindings, hydrophobic interactions and pi-pi stacking were identified between the drugs and the proteins by using the visualizers. **Discussion:** Sulfasalazine and meclocycline expressed high pharmacophore fit scores which resulted in high binding affinity scores for docking analysis. These two compounds exhibited similar pharmacophore features as well as the immunological targets with the common skin inflammatory drugs that can target TNF-α and IL-1α. **Conclusion:** The study highlights the potential of in silico drug repurposing techniques to produce FDA-approved drug candidates as alternative treatment for skin inflammatory diseases.

Keywords: Skin inflammatory diseases; *in silico* drug repurposing; TNF-α; IL-1α

ABSTRAK

PENGGUNAAN SEMULA DRUG YANG DILULUSKAN FDA SEBAGAI RAWATAN BAHARU PENYAKIT RADANG KULIT MENSASARKAN TNF-α DAN IL-1α

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Pengenalan: Penggunaan kortikosteroid topikal yang kerap sebagai rawatan utama dalam penyakit radang kulit kekal tidak mencukupi kerana kambuh dan regresi dalam kebanyakan rawatan serta kesan buruk pada tahap sistemik. TNF-α dan IL-1α adalah contoh sitokin yang memainkan peranan penting sebagai sasaran terapeutik untuk penyakit keradangan kulit. **Objektif:** Pendekatan teknik *'in silico*' telah dilaksanakan untuk mengenal pasti rawatan alternatif untuk penyakit radang kulit melalui penggunaan semula ubat-ubatan sedia ada yang diluluskan oleh FDA dengan menyasarkan TNF-α dan IL-1α. **Metodologi:** 29 ubat yang kerap dipreskripsi untuk rawatan penyakit kulit telah dipilih dan dibahagi kepada dua kumpulan berbeza iaitu 'biologike' dan 'bukan-biologike'. Model farmakofor untuk kedua-dua kumpulan ubat telah dibina melalui LigandScout 4.4.7. Seterusnya, pangkalan data ubat-ubatan yang diluluskan oleh FDA diperoleh daripada DrugBank digunakan untk melakukan proses saringan secara maya bagi mendapatkan senarai calon drug yang menepati struktur farmakofor model telah dibina melalui aplikasi LigandScout 4.4.7. Analisis 'docking' molekular menggunakan perisian AutoDock Vina telah dijalankan antara calon dadah dan TNF-α serta IL-1α untuk menentukan interaksi di tapak ikatan dan afiniti ikatan. **Keputusan:** Keputusan 'docking' menunjukan sulfasalazine dan meclocycline terikat pada ikatan afiniti yang tertinggi untuk TNF-α (-8.3 kcal/mol dan -7.6 kcal/mol) dan IL-1α (-8.1 kcal/mol dan -7.1 kcal/mol). Analisis selanjutnya menemukan sebilangan ikatan hidrogen yang kuat, sederhana dan lemah, interaksi hidrofobik dan susunan pi-pi antara ubat dan protein menggunakan alat pengimejan. **Perbincangan**: Sulfasalazine dan meclocycline menunjukkan skor farmakofor yang tinggi seterusnya menghasilkan afiniti ikatan yang tinggi bagi 'docking' analisis. Kedua-dua ubatan mempamerkan ciri farmakofor yang serupa serta sasaran imunologi yang sama dengan drug radang kulit iaitu TNF-α dan IL-1α. **Kesimpulan**: Kajian telah membuktikan potensi teknik penggunaan semula ubat *in silico* untuk menghasilkan calon drug yang diluluskan oleh FDA sebagai rawatan alternatif untuk penyakit radang kulit.

Kata kunci: Penyakit radang kulit; *in silico* penggunaan semula drug; TNF-α; IL-1α

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LIST OF ABBREVIATIONS

DALY	Disability-adjusted life years
DAMP	Damage-Associated Molecular Pattern
ELISA	Enzyme-linked Immunorbent Assay
EMA	European Medicines Agency
FDA	Food and Drug Administration
GBD	Global Burden of Disease
IFN	Interferons
IL-	Interleukin
IL-1R	Interleukin-1 Receptor
IL-6R	Interleukin-6 Receptor
IL-1Ra	Interleukin-1Receptor Agonist
MAPK	Mitogen Activated Protein Kinase
NF-κB pathways	Nuclear Factor Kappa-B
PAMP	Pathogen-Associated Molecular Pattern
PDB	Protein Data Bank
PRR	Pattern-Recognition Receptor
TCS	Topical Corticosteroid
TNF-α	Tumor Necrosis Factor Receptor- alpha
TLR	Toll-Like Receptor
TNF-R1	Tumor Necrosis Factor Receptor Type 1
TNF-R2	Tumor Necrosis Factor Receptor Type 2

CHAPTER ONE

INTRODUCTION

1.1 Background Of Study

Skin disease is widely prevalent throughout Asia countries including Malaysia. Skin disease eg. psoriasis is common, and new treatments such as biologics are costly. Financial constraints can arise because of the high prevalence of skin diseases and their treatment expenses. This is expected to have a negative impact on the average lifespan of the global population. The burden of a patient's illness often extends beyond the patient themselves to include family members, friends, and other members of society (Flohr & Hay, 2021). In parallel to all of the medications recently approved for systemic treatment in a minority of patients suffering from atopic dermatitis or psoriasis, there is an even larger demand in dermatology for new 'non-immunosuppressive' and anti-inflammatory therapies (Abels & Soeberdt, 2019). This is due to frequent application of topical corticosteroid as the primary treatment in skin inflammatory diseases remained insufficient to alleviate the effect. Relapse and regression in most of the treatment as well as the adverse effect on systemic level give rise to major drawbacks in the current treatment (Jain et al., 2021).

According to Goh et al. (2018), topical corticosteroids and emollients remain the mainstay in management of atopic dermatitis in Malaysia. The use of topical corticosteroid such as hydrocortisone (47.1%) is higher compared to emollients like calamine ointment (16.7%). This may be associated with the immediate and noticeable effect on an atopic dermatitis patient's rash, often leading to its complete disappearance in as little as one or two days. However, the repercussions of persistent application of topical corticosteroids (TCS) often lead to a condition namely as tachyphylaxis, whereas the effectiveness of a drug is reduced gradually after a certain time because the skin has developed resistance against the drug. Hence, resulting in increasing the potency of TCS as it is tempting due to the effectiveness begins to decline. Despite that, it must be kept in mind, the potency of TCS varies and the risk of adverse effects would significantly increase as we increase the dose. The local and systemic effects of TCS include skin thinning, permanent stretch mark or bruising discoloration. Apart from that, it may cause growth retardation in infants/children due to their tender skin which enhances the absorption of drugs onto the skin (Ference & Last, 2009).

There is a specific demand for locally applied, locally acting medicines for inflammatory skin conditions with better risk benefit ratios compared to TCS. Nonetheless, the de novo drug synthesis is labor-intensive and requires tedious processes for new drug discovery. Moreover, it also will be time consuming and highly expensive as the researcher needs to conduct additional study in understanding the pharmacokinetic properties and toxicity of the drug candidate. The drug rediscovery or also known as drug repurposing, drug re-profiling or drug rescuing, is now a hotly debated topic (Sethi et al., 2020). Repositioning marketed

drug candidates for alternative or new disease indications than originally proposed provides a valuable opportunity to fill pipeline gaps and boost success rates (Sekhon, 2013). Identification of existing and already-approved drug molecules for novel therapeutic reuse or of newer targets for already-approved drugs is the primary requirement for drug repurposing. The regulatory advantages include the potential bypassing of phase I and phase II clinical trials, especially when doses comparable to or lower than those used in previous validation data are employed and safety profiles from preclinical and clinical studies are available (Breckenridge & Jacob, 2018). In consequence, drug repurposing can reduce the toxicity and enhancing therapeutic efficacy of new drugs.

1.2 Problem Statement

Reduction in availability of novel therapeutic treatment against skin inflammatory disease leads to major concern on the patient's life quality and expectancy. The drawbacks in existing drugs reduce the efficacy of treatment given; thus, it is crucial to identify the alternative treatment immediately.

1.3 Justification

One of the way to seek for alternative treatment will be through drug repurposing technique which may reduce the cost and time for development of new drugs/formulation, which is hoped to subsequently increase the therapeutic efficacy.

1.4 Objectives

1.4.1 General Objective

To identify the alternative treatment for skin inflammatory diseases via in silico drug repurposing techniques of existing FDA-approved drugs that can target TNF-α and IL-1α.

1.4.2 Specific Objectives

I. To identify the common pharmacophore features of drugs used for skin inflammatory disease.

II. To determine possible drug candidates derived from FDA-approved drugs that fit the previously constructed pharmacophore features via *in silico* virtual screening method.

III. To rank and validate hit compounds that can bind to TNF-α and IL-1α as the immunological target via molecular docking method.

IV. To further analyse the docking output via ProteinPlus, Pymol and Protein Ligand Interaction Profiler.

1.5 Hypothesis

This study hypothesized that a number of existing FDA-approved drugs that share similar pharmacophore features as well as the immunological targets with the common skin inflammatory drugs that can target TNF-α and IL-1α are expected to be discovered.

CHAPTER TWO

LITERATURE REVIEW

2.1 Inflammation

2.1.1 Inflammatory Pathway

Inflammation is the physiological response to various injuries or insults, such as heat, chemical agents, or bacterial infection. Local immune, vascular, and inflammatory cell responses to infection or injury cause redness, swelling, heat, pain, and loss of tissue function during inflammation at the tissue level. During the inflammatory process, vascular permeability changes, leukocyte recruitment and accumulation, and the release of inflammatory mediators are all important microcirculatory events (Takeuchi & Akira, 2010). In the acute phase of inflammation, the response is rapid and brief. Tissue cells and inflammatory cells recruited from the bloodstream are activated simultaneously to regulate the levels of inflammatory mediators. IL-1 (interleukin 1), IL-6 (interleukin 6) and tumour necrosis factor- alpha (TNF-α) are primary inflammatory stimuli that act on the TLRs, IL-1 receptors (IL-1Rs), IL-6 receptors (IL-6Rs) and the TNF receptors (TNFRs) to cause inflammation. Mitogen activated protein kinase (MAPK) and nuclear factor kappa-B (NF-κB) pathways are two of the most important intracellular signaling pathways activated by receptor activation (Chen et al., 2017).

Pathogen invasion or cell damage is detected by innate immune cells such as macrophages, mast cells, dendritic cells, as well as by circulating leukocytes, monocytes and neutrophils. Both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are detected by pattern-recognition receptors (PRRs) expressed in both immune and nonimmune cells to set off signaling cascades, resulting in the production of mediators that attract leukocytes to the site of inflammation (Seong & Matzinger, 2004). Histamine, prostaglandins, and nitric oxide act on vascular smooth muscle to cause vasodilation, which increases blood flow and brings in circulating leukocytes, while inflammatory mediators such as histamine and leukotrienes act on endothelial cells to increase vascular permeability and allow plasma proteins and leukocytes to leave the circulation. Pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin 1 (IL1) increase the amounts of leukocyte adhesion molecules on endothelial cells, hence promoting leukocyte extravasation. Activated innate immune cells, including dendritic cells, macrophages, and neutrophils, at the site of an infection or injury remove foreign particles by phagocytosis (Newton & Dixit, 2012). The inflammatory response must be suppressed to prevent further tissue damage in order to prevent the progression from acute inflammation to persistent, chronic inflammation.

2.1.2 Cytokines

Cytokines are essential regulators of the innate and adaptive immune responses. It have a specific influence on cell connections and communication. Other names include lymphokine (lymphocyte-produced cytokines), monokine (monocyte-produced cytokines), chemokine (cytokines having chemotactic activity), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Cytokines can act on the cells that secrete them (autocrine activity), on neighboring cells (paracrine action), and in some cases, on distant cells (endocrine action) . Immune cells, such as monocytes, macrophages, and lymphocytes, are primarily responsible for releasing cytokines. Pro- and anti-inflammatory cytokines, respectively, promote and inhibit inflammation. Inflammatory cytokines are categorised as ILs, colony stimulating factors (CSF), IFNs, TNFs, TGFs, and chemokines, and are predominantly produced by cells to recruit leukocytes to the site of an infection or injury. Activated macrophages are the primary source of pro-inflammatory cytokines, which play a key role in driving inflammation. Pathological pain is thought to be caused in part by pro-inflammatory cytokines including interleukin-1, interleukin-6, and tumour necrosis factor-alpha (TNF-α) (Zhang & An, 2007). Through a complex network of interactions, cytokines modulate the immune response to infection or inflammation and regulate inflammation itself. However, excessive synthesis of inflammatory cytokines can result in tissue damage, hemodynamic abnormalities, organ failure, and eventually death (Chen et al., 2017).

2.1.2.1 Tumor Necrosis Factor- alpha (TNF-α)

Tumor necrosis factor alpha, a pro-inflammatory cytokine, is a well-known target in various inflammatory disorders. Multiple sclerosis (MS), rheumatoid arthritis, type 2 diabetes, inflammatory bowel disease, and graft-versus-host disease (GvHD), adult respiratory distress syndrome (ARDS), sepsis, cachexia, HIV infection, and insulin resistance have all been linked to high levels of TNF-α. Consequently, limiting the release of TNF-α may represent a viable treatment strategy for a variety of inflammatory diseases (Bahia & Silakari, 2010). Through two cell surface receptors, TNFR1 and TNFR2, TNF regulates apoptotic pathways, NF-κB activation of inflammation, and activation of stress-activated protein kinases via several signaling pathways (SAPKs) (Zhang & An, 2007). This cytokine is a potent inducer of the production of inflammatory cytokines like interleukin 1β, interleukin 6, and interleukin 8. Activated monocytes or macrophages function to synthesize TNF-α. For effective inflammatory response, pro-inflammatory cytokines must be produced and secreted under strict control. This is due to excessive amounts that can induce severe inflammation.

2.1.2.2 Interleukin- 1α

As a result of advancements in sequencing technologies, it has been determined that the IL-1 family has eleven members with similar

or distinct biological effects. IL-18, IL-33, IL-36α, β and γ are pro-inflammatory, in addition to IL-1α and IL-1β whereas IL-1 receptor agonist (IL-1Ra), IL-36Rα, IL-37, and IL-38 are anti-inflammatory (Fenini et al., 2017). Cytokines of the IL-1 family share a common C-terminal structure consisting of a characteristic -trefoil fold composed of 12 -strands joined by 11 loops (Palomo et al., 2015). By attaching to its respective receptors such as IL-1R1, IL-1α exerts the immune and inflammatory response. While caspase-1 cleavage of pro-IL-1β is required to generate bioactive IL-1β, both pro-IL-1α and mature IL-1α able to bind to IL-1R with equal kinetics and have comparable biological activity on epithelial and hematopoietic cells, as measured by their ability to trigger secretion of IL-6 and TNF (Kim et al., 2013). Hence, IL-1α is a powerful cytokine with a broad range of immunological and inflammatory actions (Mohan & Yu, 2011).

2.2 Skin Inflammation

Skin inflammation is a condition whereas, oftenly appears as red rash following a feeling of itchiness or burning sensation including formation of blisters as well as skin thickening depending on the causes and severity of the inflammation (Williams, 2005). Some of the potential causes of skin inflammation are immune system dysfunction, allergic reaction and bacterial or fungi infection. Eczema or also known as atopic dermatitis is an example of skin disease that is associated with all the possible causes mentioned before. The skin is the body's outermost layer and

the largest organ. It protects the host by acting as a main barrier between the host and the external environment (Benson, 2012). The skin also has great defenses against viruses and other environmental pollutants (Wang H. et al., 2013). The epidermis, dermis, and subcutaneous fat tissue are the three layers of the skin. The epidermis, the skin's outermost layer, is divided into four layers: the stratum corneum, the stratum lucidum, the stratum granulosum, and the stratum basale (Menon et al., 2012). The stratum corneum is made up of three layers that serves as both an outside-in barrier to keep foreign substances and bacteria out and an inside-out barrier to retain the water (Kubo et al., 2013).

However, the key feature of skin diseases is defective skin barrier that affects both lesional and non-lesional skin areas, as seen by increased transepidermal water loss. Hence, it facilitates the penetration of external substances like allergens, microbes, and viruses through the defective barrier (Bieber, 2010). Upon the invasion of chemical, physical or microbiological stressor the keratinocytes release a large quantity of soluble mediators including proteases, neuropeptides, neurotrophins, cytokines, and prostanoids. These mediators may stimulate sensory nerve fibers, resulting in classic clinical presentation of skin inflammation such as itching and redness. Thus, keratinocytes are at the forefront of skin homeostasis, leading communication with neighboring IENF and other epidermal cells upon encounter with exogenous noxious chemicals (Lauria & Lombardi, 2012). The epidermis and dermis are linked, these anatomical compartments, which include sensory nerves and cells of various origin, mast cells, fibroblasts, endothelial cells, and leucocytes, communicate in a coordinated manner to directly buffer noxious agents or neutralise the elicited local reactions to avoid a systemic response, such

as vascular and immune reactions, which may result in tissue injury or dysfunction of this barrier organ (Abels & Soeberdt, 2019).

2.2.1 Global Statistic of Skin Inflammatory Diseases

Skin inflammatory disease is greatly known as the most frequent type of disease affecting the population worldwide contributing to the global disease burden. As reported by Karimkhani et al. (2017) the percentage of skin and subcutaneous diseases in global burden of disease (GBD) was 1.79% and ranked as the fourth leading cause of nonfatal disease burden and disability in 2013. A cross sectional study of the global burden of skin disease 1990-2017 based on the socioeconomic status conducted by Urban et al. (2021) revealed Asian countries including Malaysia as the most common region where the population present with skin disability such as psoriasis, atopic dermatitis or contact dermatitis. The significant increase in prevalence of atopic dermatitis in Asian region is associated to progressive urbanization, increased family income, better education available and increased allergen exposure leading to frequent bathing and soap daily usage. To add, Laughter et al. (2021) revealed atopic dermatitis as the highest disease burden among the skin diseases obtained from DALYs database and ranked 15th among the non-fatal diseases. The impact of psoriasis is correlated with gradual increase over a period of time put on the additional burden of skin diseases (Iskandar et al., 2020). Apart from that, acne also can be categorized as the most

common inflammatory dermatosis affecting adolescents and women (Layton et al., 2020).

2.2.2 Current Treatment of Skin Inflammatory Diseases

The fundamental of skin inflammatory diseases treatment is to eliminate inflammation and infection, preserve and restore barrier function, and reduce inflammatory variables. Consequently, future strategy in skin diseases such as atopic dermatitis will be to regulate skin inflammation through more proactive treatment in order to potentially prevent the establishment of sensitization and to design personalised therapy based on genetic and pathophysiologic knowledge (Bieber, 2010). Essentially, the treatment of inflammatory skin conditions like psoriasis, atopic dermatitis, chronic spontaneous urticaria, and hidradenitis suppurativa has primarily focused on symptom management with topical ointments and/or selected oral antibiotics, antihistamines, corticosteroids, and retinoids. To date, topical medication such as corticosteroid have been the mainstay of treatment in atopic dermatitis over the past 40 years due to its rapid and spectacular effect that can be seen within a few days after the application (Atherton, 2003). The use of topical corticosteroid is also favored in mild to moderate psoriasis cases considering the limited systemic effect as it is applied locally (Schadler et al., 2019).

Deeper understanding of the pathophysiology of these diseases has recently resulted in the creation of a cascade of innovative therapy options

targeting critical immune system molecules namely the cytokines and pro-inflammatory cytokines. In the 1990s, the discovery of tumor necrosis factor and the subsequent development of its antagonists altered the paradigm of treating chronic skin disorders towards increased specificity. Since then, various biological agents for the treatment of chronic skin disorders have been created, and more are now in development (Yao et al., 2019). The biologic drugs can be further classified into 4 major classes: TNF inhibitor, IL-12/23 inhibitor, IL-17 inhibitor as well as IL-23 inhibitor. Etanercept, adalimumab, infliximab, ixekizumab, secukinumab, brodalumab, guselkumab, ustekinumab, and tildrakizumab are some examples of commonly used biologics (Armstrong & Read, 2020).

2.2.3 Drawbacks of Current Skin Inflammatory Diseases Treatment

Despite the "hype" surrounding these systemic medicines, the majority of dermatological patients with atopic dermatitis or psoriasis are still treated topically. If emollient therapy is insufficient, topical corticosteroids and calcineurin inhibitors (tacrolimus FDA 2000, EMA 2002; pimecrolimus FDA 2001, EMA 2002) remain the basis of dermatological care (Abels & Soeberdt, 2019). Phototherapy and non-biologic systemic drugs have proven effective therapies; however, phototherapy is time intensive, and non-biologic systemic medications have relatively small response rates (Schadler et al., 2019). Apart from that, systemic drugs can be burdensome primarily because they are expensive and the adverse effects that are associated after

employment of the drugs in treatment can threaten the lives of patients. These biologics can cause a variety of serious complications, including malignancy or lymphoma in the case of alefacept. They can also induce immunosuppression or congestive heart failure in severe circumstances. These systemic biologics may also cause bacterial infections or TB, as well as a variety of other respiratory tract disorders (Egeberg et al., 2018).

Moreover, the fact that atopic dermatitis is remitting and relapsing, parents of affected children are concerned about the long-term repercussions of TCS use, which has led them to seek alternatives (Koo et al., 2020). This is due to children being more susceptible to the systemic adverse effects because of enhanced percutaneous absorption through their tender skin. Particularly, topical corticosteroids can be distinguished into seven different classes according to their potency ranging from ultra-high potency to the lowest potency. Consequently, the lowest potent type of topical corticosteroid would be chosen as the first stage of treatment (Ference & Last, 2009). However, the long term use of a low potent type of topical corticosteroid can lead to a condition known as tachyphylaxis whereas the effectiveness would gradually diminish after progressive use. This phenomenon can result in an increased risk of adverse effects since it is tempting, when faced with declining effectiveness, to respond by increasing the potency of the topical corticosteroid (Atherton, 2003). Furthermore, frequent application of low potent topical corticosteroid gives risk of skin thinning, permanent stretch mark, bruising and discoloration as well as growth retardation in infants/children.

2.3 Drug Repurposing and Its Benefit

The aim of drug repurposing is to develop new uses of drugs from already existing drugs. It makes use of well-developed drugs that either had approved for its safety or passed during the clinical trials to assist in identifying the efficacy of drugs in disease other than the one originally proposed. For example, minoxidil is commercially known as anti-hypertensive drugs yet new indications revealed the therapeutic effect on the hair growth (Abels & Soeberdt, 2019). The repurposing or repositioning of approved or investigational drugs can be beneficial for new drug discovery (Baker et al., 2018). First and foremost, the risk of failure is lowered as the drug showed safety in preclinical models or in human models during the clinical development. Aside from that, the existing data can reduce the development time since the formulation has been completed. The data available from preclinical testing and assessment is sufficient to exclude the needs for additional study in order to understand the pharmacokinetic properties as well as the toxicity level of the drug. As mentioned by Strittmatter (2014), drug repurposing will not only reduce toxicity and improve therapeutic efficacy, but it will also reduce the time necessary to develop new formulations.

Moreover, following the stage of development, the cost of repurposed drugs may be shortened. The regulatory and phase III costs for a repurposed drug may be similar to those for a new drug discovery in the same indication, but there may be significant savings in preclinical and phase I and II expenditures (Pushpakom et al., 2018). Essentially, result in a less risky and rapid return on investment in the development of repurposed drugs, with lower average related costs. In addition, those repurposed drugs can enhance new discoveries on targets and pathways

involved for further invention. For instance, from a rare disease point of view such as some genetic abnormalities and autoimmune diseases. Since standard ways to treat rare diseases are prohibitively expensive and time-consuming, drug repurposing is advantageous. Hence, drug repurposing is a viable strategy for overcoming hurdles in the development of novel drugs, as well as for discovering and reinvestigating the therapeutic effectiveness of existing marketed drugs in new indications (Sekhon, 2013).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Identification of Commonly Prescribed Drugs in Skin Inflammatory Diseases

The identification of frequently prescribed drugs were based on the literature review of medication articles focusing on skin inflammatory drugs. The articles were gathered from various trustable sources using keywords such as “skin disease”, “skin inflammation”, “drug”, “TNF-α” and “Interleukin-1α”. The trustable sources that had been used are scientific search engine which were PubMed, Scopus, Google Scholar and Science Direct. The drugs are then further classified in two different groups which are the biologic and non-biologic drugs.

3.2 Determination of Pharmacophore Model

International Union of Pure and Applied Chemistry (IUPAC) defines pharmacophore as "the ensemble of steric and electronic features required to ensure optimal supramolecular interactions with a specific biological target structure and to trigger (or block) its biological response". Rather than specific functional groups, interaction patterns of bioactive molecules with their targets are represented by a three-dimensional (3D) arrangement of abstract features that define interaction types. These interaction types may include the formation of hydrogen bonds, charged interactions, metal interactions, and hydrophobic (H) and aromatic (AR)

contacts, among others (Kaserer et al., 2015). Ultimately, the construction of a pharmacophore model may be beneficial for new drug discovery as it facilitates the preliminary selection of compounds to be discovered. It can either be generated through a structure-based approach or ligand-based approach.

In this study, a ligand-based modeling was used to represent the binding mode of ligands with a target. LigandScout 4.4.7 software was used to construct the pharmacophore model. Initially, the three-dimensional structure (3D) of frequently prescribed drugs for skin inflammatory disease were downloaded from the PubChem database and Protein Data Bank (PDB). Next, one of the three-dimensional structure (3D) of the drug was selected and added in the LigandScout software to kick off the pharmacophore design processes. Simply select 'add molecule to alignment view' from the data exchange widget to add the drug. Essentially, the pharmacophore of an individual drug must be generated first before adding up the other drugs in the alignment view. The selected individual pharmacophore was then proceeded with clicking the 'add pharmacophore to alignment view' section. The steps were further repeated with other drugs until all the pharmacophores of drugs were aligned together. Finally, the 'shared feature pharmacophore' button was selected to determine the common pharmacophore features shared among these sets of drugs.

3.3 Performing Virtual Screening

The main objective of pharmacophore-based virtual screening is to yield the potential hit compounds that fits the pharmacophore model constructed previously by using the LigandScout 4.4.7 software. A higher pharmacophore fit score would

indicate a better geometric alignment of the features of the hit compounds to the 3D-pharmacophore model. Like pharmacophore model construction, LigandScout 4.4.7 software was used to perform the virtual screening. The software provides virtual screening from a multi-conformational database with known active compounds which in this study, FDA-approved drug database was employed to yield the possible hit compounds. The hit compounds were then distinguished as drug candidates obtained from DrugBank.

To begin with, the previously constructed pharmacophore was added in the LigandScout software. Next, 'Screen pharmacophore against external library' located in the pharmacophore menu was clicked to initiate the virtual screening. It is vital to ensure the selected database is available in the library file. Subsequently, a list of hit compounds along with their pharmacophore fit scores was determined. Hit compounds with high pharmacophore fit scores were chosen and their three-dimensional structure (3D) were downloaded from DrugBank to proceed with molecular docking.

3.4 Molecular Docking Analysis

3.4.1 Preparation of Receptor and Ligand Inputs

The receptors, TNF-α and IL-1α, were obtained from the Protein Data Bank (PDB) database in '.pdb' format file with PDB ID of 4g3y for TNF-α and 2l5x for IL-1α. The manipulation and visualization of receptors were operated using AutoDock Vina tool. First, the unwanted chains and ligand were eliminated from the crystal structure of the protein, leaving only the target

protein. The crystal waters were subsequently removed from the structure, as their presence could complicate docking processes and calculations of binding affinities. Hydrogen atoms were subsequently added to the structure. The crystal structure was subsequently saved as '.pdb' files. The structure of ligands, which are hit compounds resulting from virtual screening, were obtained from the DrugBank database in '.pdb' format.

3.4.2 Set Up of Docking Grid Box and Configuration Files

The arrangement of the docking grid box that includes the desired binding site is prominent to establish a good docking result. By employing the AutoDock Vina tool software, the protein's binding residues were highlighted, and the grid box was then adjusted to encompass all of the binding residues. The reported binding residues for TNF-α comprises of leucine57, tyrosine59, serine60, glutamine61, tyrosine119, leucine120, glycine121, glycine122 and tyrosine151 (Halim et al., 2021). In the meantime, a total of 11 residues were selected as active site for ligand binding of IL-1α. The binding residues were isoleucine33, arginine34, asparagine47, aspartic acid49, alanine51, glutamic acid50, lysine53, isoleucine74, lysine76, leucine79 and valinel52 (Gao et al., 2021). The size and coordinates of the grid box were recorded for use as molecular docking parameters. The adjusted parameters were then typed in a text editor using Crimson Editor and saved as configuration files. The configuration files need to be saved in ‘txt’ format.

Protein	Size		Coordinate	
TNF-α	x-dimension	28	x	5.303
	y-dimension	42	y	-2.721
	z-dimension	50	z	-16.28
IL-1α	x-dimension	48	x	36.263
	y-dimension	56	y	4.196
	z-dimension	40	z	11.28

Table 3.1: The adjusted parameters of TNF-α and IL-1α

3.4.3 Molecular Docking

Molecular docking aims to predict the structure of the ligand-receptor complex using computational methods. It involves the prediction of the ligand conformation as well as its position and orientation within the active sites and the evaluation of the binding affinity (Meng et al., 2011). The docking analysis of hit compounds against TNF-α and IL-1α were executed by using AutoDock Vina tool software. The command prompt was used to run AutoDock Vina and the docking job was then performed. All the prepared PDB files, configuration file and software were gathered in a folder. If the files needed were insufficient, it will prevent the docking job to be done. An output file with the calculated binding affinity was generated at the end of the docking process. The binding affinity was calculated and measured by using the scoring function.

3.5 Analysis on Docked Complexes

The AutoDock Vina-predicted binding poses with the highest binding affinity were further analysed using PoseView from Protein Plus web server, Protein-Ligand Interaction Profiler and Pymol software. Using Poseview and Protein-ligand Interaction Profiler, information on the interactions of the docked complex was obtained, followed by PyMol visualisation and distance measurement between hit compounds and interacting residues on the protein TNF-α and IL-1α.

CHAPTER 4

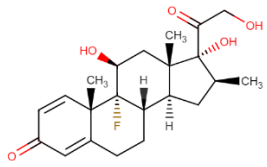
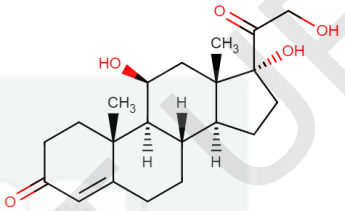
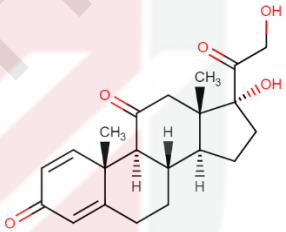
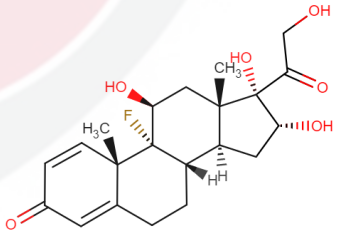
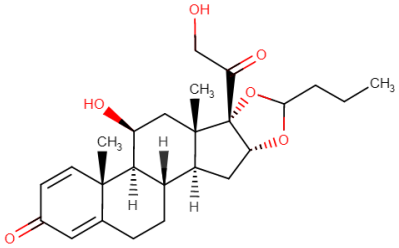
RESULTS

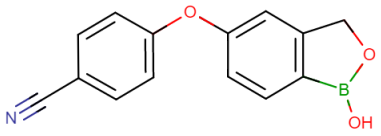
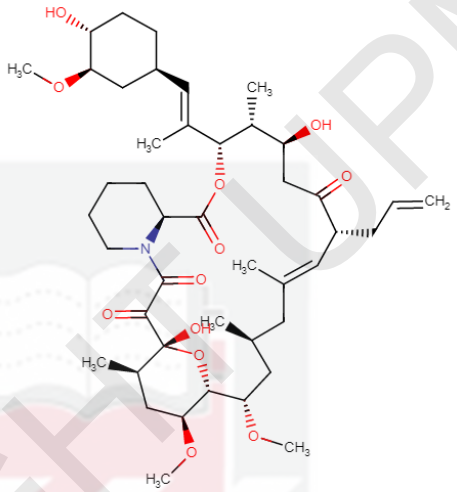
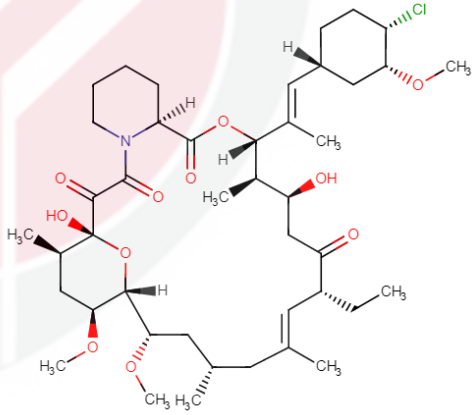
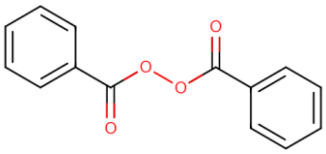
4.1 List of Drugs Commonly Prescribed for Skin Inflammatory Diseases

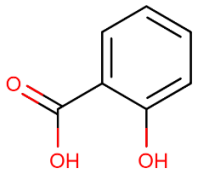
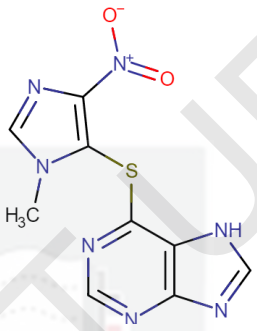
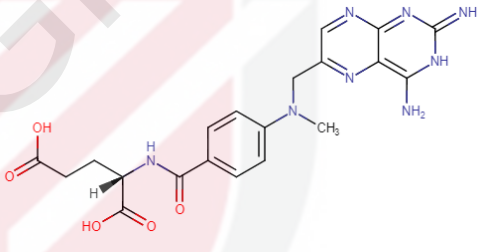
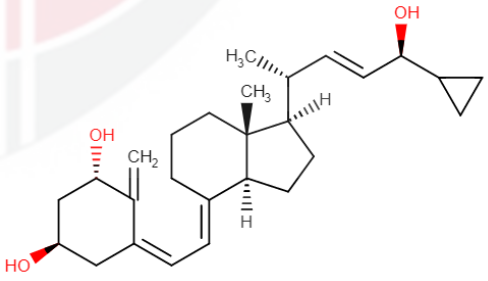
A total of 29 drugs that are frequently prescribed for treatment of skin inflammatory diseases are obtained. Table 4.1 and Table 4.2 showed the drugs commonly prescribed for skin inflammatory diseases.

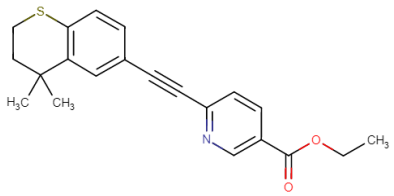
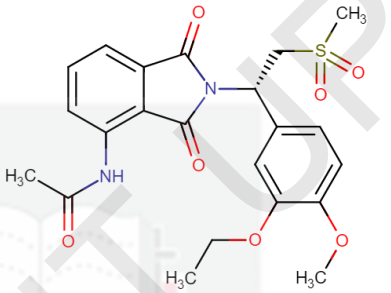
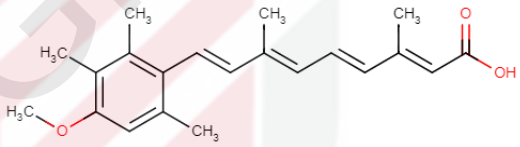
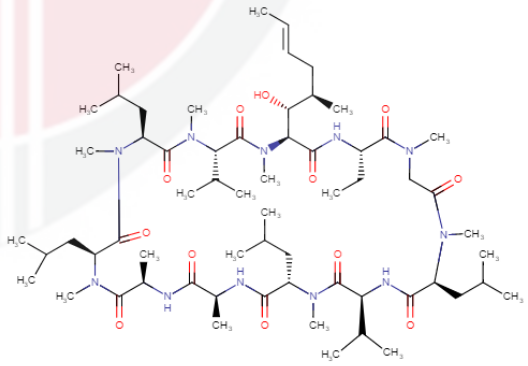
Biologic Drugs	
1.	Adalimumab
2.	Etanercept
3.	Infliximab
4.	Certolizumab pegol
5.	Ixekizumab
6.	Secukinumab
7.	Brodalumab
8.	Ustekinumab
9.	Guselkumab
10.	Tildrakizumab
11.	Risankizumab

Table 4.1: Biologic drugs for skin inflammatory diseases

Non-biologic Drugs		
1.	Betamethasone	
2.	Hydrocortisone	
3.	Prednisone	
4.	Triamcinolone	
5.	Budesonide	

6.	Crisaborole	
7.	Tacrolimus	
8.	Pimecrolimus	
9.	Benzoyl peroxide	

10.	Salicyclic acid	
11.	Azathioprine	
12.	Methotrexate	
13.	Calcipotriene	

14.	Tazarotene	
15.	Apremilast	
16.	Acitretin	
17.	Cyclosporine	

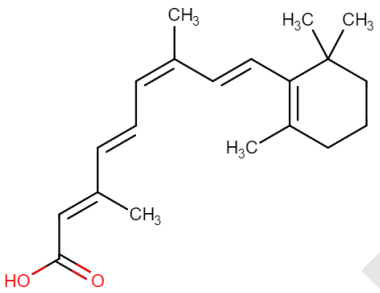
18.	Alitretinoin	

Table 4.2: Non-biologic drugs for skin inflammatory diseases

4.2 The Pharmacophore Model of Skin Inflammatory Diseases

The pharmacophore model of non-biologic drugs group constructed by using LigandScout 4.4.7 software was shown in **Figure 4.1**. The pharmacophore model highlights the similarity of pharmacophore features as well as the immunological targets shared within the drugs.

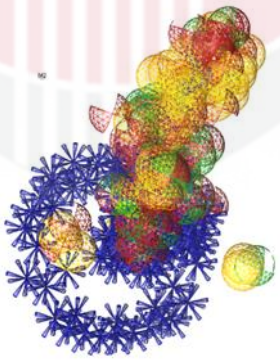


Figure 4.1: Pharmacophore model for non-biologic drugs

However, the pharmacophore model of biologic drugs was unable to be generated. This was due to the non-existence of three-dimensional (3D) structure for biologic drugs in both databases. Moreover, to perform homology modelling as an attempt to predict the crystal structures will be time consuming due to the complexity of the biologic drug structures which is beyond the scope of the current study. Hence, this study was focusing on the non-biologic drugs only.

4.3 List of Hit Compounds Resulted from Virtual Screening

Based on the previously constructed pharmacophore model, a virtual screening was conducted by utilising the LigandScout 4.4.7 software. A total of 16 hit compounds were executed from FDA-approved drug databases obtained from DrugBank.

No.	FDA-approved drug	Pharmacophore Fit Score
1.	Lisinopril and Hydrochlorothiazide; **Lisinopril**	86.72
2.	Adderall 10; **Amphetamine** Aspartate	86.50
3.	Enalaprilat; **Enalaprilat**	86.14
4.	Bexarotene; **Bexarotene**	86.01
5.	Kinevac; **Sincalide**	85.94
6.	Reclast; **Zoledronic Acid**	85.73
7.	Neopasalate; **Aminosalicylic Acid**	78.67
8.	Apriso; **Mesalazine**	78.15
9.	Azulfidine En-Tabs; **Sulfasalazine**	78.12
10.	Meclan; **Meclocycline** Sulfosalicylate	77.37
11.	Diflunisal; **Diflunisal**	76.66
12.	Ticar; **Ticarcillin** Disodium	76.62

13.	Carbidopa and Levodopa; **Carbidopa**	76.16
14.	Aldoclor-150; **Methyldopa**	75.98
15.	Promacta; **Eltrombopag** Olamine	75.95
16.	Bendopa; **Levodopa**	75.54

Table 4.3: Pharmacophore fit score of hit compounds derived from FDA-approved drug database

4.4 Molecular Docking of Hit Compounds Against TNF-α and IL-1α

4.4.1 Crystal Structure and Hydrophobic Pocket Residues of TNF-α

Crystal structure of TNF-α was accessed from Protein Data Bank (PDB) with PDB ID: 4g3y. The crystal structure of TNF-α complex was bounded to infliximab fragment and shown at 2.6 resolution. The crystallographic 3-fold axis revealed a core TNF-α trimer bound by three infliximab Fab molecules organised in a symmetrical arrangement Figure 4.2. Also, comparing the TNF-α in the TNF-α-infliximab Fab complex with wild-type TNF-α gave a root mean square deviation (r.m.s.d.) of 1.4 Å for the C^{α} atoms of all residues. This showed that there were no significant structural differences between free TNF-α and TNF-α in complex, except for the residues at the antibody-antigen interface (Liang et al., 2013). Therefore, molecular docking studies was conducted only on chain C.

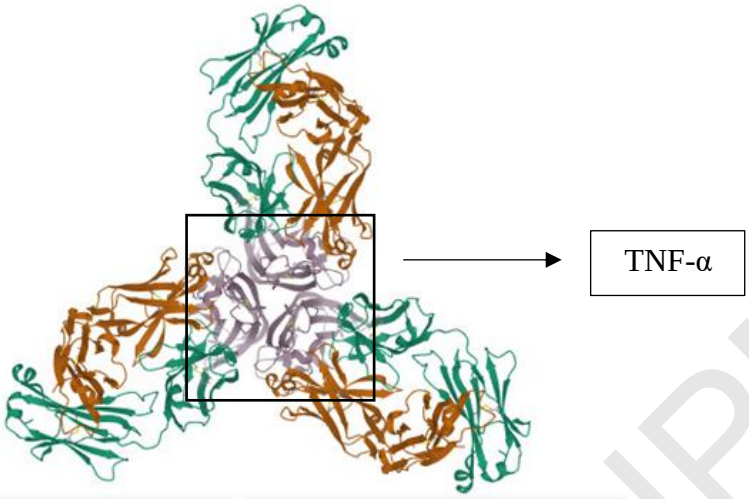


Figure 4.2: Crystal structure of TNF-α obtained from PDB (PDB ID: 4g3y)

According to Halim et al. (2021), nine residues were found on chain A and seven residues were found on chain B out of the total of 16. These residues may serve as ligand-binding sites. Leucine57(A/B), tyrosine59(A/B), serine60(A/B), glutamine61(A), tyrosine119(A/B), leucine120(A/B), glycine121(A), glycine122(A/B) and tyrosine151(A/B) are the receptor binding residues. TNFR binding site was stabilised by the hydrophilic and hydrophobic interactions provided by these residues. Since chain c of TNF-α retrieved from the PDB was similar with the wild-type of TNF-α, hence these binding residues could be used as potential target for ligand binding in this study.

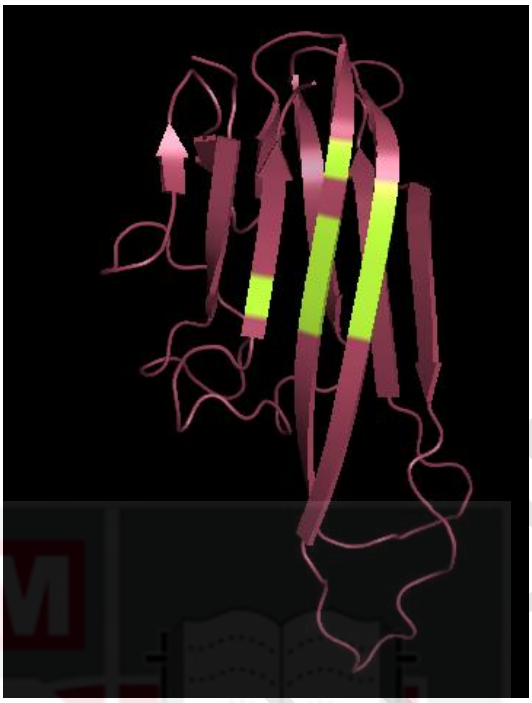


Figure 4.3: Crystal structure of TNF-α visualized using PyMol. The binding residues are highlighted (green).

4.4.2 Crystal Structure and Hydrophobic Pocket Residues of IL-1α

Crystal structure of IL-1α was obtained from PDB with PDB ID: 2l5x (Figure 4.4). The crystal structure represents an IL1α-S100A13 complex that consists of 4 chains. Chain A and D depicted as IL-1α while chain B and C stand for S100A13 protein. The tetrameric IL1α-S100A13 complex is a homodimer. Each monomer in this complex binds one IL-1α molecule, and the complex consists of two symmetric units. Therefore, chain A and D are exact copy of IL-1α molecule. Hence, only chain A was retained to proceed with molecular docking analysis.

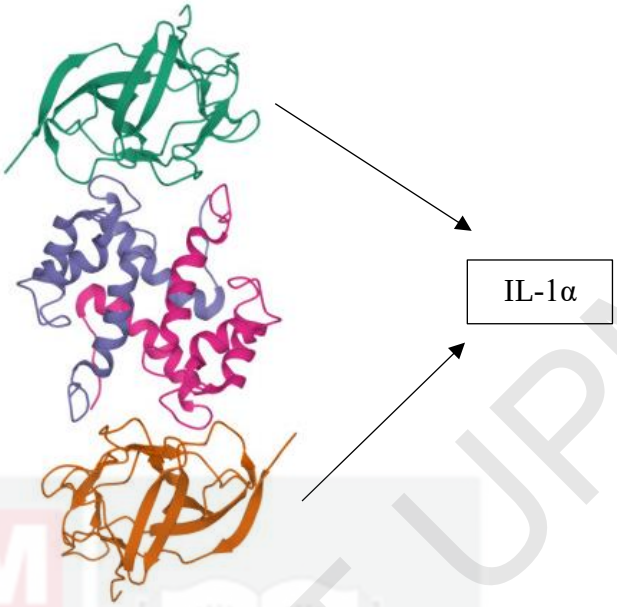


Figure 4.4: Crystal structure of IL-1α obtained from PDB (PDB: 2l5x)

As stated by Gao et al. (2021), several amino acid residues such as isoleucine33, alanine51, valinel52, isoleucine74, and leucine79 interact hydrophobically at the binding pocket of the IL-1α. To add, aspartic acid49, glutamic acid50, arginine34, lysine53, and lysine76 are amino acid residues associated with the active site via electrostatic interactions. Aside from that, significant interaction is reported located at arginine34, asparagine47, aspartic acid49 and glutamic acid50 residues of IL-1α (Xu et al., 2018). Consequently, a total of 11 residues were selected as ligand binding sites.

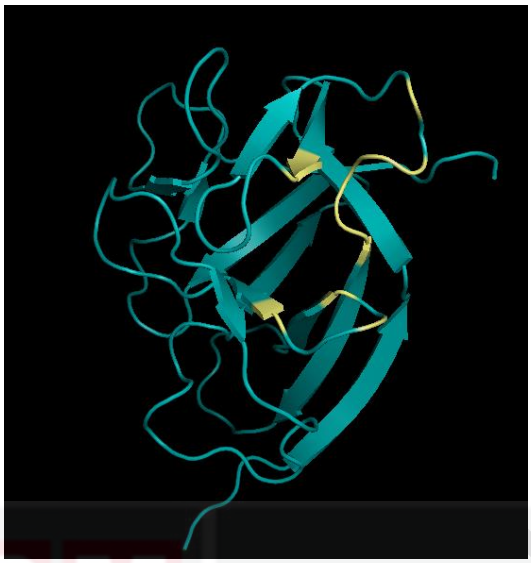


Figure 4.5: Crystal structure of IL-1α visualized using PyMol. The binding residues are highlighted (yellow).

4.4.3 AutoDock Vina Result

The docking results of 16 hit compounds against TNF-α and IL-1α were presented in Table 4.4 and Table 4.5. The highest binding affinity (kcal/mol) belongs to sulfasalazine with -8.3 kcal/mol, followed by meclocycline with -7.6 kcal/mol and next is eltrombopag with -7.4 kcal/mol respectively for TNF-α. Meanwhile, docking result against IL-1α, the first rank belongs to bexarotene with -8.8 kcal/mol. Sulfasalazine and meclocycline ranked as the second and third highest with -8.1 kcal/mol and -7.1 kcal/mol respectively. However, considering their strong affinity for binding to TNF-α and IL-1α, the next stage would only focus on sulfasalazine and meclocycline as the main drug candidates.

No	FDA Drug Names	Binding affinity (kcal/mol)
1.	Azulfidine En-Tabs; **Sulfasalazine**	-8.3
2.	Meclan; **Meclocycline** Sulfosalicylate	-7.6
3.	Promacta; **Eltrombopag** Olamine	-7.4
4.	Bexarotene; **Bexarotene**	-6.9
5.	Kinevac; **Sincalide**	-6.9
6.	Enalaprilat; **Enalaprilat**	-6.7
7.	Lisinopril and Hydrochlorothiazide; **Lisinopril**	-5.9
8.	Diflunisal; **Diflunisal**	-5.6
9.	Ticar; **Ticarcillin** Disodium	-5.5
10.	Carbidopa and Levodopa; **Carbidopa**	-5.0
11.	Reclast; **Zoledronic Acid**	-4.9
12.	Apriso; **Mesalazine**	-4.8
13.	Aldoclor-150; **Methyldopa**	-4.8
14.	Bendopa; **Levodopa**	-4.5
15.	Neopasalate; **Aminosalicylic Acid**	-3.9
16.	Adderall 10; **Amphetamine Aspartate**	-3.8

Table 4.4: Molecular docking of hit compounds against TNF-α

No	FDA Drug Names	Binding affinity (kcal/mol)
1.	Bexarotene; **Bexarotene**	-8.8
2.	Azulfidine En-Tabs; **Sulfasalazine**	-8.1
3.	Meclan; **Meclocycline** Sulfosalicylate	-7.1
4.	Promacta; **Eltrombopag** Olamine	-6.7
5.	Kinevac; **Sincalide**	-6.6
6.	Enalaprilat; **Enalaprilat**	-6.2
7.	Diflunisal; **Diflunisal**	-5.8
8.	Lisinopril and Hydrochlorothiazide; **Lisinopril**	-5.7
9.	Adderall 10; **Amphetamine** Aspartate	-5.7
10.	Carbidopa and Levodopa; **Carbidopa**	-5.3
11.	Ticar; **Ticarcillin** Disodium	-5.2
12.	Aldoclor-150; **Methyldopa**	-4.7
13.	Neopasalate; **Aminosalicylic Acid**	-4.7
14.	Reclast; **Zoledronic Acid**	-4.7
15.	Bendopa; **Levodopa**	-4.6
16.	Apriso; **Mesalazine**	-4.5

Table 4.5: Molecular docking of hit compounds against IL-1α

4.5 Binding of Sulfasalazine to Tumor Necrosis Factor-α

4.5.1 AutoDock Vina Result

The docking of sulfasalazine to TNF-α yielded the outcomes detailed in Table 4.6. AutoDock Vina had predicted nine optimum binding modes of sulfasalazine to TNF-α, ranging from highest to lowest binding affinity. The optimal docked position with the highest binding affinity was chosen for further analysis

Mode	Binding affinity (kcal/mol)
1.	-8.3
2.	-7.9
3.	-7.8
4.	-7.7
5.	-7.7
6.	-7.7
7.	-7.7
8.	-7.6
9.	-7.6

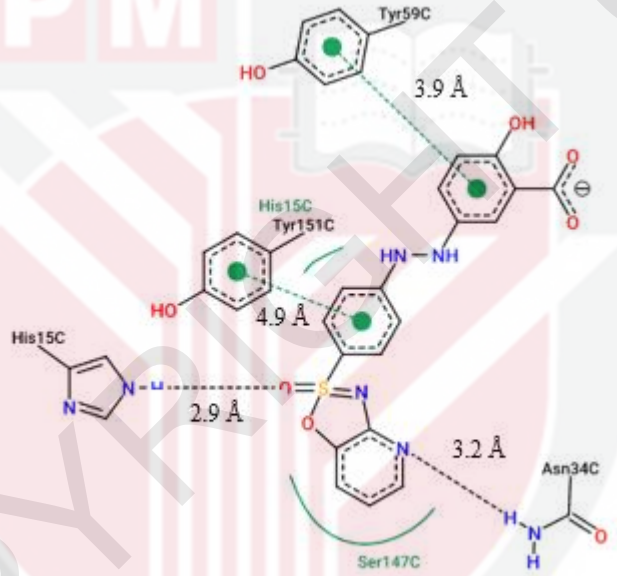
Table 4.6: Predicted binding poses of sulfasalazine to TNF-α

4.5.2 Analysis of Best Docked Complex

The best theoretical binding results of sulfasalazine to TNF-α are represented in Figure 4.6. Sulfasalazine fit the active site of the binding pocket of TNF-α with binding affinity of -8.3 kcal/mol. Detailed analysis viewed from Poseview by ProteinPlus web server showed that benzene ring of sulfasalazine bind at the hydrophobic pocket region of TNF-α at serine147 in chain C. Subsequently, two weak hydrogen bonds were formed from the

4-OH residue of sulfasalazine, of which were one with asparagine34 in chain C (distance 3.2 Å) and another one with histidine15 in chain C (distance 2.9 Å). At the same time, two π–π stacking were observed between the aromatic rings of sulfasalazine at tyrosine 59 residue in chain C at a distance 3.9 Å while, another π–π stacking was residing at tyrosine151 in chain C (distance 4.9 Å).

A



B

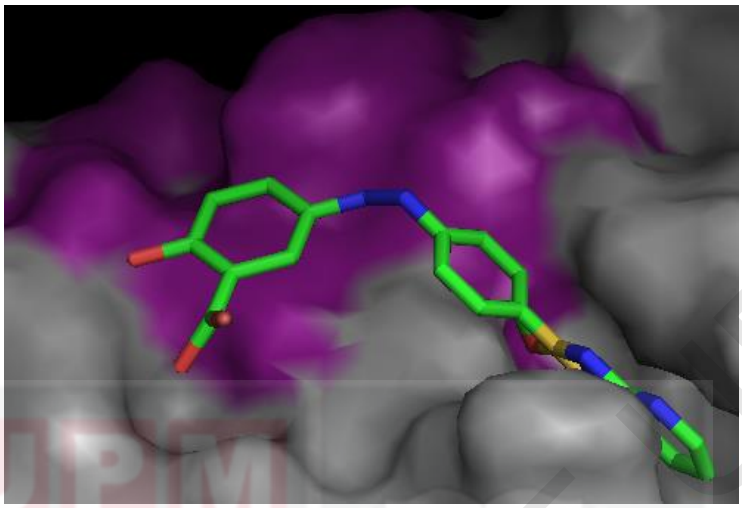


Figure 4.6: Binding mode of sulfasalazine to TNF-α. Sulfasalazine were docked to TNF-α with binding affinity of -8.3 kcal/mol. **(A)** Visualisation of protein-ligand interaction generated by PoseView. A few weak hydrogen bondings (black dashed lines) and pi-pi stacking (green dashed lines) were identified. Hydrophobic contact areas between ligand and protein are represented by green spline segments with green residue labels. **(B)** PyMol visualisation of sulfasalazine bind to TNF-α.

4.6 Binding of Meclocycline to Tumor Necrosis Factor-α

4.6.1 AutoDock Vina Result

AutoDock Vina has predicted the best nine binding modes of meclocycline to TNF-α. Table 4.7 shows the predicted binding poses of meclocycline to TNF-α ranked from highest binding affinity to the lowest. The best docked pose with the highest binding affinity was selected for further analysis.

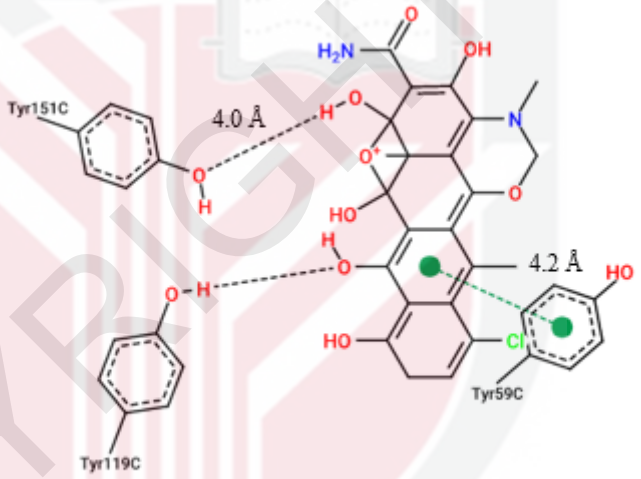
Mode	Binding affinity (kcal/mol)
1.	-7.6
2.	-7.6
3.	-7.4
4.	-7.4
5.	-7.4
6.	-7.4
7.	-7.3
8.	-7.3
9.	-7.2

Table 4.7: Predicted binding poses of meclocycline to TNF-α

4.6.2 Analysis of Best Docked Complex

In **Figure 4.7** meclocycline showed best theoretical binding result which fit the hydrophobic active site of TNF-α with binding affinity of -7.6 kcal/mol. The 4-OH residue also had a weak hydrogen bond at distance 4.0 Å with tyrosine151. A π–π stacking was formed with tyrosine59 in chain C at a distance of 4.2 Å.

A



B

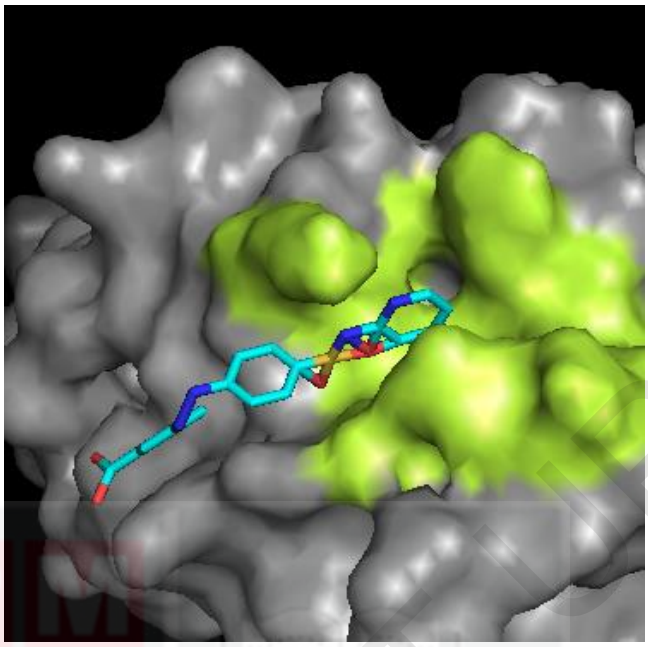


Figure 4.7: Binding mode of meclocycline to TNF-α. Meclocycline were docked to TNF-α with binding affinity of -7.6 kcal/mol. **(A)** A 2D diagram of protein-ligand interaction generated by PoseView. Weak hydrogen bondings (black dashed lines) and pi-pi stacking (green dashed lines) were identified. **(B)** PyMol visualisation of meclocycline bind to TNF-α.

4.7 Binding of Sulfasalazine to Interleukin-1α

4.7.1 AutoDock Vina Result

The result of the docking of sulfasalazine to IL-1α is outlined in Table 4.8 AutoDock Vina had predicted nine best binding modes of sulfasalazine against IL-1α that were ranked from the highest binding affinity to the lowest. The best docked pose with the highest binding affinity was selected for further analysis.

Mode	Binding affinity (kcal/mol)
1.	-8.1
2.	-7.9
3.	-7.6
4.	-7.6
5.	-7.5
6.	-7.3
7.	-7.3
8.	-7.2
9.	-7.1

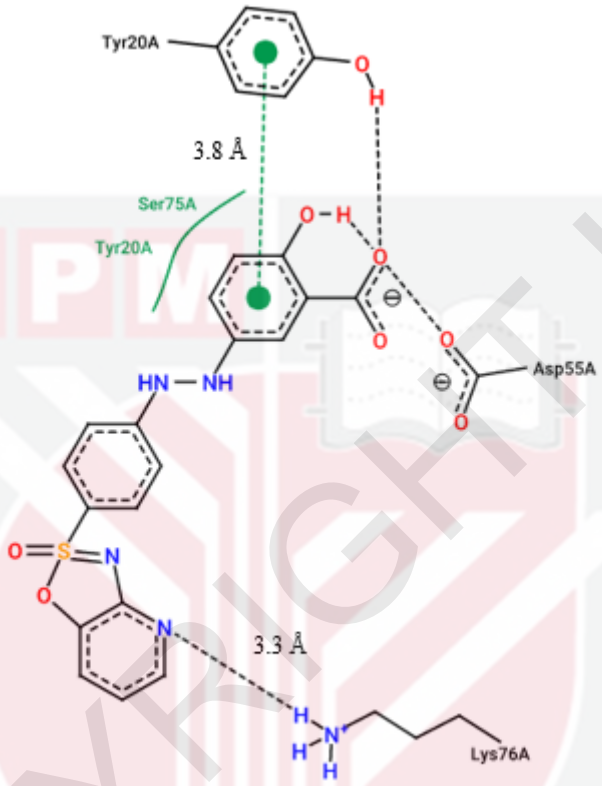
Table 4.8: Predicted binding poses of sulfasalazine against IL-1α

4.7.2 Analysis of Best Docked Complex

The best theoretical binding result of sulfasalazine to IL-1α is summarised in Figure 4.8. Sulfasalazine fit the hydrophobic active site of IL-1α with binding affinity of -8.1 kcal/mol. Sulfasalazine can have a Van Der Waals interaction and occupy the hydrophobic pocket region of tyrosine20 and serine75 in chain A. One moderate hydrogen bond was formed at

lysine76 residue in chain A at a distance of 3.3 Å. Aside from that, a π–π stacking interaction was observed at tyrosine20 in chain A at a distance of 3.8 Å.

A



B

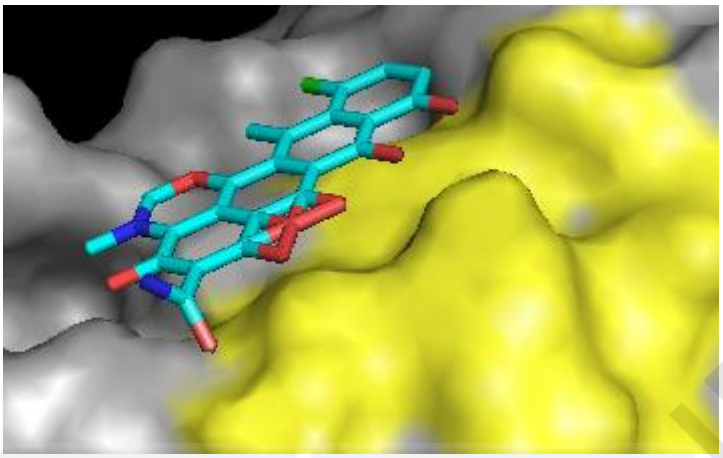


Figure 4.8: Binding mode of sulfasalazine to IL-1α. Sulfasalazine were docked to IL-1α with binding affinity of -8.1 kcal/mol. **(A)** A 2D diagram of protein-ligand interaction generated by PoseView. A moderate hydrogen bondings (black dashed lines) and pi-pi stacking (green dashed lines) were identified. Hydrophobic contact areas between ligand and protein are represented by green spline segments with green residue labels. **(B)** PyMol visualisation of sulfasalazine bind to IL-1α.

4.8 Binding of Meclocycline to Interleukin-1α

4.8.1 AutoDock Vina Result

AutoDock Vina had predicted the top nine binding modes between IL-1α and meclocycline. Table 4.9 displays the expected binding poses of meclocycline to IL-1α in ascending order of binding affinity. The optimal docked conformation with the highest binding affinity was chosen for further analysis.

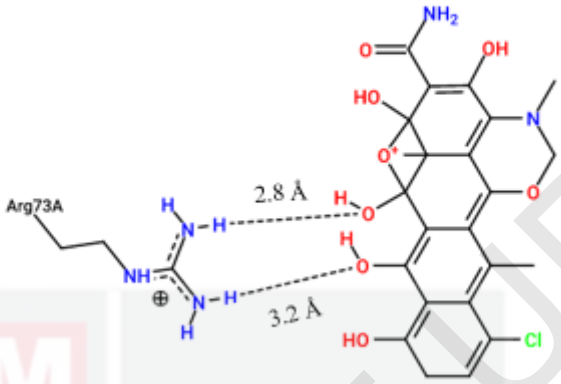
Mode	Binding affinity (kcal/mol)
1.	-7.1
2.	-6.6
3.	-6.5
4.	-6.5
5.	-6.4
6.	-6.4
7.	-6.1
8.	-6.1
9.	-6.0

Table 4.9: Predicted poses of meclocycline bind to IL-1α

4.8.2 Analysis of Best Docked Complex

Meclocycline docked to IL-1α with binding affinity of -7.1 kcal/mol. Two moderate hydrogen bonds were assembled at arginine73 in chain A. The moderate hydrogen bonds formed at a distance of 3.2 Å and 2.8 Å respectively.

A



B

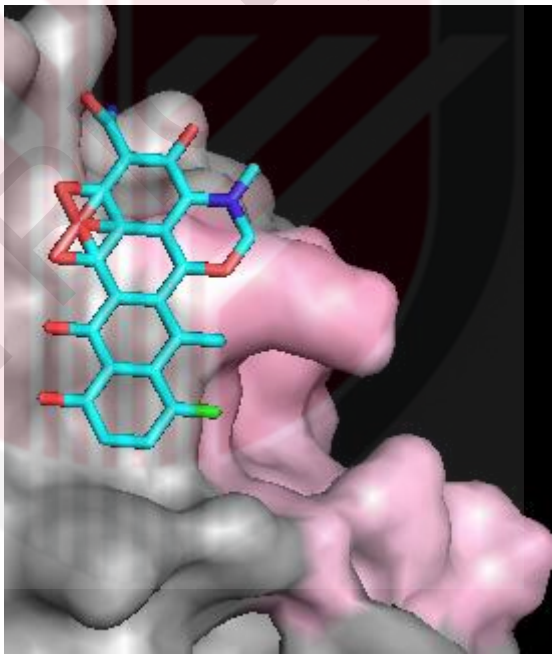


Figure 4.9: Binding mode of meclocycline to IL-1α. Meclocycline were docked to IL-1α with binding affinity of -7.1 kcal/mol. **(A)** Visualisation of protein-ligand interaction generated by PoseView. A number of moderate hydrogen bondings (black dashed lines) were identified. **(B)** PyMol visualisation of sulfasalazine bind to IL-1α.

CHAPTER FIVE

DISCUSSION

Inflammation is a body defense mechanism against harmful elements and pathogens invading the human body. The defense system is mediated by chemical mediators such as pro-inflammatory cytokines, chemokines, histamine, and prostaglandin. The focus of this study was on TNF-α and IL-1α, which are pro-inflammatory cytokines that plays crucial role as the hallmark of many inflammatory diseases including skin inflammatory diseases such as psoriasis. The over-expression of both pro-inflammatory cytokines due to progessive and continuous acute inflammation may result in chronic inflammatory response. Hence, by targeting both TNF-α and IL-1α the inflammation can be controlled and meditate effectively.

New and improved immunologically active drugs with higher target specificity provide better management of skin inflammatory diseases. Rapid action and diminishing of skin lesion or rash at early application are the benefits of these systemic drugs. However, the adverse effect outweighs the goodness in it. In the case of alefacept, malignancy or lymphoma can develop as a result of the use of these biologics. Immune suppression and congestive heart failure can also occur in extreme circumstances (Egeberg et al., 2018). Not to mention, the wide use of topical corticosteroid as alternative regimens in dermatological therapy in exchange of systemic drugs lead to tachyphylaxis highlights the drawbacks with current

treatment for skin inflammatory diseases. Hence, drug repurposing is proposed to meet the demands of new treatments for skin inflammatory diseases.

In this study, *in silico* technique approaches is implemented to identify alternative treatment for skin inflammatory diseases via drug repurposing of existing FDA-approved drugs by targeting the TNF-α and IL-1α. The pharmacophore model of frequently prescribed drugs for skin inflammatory diseases mainly focussed on non-biologic drugs was constructed to yield possible hit compounds as drugs candidates via virtual screening. The pharmacophore model highlights the similarity of pharmacophore features as well as the immunological targets shared within the drugs. A higher pharmacophore fit score indicates a better geometric alignment of the features of the hit compounds to the 3D-pharmacophore model. Molecular docking analysis was performed to validate the binding of ligand-receptor by using the scoring function. Both sulfasalazine and meclocycline showed best binding affinity to TNF-α and IL-1α.

5.1 Molecular Docking of Sulfasalazine and Meclocycline to Hydrophobic Pocket Residues of TNF-α and Interleukin-1α

Sulfasalazine and meclocycline were docked to the hydrophobic pocket residues of TNF-α to determine their anti-inflammatory potential. A lower vina score implies that the interaction between the drug and receptor is more robust and sustained (Meng et al., 2011). From docking analysis, the binding affinities of sulfasalazine to TNF-α ranging between -7.6 and -8.3 kcal/mol and meclocycline to TNF-α was between -7.2 and -7.6 kcal/mol respectively. Both ligand-receptor

interactions are considered as good interactions due to the lower vina scores which would indicate more stable ligand-receptor binding. Similarly, best interaction of protein ligand complexes also can be observed when both of the drugs bind to IL-1α. Sulfasalazine maintains stronger binding interactions to IL-1α which ranging between -7.1 and -8.1 kcal/mol. Along with that, meclocyclne also sufficiently bind to hydrophobic residues of IL-1α resulting in binding affinity ranging between -6.0 and -7.1 kcal/mol.

The presence of hydrogen bonds as well as the hydrophobic and pi-pi stacking interactions significantly influence the ligand binding affinity. The availability of hydrogen bonds was not only essential for maintaining the stability of molecular binding but it also influences the specificity of binding (M. A. Williams & Ladbury, 2003). Jeffrey (1995) classifies hydrogen bonds strength according to donor-acceptor lengths. For a distance between 2.2 Å and 2.5 Å as "strong, predominantly covalent," 2.5 Å to 3.2 Å as "moderate, predominantly electrostatic," and 3.2 Å to 4.0 Å as "weak, electrostatic". A number of strong, moderate and weak hydrogen bondings, hydrophobic interactions and pi-pi stacking are identified between the drugs and the proteins by using the visualizers. Accordingly, these interactions suggest good structural stability, which could result in potent TNF-α and IL-1α inhibitor. Thus, the ability of sulfasalazine and meclocycline to modulate the inflammatory actions can be assured.

5.2 The Therapeutic Potential of Sulfasalazine and Meclocycline

Recent studies have demonstrated the efficacy of sulfasalazine in the treatment of rheumatoid arthritis, although this medication has proven to be most useful in the treatment of inflammatory bowel disease. The drug has proven to be useful in the long-term therapy of ulcerative colitis, and it may also be beneficial in the treatment of Crohn's disease (Taffet & Das, 1983). The possibility of benefit in the case of alopecia areata has also been mentioned (Chairerg et al., 2012). Anorexia, nausea, vomiting, dyspepsia, and abdominal discomfort are the most commonly reported gastrointestinal side effects of sulfasalazine therapy. Therefore, sulfasalazine is prescribed as enteric coated tablets for the majority of patients, in order to reduce the risk of gastrointestinal side effects. Less frequently and more transiently, elevated liver enzymes and hepatic dysfunction occur (Mushtaq & Sarkar, 2020). Typically, discontinuing sulfasalazine would resolve the undesirable effects.

Historically, tetracyclines were commonly prescribed to treat a wide range of infections in the respiratory, gastrointestinal, and genitourinary systems (Moffa & Brook, 2015). Meclocycline is a tetracycline antibiotic synthesized from oxytetracycline that is frequently applied topically to treat acne vulgaris as meclocycline sulfosalicylate (Gelmetti, 2008). Aside from that, it also used to treat urinary tract infections, bacterial vaginitis, vulvovaginitis, and cervicitis. To stop the growth of bacteria, meclocycline blocks the production of new protein. It also limits bacterial adherence to human cells, which reduces pathogenicity of bacteria. This may be accomplished by preventing the bacterial cell surface from producing a specific protein. The common side effects associated with meclocycline include

diarrhea, loss of appetite, nausea, vomiting, headache, dizziness, light headedness, or rectal discomfort (Moffa & Brook, 2015).

Therefore, both sulfasalazine and meclocycline can be considered as safe due to their less severe side effects as compared to systemic drugs or TCS used to treat most skin inflammatory diseases. Moreover, the efficacy and therapeutic target of both drugs in managing skin inflammatory disease can be assured which subsequently increase the chances of effectiveness in managing the inflammation. This is due to antibacterial and anti-inflammatory properties that can be found in topical antibiotics like meclocycline resulting in suppression of leukocyte chemotaxis and reduction of pro-inflammatory free fatty acids in skin surface lipids (Moffa & Brook, 2015). Moreover, in addition to its anti-inflammatory and anti-proliferative properties, sulfasalazine provides a low-cost medication with little side effects (Willsteed et al., 2005). Not to mention, both of the drugs are actually widely used as off-label to treat certain skin inflammatory diseases such as psoriasis and acne (Mushtaq & Sarkar, 2020). However, due to lack of randomized trials as well as literature available on its use in dermatology lead to a limited number of people acknowledging the potential of both drugs in the dermatology field (Mushtaq & Sarkar, 2020). The wide spread of resistance, as well as newer, more bactericidal medications, has reduced the number of diseases for which they are now considered the best treatment (Moffa & Brook, 2015).

CHAPTER SIX

CONCLUSION

6.1 Conclusion

This study demonstrates the ability of sulfasalazine and meclocyline to bind to the binding sites of two pro-inflammatory cytokines, TNF-α and IL-1α thereby suggesting their potential to modulate the inflammatory activities of the cytokines and act as a skin anti-inflammatory agent. Sulfasalazine and meclocycline both exhibited good binding affinity as well as the pharmacophore fit score consequently increase the chances in recruitment of new treatment for skin inflammatory diseases. Moreover, with fewer side effects, these drugs have a bright future to be repurposed as alternate regimens in dermatology

6.2 Limitation

The study is restricted by the reliability of docking software itself to predict the interaction between ligand and receptor. Lack of confidence in the capacity of scoring functions to provide accurate binding energies is the most significant restriction of molecular docking (Sethi et al., 2020). This is because some intermolecular interaction parameters, such as solvation effect and entropy change, are difficult to forecast precisely. Moreover, different docking software resulted in

varying ranges of binding affinity calculated (Ramírez & Caballero, 2016). Not to mention, the majority of software performs molecular docking between a flexible ligand and a rigid receptor, while receptors are naturally flexible and undergo continual conformational changes (Sethi et al., 2020). Hence, the flexibility of receptors must be taken into account in order to effectively forecast binding events. There are, of course, a number of issues that need to be addressed. However, there is currently more than enough evidence to demonstrate the utility of computational tools in drug design, and there is no room for discussion regarding the effectiveness and advantage of computational tools in the drug discovery process (Sethi et al., 2020).

6.3 Future Recommendation

Several recommendations are proposed to establish the therapeutic potential of drug candidates in the field of dermatology.

1. To perform a Molecular Dynamic (MD) simulation studies on both sulfasalazine and meclocycline by employing GROMACS software to predict the interaction of these drugs to protein targets in aqueous solution as the stability and flexibility of these drugs in biological environment can be assured.

2. To conduct an *in-vitro* studies of both sulfasalazine and meclocycline on skin cells induced with skin inflammation such as A431 skin carcinoma cell line utilising:
    a. Cytotoxicity test
    b. Skin inflammation assay
    c. ELISA test
3. To execute *in-vivo* studies of both sulfasalazine and meclocycline using an animal model such as mice induced with psoriasis to predict the effectiveness of both drugs in controlling the inflammation.

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