SYNTHESIS AND SAR OF ACNE MEDICATION

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelors of Pharmacy

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis/project titled "Synthesis and SAR of Acne Medication" submitted by Tasnia Naureen (19146043) of Summer, 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy.

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Ethics Statement

The study has been conducted ethically. It did not involve any human or animal trial.

Abstract

This undergraduate research thesis explores the synthesis and structure-activity relationships (SAR) of drugs used for the treatment of acne vulgaris, which affects approximately 85% of individuals between the ages of 12 and 25. The objective of this review is to investigate the etiology of acne, current therapies, drugs used for treatment, their SAR, and synthesis. The pathophysiology of acne is multifactorial and includes increased sebum production, aberrant follicular keratinization, an increase in Propionibacterium acnes, and inflammation. The methodology used for this study involved searching for relevant articles on Google Scholar, PubMed, and Web of Science. Additionally, the study delves into the enzyme 5α-reductase, which may be the root cause of androgen production and consequently the development of acne. The review article indicates that an excess of precursor androgens, particularly in individuals with severe acne, can lead to hormonal imbalances and the development of the condition. The synthesis of newer acne medications and further research in this field will provide a better understanding of the varying degrees of effectiveness of acne medication from person to person.

Keywords: Acne vulgaris, Synthesis, SAR, Androgen, 5α-reductase, Inhibitors

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List of Acronyms

A	Androstenedione
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone sulphate
T	Testosterone
DHT	Dihydrotestosterone
3α-diol G	3α-diol glucoronide
S5αR	Steroid 5α-reductase
17β-HSD	17β-hydroxysteroid dehydrogenase
3BHSD	Adrenal 3*β*-hydroxy-Δ^5-steroid dehydrogenase/3-keto-Δ^5-steroid isomerase

Chapter 1

Introduction

Acne vulgaris, the most common form of acne, affects around 85 percent of persons between the ages of 12 and 25, making it the most prevalent skin illness. Acne, on the other hand, is not in any way restricted to adolescents and young people. Patients in their late twenties, sometimes in their thirties, and even in their forties are being seen in ever-increasing numbers. (Leyden & Shalita, 1986)

Acne vulgaris is a chronic disease of the skin, generating in the pilosebaceous region. It is an inflammatory condition which eventually clears up on its own. This disorder can develop by itself but has a long course. During puberty, Propionibacterium acnes, in conjunction with the presence of normal levels of circulating dehydroepiandrosterone (DHEA), are responsible for the development of acne vulgaris. It is a relatively common skin condition that can manifest itself in inflammatory or non-inflammatory lesions, most commonly on the face, but it can also take place on various other parts of the body such as the arms and back.

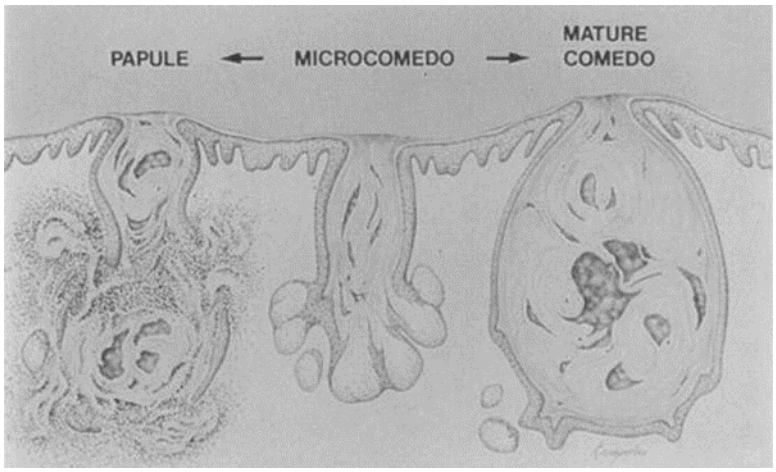


Figure 1 Microcomedo consisting of sebum and keratinous debris which develop into mature comedo (non-inflammatory) or papule (inflammatory). (Berson & Shalita, 1995)

Acne vulgaris pathophysiology is multifactorial. During puberty, higher levels of androgen cause the sebaceous glands to expand and create more sebum in the sebaceous follicle (Figure 1). Following aberrant keratinization and hyperkeratosis of the follicular epithelium, a horny plug obstructs the duct. Sebum and keratin fill up the duct, generating a microcomedo, the forerunner to all abrasions of acne. Further exacerbation results in the formation of non-inflammatory, open and closed comedones. The abundant sebum in the microcomedo gives

Propionibacterium acnes a medium where the bacteria can freely grow in anaerobic conditions. Bacterial lipases break down sebum triglycerides into free fatty acids that can cause further irritation and comedones. *P. acnes* also releases neutrophil-attracting chemotactic factors. As shown in Figure 2, lysosomal enzymes secreted by neutrophils breach the follicular wall, therefore secreting proinflammatory mediators, such as keratin and lipids, into the neighbouring dermis. The consequence is inflammatory papules and pustules. Cysts and nodules result from macrophage- and foreign-body-induced inflammation. Increased sebum production, aberrant follicular keratinization, an increase in *P. acnes*, and inflammation are, thus, the four key components in the pathophysiology of acne. (Berson & Shalita, 1995)

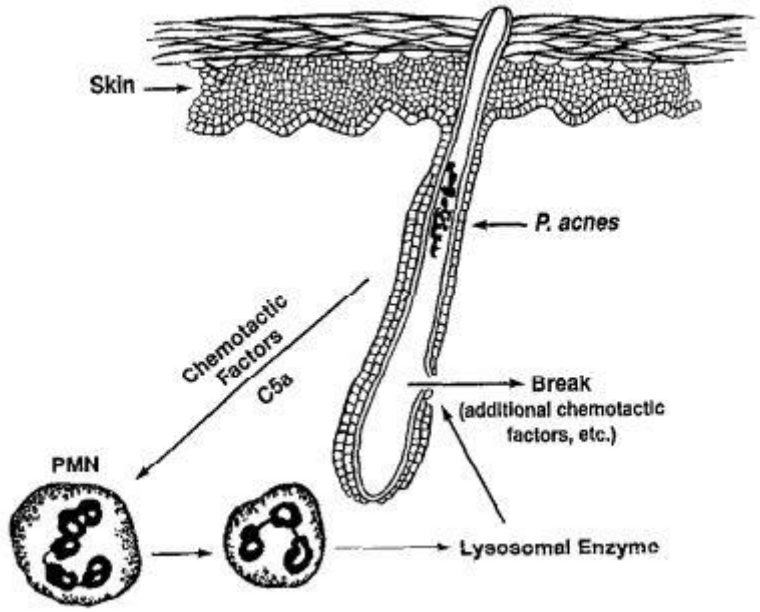


Figure 2 Release of chemotactic factors by *P. acnes*, causing polymorphonuclear leukocytes to release lysosomal enzymes. (Berson & Shalita, 1995)

1.1 Objective

Acne vulgaris affects 85% of the population, making it one of the most common disorders affecting young persons. It affects their physical, as well as mental, wellbeing and greatly reduces their quality of life. For many patients of acne, finding the correct medication and dosage becomes a long and strenuous endeavour. Therefore, it is important to study the details of the pathology of acne and its available medications. This review article focuses on the etiology of the disease, current acne therapy, the drugs used to treat the disease, their structure-activity relationship, as well as synthesis.

Although several synthetic methods are currently available for the preparation of drugs for acne therapy, as well as availability of SARs of several different compounds, an assessment of the current technology in the field can help us better understand the varying degrees of effectiveness of acne medication from person to person. It can also provide a basis for future synthetic work and newer acne medications.

1.2 Methodology

The methodology used for this thesis was finding relevant articles on Google Scholar, PubMed and Web of Science. Relevant articles were looked into at first to find gaps in knowledge for the treatments available for acne. References were found from forward citations of highly referenced articles, as well as cross references within the articles for further literature study.

From the research, looking further into the enzyme 5α-reductase proved to be interesting. It was found that this enzyme may be the root cause of androgen production, and consequently development of acne. Therefore, this approach has been taken for further study in this paper.

1.3 Androgen production and incidence of acne vulgaris

A surplus of androgen is an etiologic factor linked to patients suffering from acne. Androstenedione (A), dehydroepiandrosterone sulphate (DHEA-S), and testosterone (T) are the three main plasma precursor androgens found in females. DHEA-S and A are prehormones that can be converted peripherally to more active androgens. (Figure 3) They are derived primarily from secretion occurring from the glands (DHEA-S is released from the adrenal glands and A is released from both the ovaries and the adrenal glands). (Lookingbill et al., 1985) T is produced by the peripheral conversion process, which takes place in the fat, muscle, and other tissues, as well as direct glandular secretion in women. Women with severe acne have elevated levels of T and/or DHEA-S. Therefore, it can be seen that the development of acne is influenced by excess precursor androgens, especially in those with severe acne. The target tissues produce more androgens which may be the cause behind the hormonal imbalance in such patients. (Lookingbill et al., 1985) According to this hypothesis, affected skin has a higher level of activity of the steroid 5α-reductase (S5αR). This enzyme converts testosterone into dihydrotestosterone (DHT), which is a more powerful tissue androgen. (Lookingbill et al., 1985) After that, dihydrotestosterone (DHT) can undergo additional metabolization to produce 3α-androstanediol (3α-diol), after which the inactive metabolite, 3α-diol glucoronide (3α-diol G), is formed from a coupling reaction with glucoronide. After this step, 3α-diol G is

transformed into a more polar compound before being released into the circulation. An elevated level of 5α-reductase in vitro in the skin of acne patients was shown, which further adds evidence to the theory that tissue androgen production is responsible for acne. (Lookingbill et al., 1985)

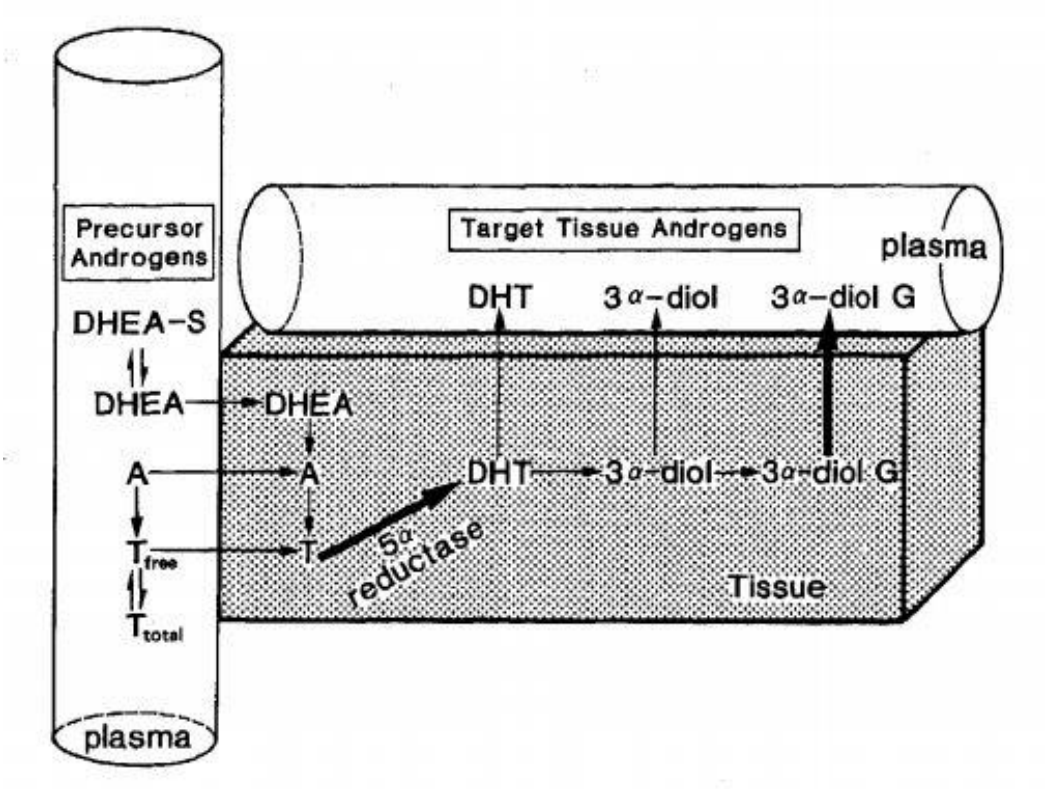


Figure 3 Production of tissue androgen in women. (Lookingbill et al., 1985)

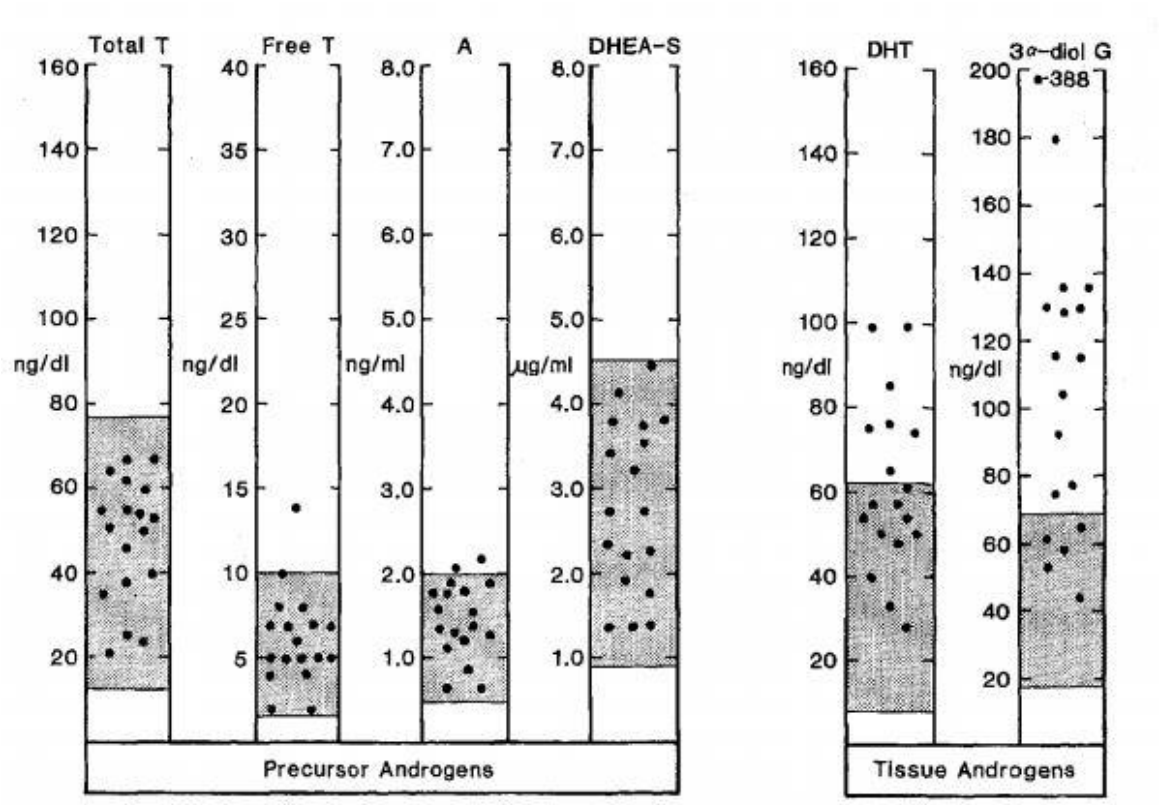


Figure 4 Eighteen acne-prone women's plasma androgen. Total T and DHEA-S are normal. 3α-diol G is elevated more often than DHT among tissue-produced androgens. (Lookingbill et al., 1985)

As androgens have been found to have been found to be a leading cause in the incidence of acne, the approach for effective treatment can be shifted towards compounds that produce anti-androgenic action. DHT is a potent androgenic hormone, which mainly comes from reduction by S5αR. However, it has higher affinity towards the single androgen receptor. (Srivilai et al., 2017) Therefore, curcuminoids can be used to treat acne as curcumin analogues were found to have androgenic receptor antagonist activity. In Asian traditional medicine, the plant species Curcuma longa L., which is a member of the Zingiberaceae family, has been utilised to treat a wide variety of conditions, including liver problems, peptic ulcers, biliary disorders, flatulence, and skin diseases. Curcumin is one of its primary components, known as curcuminoids. Two other molecules, demethoxycurcumin and bisdemethoxycurcumin, are considered to be minor components. Antioxidants, anti-inflammatories, antimicrobials, and anticancer properties can all be attributed to curcuminoids' pharmacological activity. (Srivilai et al., 2017)

1.4 Relationship between steroid 5α-reductase in the development of acne

As one of the pathogenesis of acne involves the enlargement of sebaceous glands, which is caused by androgens during puberty, the enzyme causing androgen production can be studied. The sebaceous glands and whole skin collected from the face, scalp, and other areas of the body that are not prone to acne have been discovered to largely contain type 1 S5αR. This enzyme's activity is concentrated in these regions, although more activity was identified in the skin of the face and scalp, which are also areas that are more prone to acne. In addition, it has been discovered that the sebaceous glands are in control of the conversion of T to DHT via the type 1 S5αR. Skin that has developed acne had higher levels of DHT than normal skin. This suggests that people with acne may be more sensitive to androgens at the end-organ level, which could be due to the rate at which testosterone is converted to DHT. (Thiboutot et al., 1995)

Profiles created using pH can be used to investigate the amounts of each isoenzymes, as they show different activities in different pH. Figure 5 depicts the pH dependency of the S5αR in a sebaceous gland homogenate taken from the scalp. At a pH between 6.0 and 8.0, the activity level is at its peak. At a pH of 8, 17β-hydroxysteroid dehydrogenase (17β-HSD) was seen to convert testosterone into androstenedione at a higher rate than before. The generation of DHT followed a linear time course that ranged from ten minutes to one hour. Because of the extended incubation durations, the 17β-hydroxysteroid dehydrogenase (17β-HSD) enzyme was able to convert a greater quantity of the substrate, testosterone, into androstenedione. (Thiboutot et al., 1995)

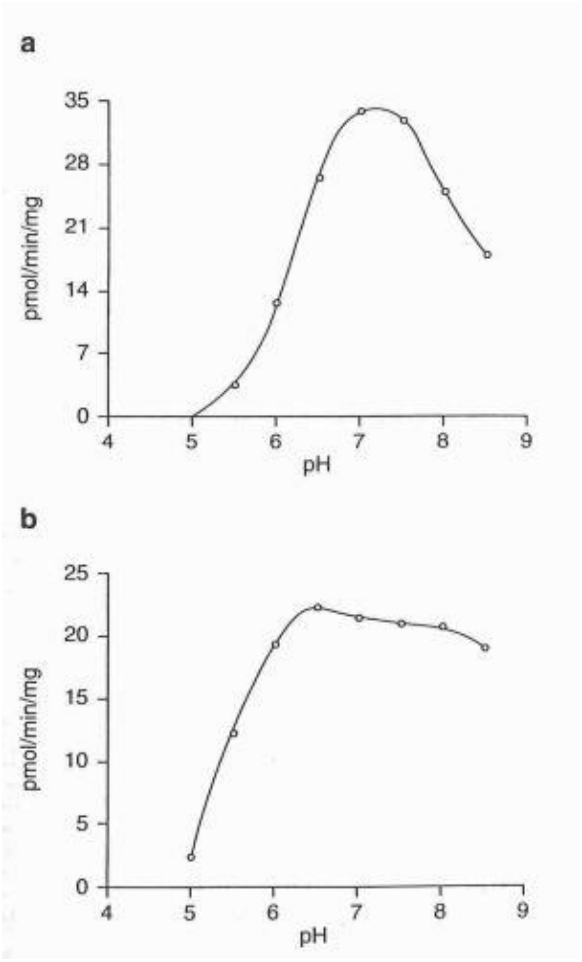


Figure 5 a. In individual scalp sebaceous glands. At pH 7, where 5α-reductase activity peaks, the type 1 isozyme is present. The activity of 17β-hydroxysteroid dehydrogenase increased at higher pH levels. b. 5α-reductase exhibits peak activity in the entire scalp tissue at pH 6 through 8, further demonstrating the presence of the type 1 isozyme. (Srivilai et al., 2017)

In order to determine whether or not L-733,692 and L-652,918 were able to block S5αR activity, they were tested. L-733,692 was a powerful inhibitor of the type 1 isoenzyme and enzyme activity in the sebaceous glands, whereas L-652,918 was a selective inhibitor of the type 2 isoenzyme and showed poor inhibitory activity in the sebaceous glands. Both compounds were subject to tests for their ability to inhibit enzyme activity in the sebaceous glands. Isotretinoin was also examined for its capacity to suppress enzyme activity, although the results indicated that it had a weak effect on either type of isoenzyme. (Thiboutot et al., 1995)

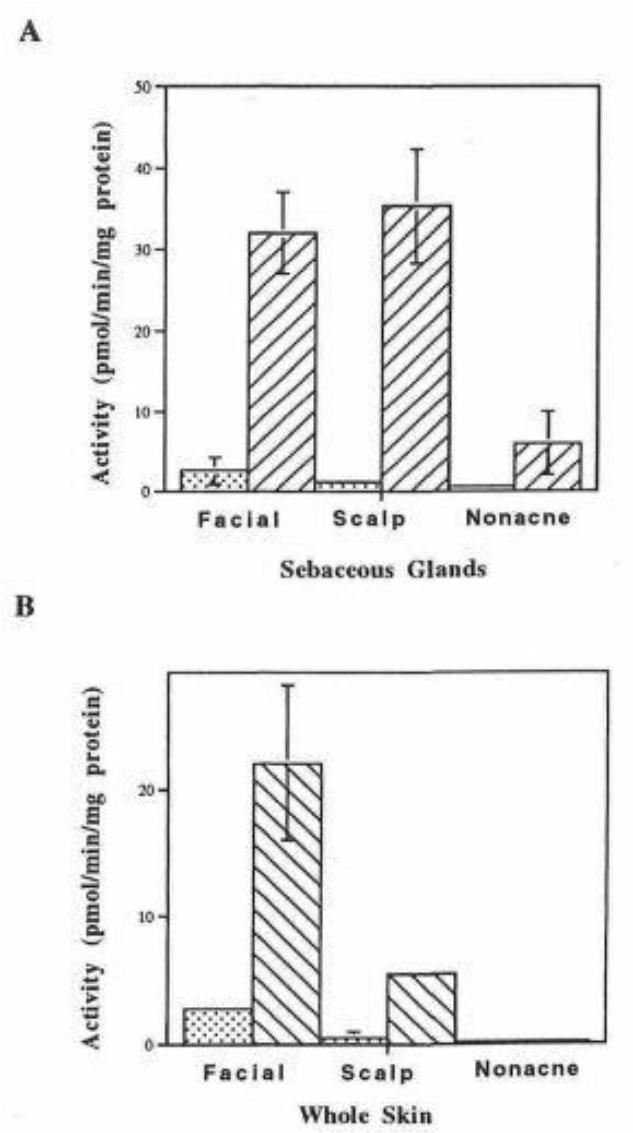


Figure 6 A. Activity of 5α-reductase in isolated sebaceous glands. B. Activity of 5α-reductase in samples taken from whole skin. Activity of type 1 isoenzyme is more in both sebaceous glands and whole skin. (Srivilai et al., 2017)

Samples were taken from face, scalp and non-acne prone skin. These samples were incubated with testosterone to determine the activity of each type of isoenzymes. Figure 6 shows activity of samples of homogenates of isolated sebaceous glands, type 1 isoenzyme showed higher activity in all the samples taken. Samples of whole skin were also taken, with higher activity of type 1 isoenzyme as well. (Thiboutot et al., 1995)

1.5 Available treatment for acne vulgaris

1.5.1 Tretinoin

Tretinoin, also known as all-trans retinoic acid, is a comedolytic agent that has proven to be the most successful. The beneficial effects of tretinoin include influencing the desquamation of irregular epithelium, changing the microclimate of microcomedones, altering the environment of individual follicles, resolving developed comedones, preventing fresh lesions from developing, and enhancing the penetration of other drugs. (Berson & Shalita, 1995)

As a result of tretinoin's photoirritant properties, sunscreens should also be worn concurrently. Often, six weeks of continuous therapy are necessary for significant clinical improvement (Figure 7). Three to four months may be required for effective improvements. (Berson & Shalita, 1995)

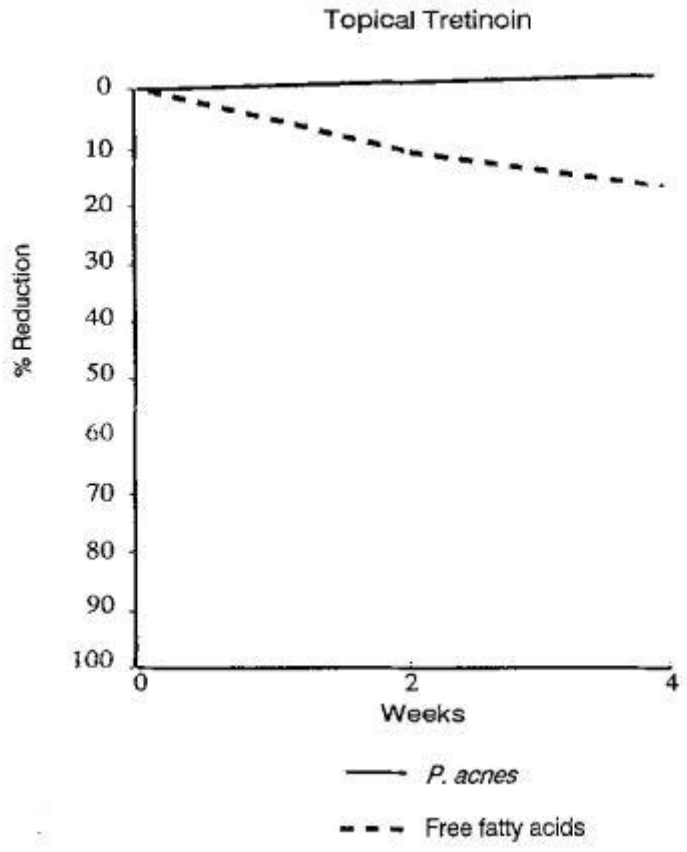


Figure 7 Effect of topical tretinoin on the population of *P. acnes* and concentration of free fatty acids. It is shown here that application of topical tretinoin causes both to reduce. (Berson & Shalita, 1995)

1.5.2 Benzoyl peroxide

Benzoyl peroxide is a highly effective bactericidal medication. It decreases the growth of *P. acnes* in the sebaceous follicle by producing reactive oxygen species. Additionally, the free fatty acids that initiate the alterations which cause retention hyperkeratosis and microcomedo development decrease. (Berson & Shalita, 1995)

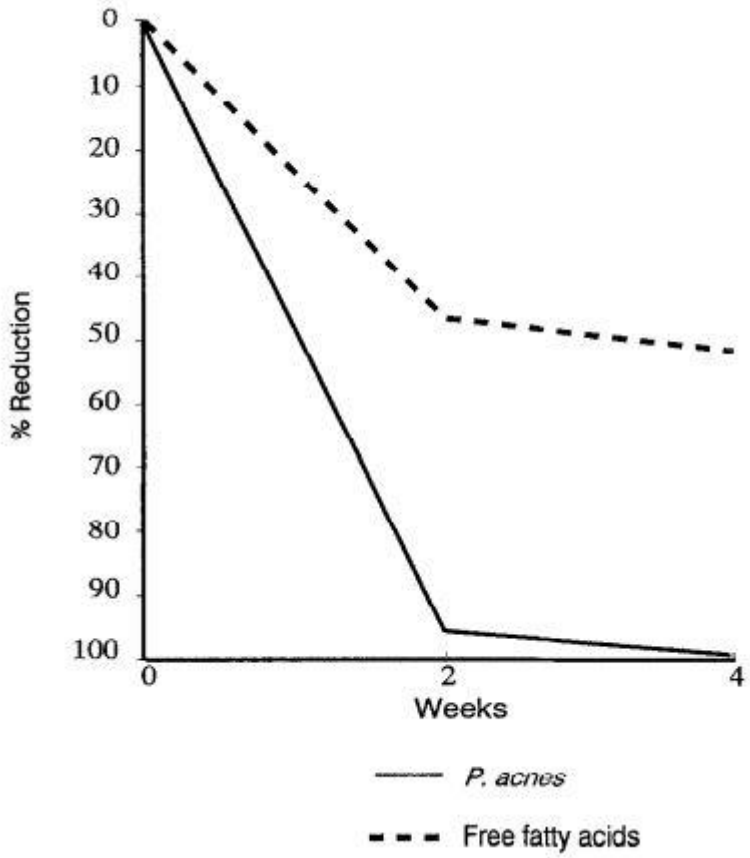


Figure 8 Percentage reduction in population of *P. acnes* and concentration of free fatty acids due to topical benzoyl peroxide. (Berson & Shalita, 1995)

1.5.3 Topical antibiotics

Topical antibiotics that have shown effectiveness in treating acne include erythromycin and clindamycin. These medicines decrease the number of *P. acnes* on the surface of the skin and in the hair follicles. Antibiotics exhibit anti-inflammatory characteristics through inhibition of chemotaxis and lowering the proportion of free fatty acids that are capable of causing inflammation in lipids found on the surface, as well as antibacterial capabilities. Antibiotics

lack comedolytic properties and, hence, have no impact on established comedones. (Berson & Shalita, 1995)

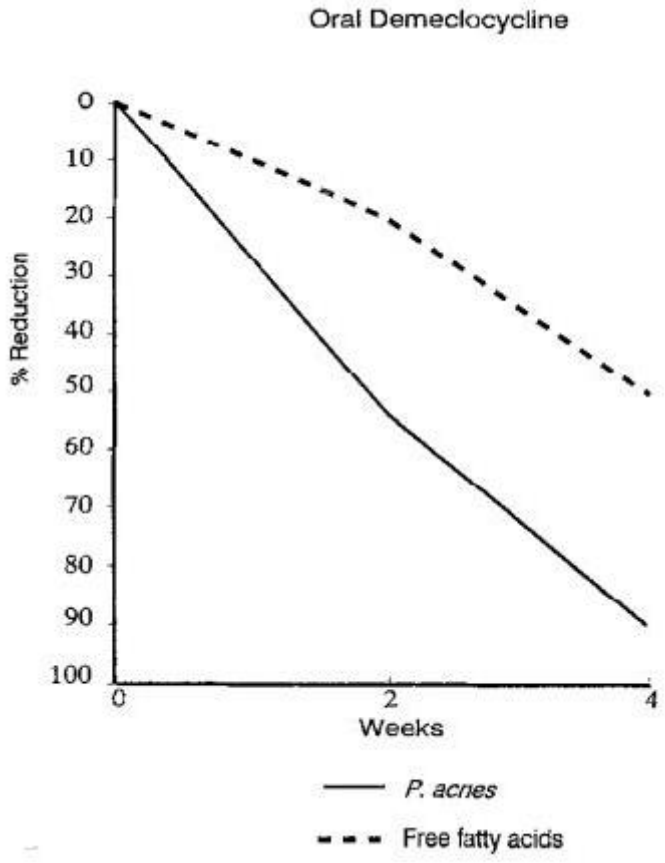


Figure 9 Percentage reduction in population of *P. acnes* and concentration of free fatty acids due to oral antibiotic. (Berson & Shalita, 1995)

1.5.4 Combination topical therapies

The effectiveness of topical antibiotics is improved when they are used in combination with tretinoin, as it aids in better penetration of the agents. Tretinoin is effective at lowering hyperkeratosis, which in turn lessens the degree to which bacteria is able to grow in the anaerobic medium created within the follicle. Topical antibiotics and benzoyl peroxide suppress *P. acnes*, chemotaxis of white blood cells such as neutrophils, and further production of free fatty acids. This is how the concurrent use of tretinoin, as well as, a topical antibiotic can reduce keratinization, P. acnes growth, and papule formation. (Berson & Shalita, 1995) When all types of lesions are evaluated, the concomitant use of tretinoin and topical clindamycin is clinically more beneficial than the use of either drug alone. With the addition

of clindamycin, the irritating effects of tretinoin are reduced, making it more tolerable. When clindamycin and benzoyl peroxide are combined, the number of lesions and irritation are also reduced. The successive use of tretinoin and topical erythromycin improves papules, pustules, and comedones more efficiently than either monotherapy for treating mild inflammatory acne. Because it reduces inflammation, erythromycin may decrease the premature flare that is caused by tretinoin therapy in most cases. This combination may reduce the requirement for long-term oral antibiotics. (Berson & Shalita, 1995)

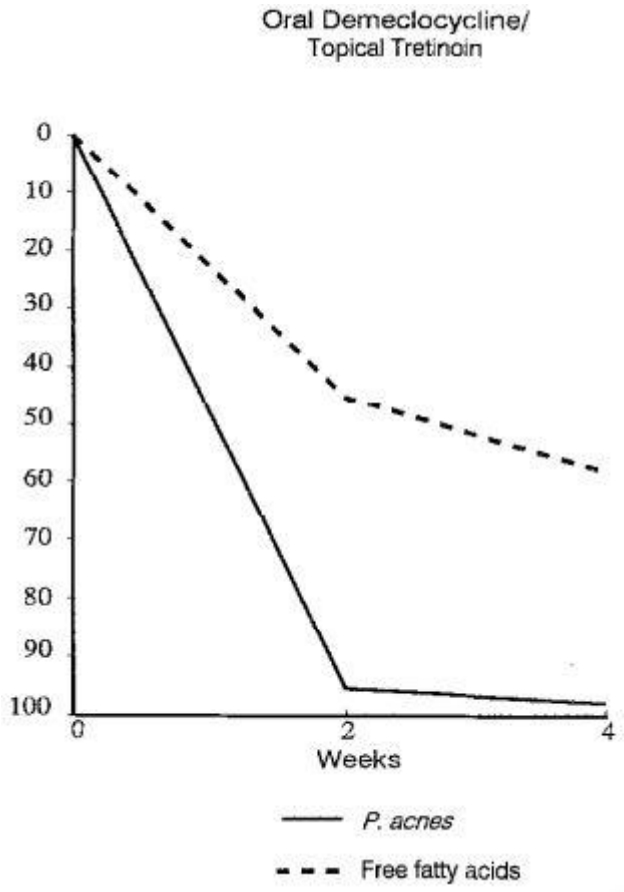


Figure 10 Percentage reduction in population of *P. acnes* and concentration of free fatty acids due to combination of oral antibiotic and topical tretinoin. (Berson & Shalita, 1995)

1.5.5 Systemic therapies

When inflammatory acne fails to show any response to topical combinations, systemic medicines might be added to the regimen, such as tetracycline, erythromycin, minocycline, doxycycline, and trimethoprim-sulfamethoxazole. (Berson & Shalita, 1995) They inhibit the growth of *P. acnes* and reduce the proportion of free fatty acids in surface lipids at suitable

doses. Tetracycline and oral erythromycin lower the number of papules and pustules in inflammatory acne. (Berson & Shalita, 1995) Tetracycline blocks lipase, chemotactic factors, and neutrophils and reduces *P. acnes*, while erythromycin inhibits *P. acnes* and reduces inflammation. Although *P. acnes* has a tendency to acquire resistance to both tetracycline and erythromycin, erythromycin resistance is stronger. Additionally, its usage is typically restricted by adverse effects in the GI tract, but it is an excellent choice for photosensitive people. Minocycline is a powerful antibacterial antibiotic that can permeate the sebaceous gland and reduce *P. acnes* growth. It is less likely to cause gastrointestinal distress and phototoxic responses. Gradual increases in the drug's dosage can effectively manage the vertigo-like symptoms. Although equally suitable for treating acne, doxycycline can be more sensitive to light. Trimethoprim-sulfamethoxazole might be used to treat the infection when other antibiotic treatments are ineffective. It works for severe acne and gram-negative folliculitis because it is fat soluble. This agent is responsible for causing serious but uncommon side effects, such as drug eruptions and bone marrow suppression. Antibiotics are often used regularly for extended durations (4 to 6 months). Adverse effects can be reduced by changing the dose and by introducing tretinoin treatment after the withdrawal of the antibiotics. The combination increases effectiveness and accelerates due to the improved penetration of an antimicrobial agent by the comedolytic agent. (Berson & Shalita, 1995)

1.5.6 Isotretinoin

Isotretinoin (13-cis retinoic acid) is suitable for individuals with nodulocystic acne and severe inflammatory illnesses who have failed to respond to therapy with the combinations mentioned thus far. Isotretinoin affects sebaceous gland activity and directly affects irregular follicular keratinization, which no other drug is able to do. Therefore, the growth of *P. acnes* and the subsequent production of proinflammatory mediators are reduced, resulting in the successful treatment of all four pathogenic factors influencing acne vulgaris. During therapy with isotretinoin, the size as well as the number of cysts decrease significantly, with optimum improvement seen during post treatment. Nonetheless, this medication is teratogenic. Women of reproductive age are instructed to begin taking the medication on the second day of their menstrual cycle, and additional contraception must be provided for the duration of therapy and one month afterward. Moreover, the contraception must be carefully prescribed so as not to cause interactions.

Isotretinoin can also be given systemically, which is a highly effective medication for managing severe types of acne, resulting in long-lasting remissions even when treatment is discontinued. The majority of patients require only a single session of isotretinoin medication; however, about 25% of individuals need further treatment. (Berson & Shalita, 1995)

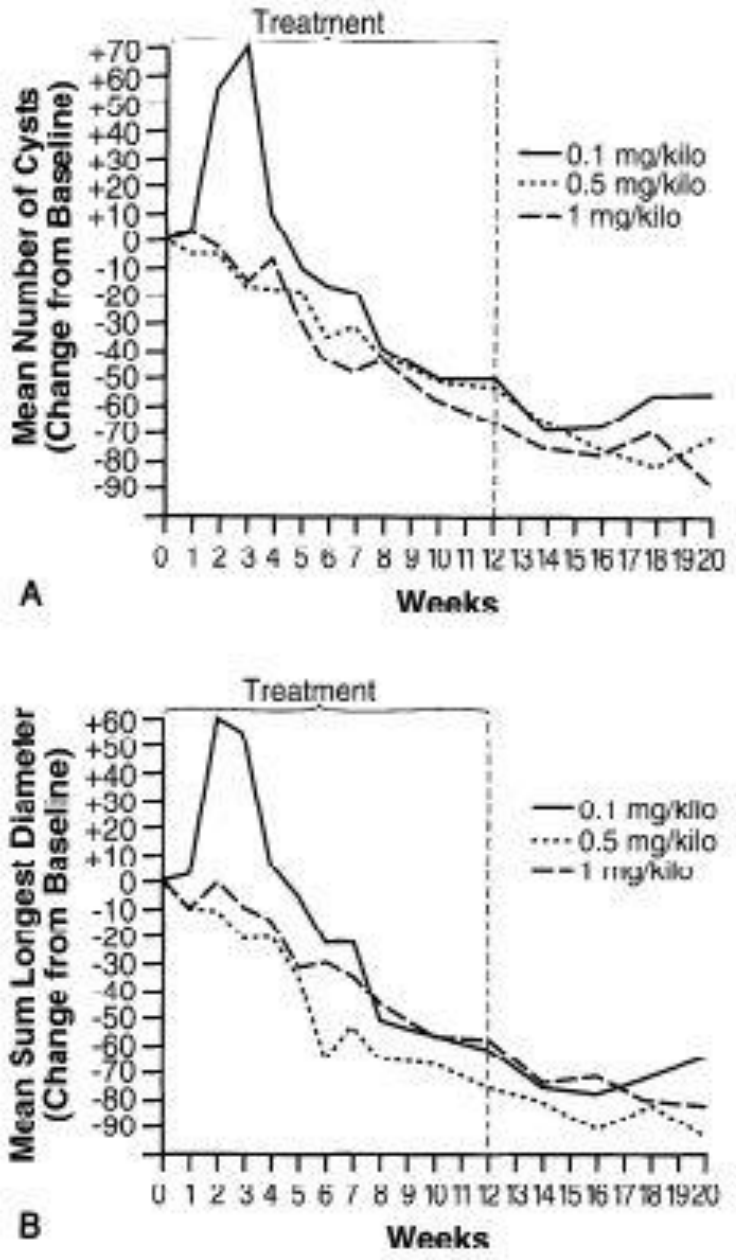


Figure 11 A. How production of sebum changes due to usage of 0.1, 0.5, and 1.0 mg/kg per day of 13-cis retinoic acid. B. How diameter changes from usage of 0.1, 0.5, and 1.0 mg/kg per day of 13-h retinoic acid. (Berson & Shalita, 1995)

1.5.7 Hormonal therapy

Patients who suffer from severe acne that is resistant to treatment and are not able to receive Isotretinoin treatment may benefit from hormone replacement therapy. Indications of hormonal involvement include the presence of adult-onset, chronic, and inflammatory acne; premenstrual flares; development of acne on the lower face, jawline, and chin; increased oiliness in the face. (Berson & Shalita, 1995) The blood levels of dehydroepiandrosterone sulphate and free testosterone may be tested in the laboratory to determine whether or not hirsutism, increased frequency of hair fall, or monthly irregularities are present. Systemic hormonal treatment diminishes sebum production by preventing the effects of androgens, which are male hormones, from having an impact on the sebaceous gland. (Berson & Shalita, 1995) At the moment, hormone therapies are only used for treating systemic acne in female patients. Oestrogens, glucocorticoids, and systemic antiandrogens are the three alternatives available. Oestrogens suppress adrenal androgen, glucocorticoids reduce ovarian androgen, and systemic antiandrogens mostly function in the peripheral. Oral contraceptive pills include low amounts of oestrogens; studies have shown that dosages ranging from 35 to 50 g may reduce the amount of sebum produced by the body. When used with low-dose corticosteroids, such as 5 mg of prednisone, oestrogen has the ability to provide a greater degree of suppression. Prednisone and dexamethasone are two examples of low-dose glucocorticoids that have the potential to block and suppress testosterone production in addition to their anti-inflammatory effects. Spironolactone is a potent steroid derivative that also acts as an anti-androgen. It does this by competing with androgens for the receptor sites and by acting on the sebaceous follicle to reduce sebum production. Spironolactone inhibits the production of androgen in the adrenal glands as well as the ovaries. (Berson & Shalita, 1995) However, lower amounts may be used in combination with other drugs as an adjuvant therapy if necessary. The dosage varies from 50 to 200 milligrammes per day. Cyproterone acetate, flutamide, cimetidine, and ketoconazole are additional anti-androgens. Cyproterone acetate is in competition with androgens at the sites where they bind to receptors. It is useful for acne treatment at low dosages (2 mg) when taken with oestrogen (35 micrograms). When it comes to treating hirsutism, flutamide is the nonsteroidal androgen antagonist that has shown to be the most successful. (Berson & Shalita, 1995)

1.6 Adapalene for the treatment of acne

The synthetic naphthoic acid derivative known as adapalene, which exhibits retinoid activity, has been shown to be capable of reversing the abnormal follicular desquamation and inflammatory reactions that are involved in the formation of acne. Adapalene has been demonstrated to be successful in treating acne in a vast number of clinical investigations, both on its own as a monotherapy and in combination with other antibiotics that are either topically applied or taken orally. These antibiotics may be applied to the skin or taken by mouth. Additionally, it has been shown that adapalene is better tolerated than other topical retinoids, including all types of tazarotene and tretinoin. This is an important benefit of using adapalene. There are a number of different formulations of adapalene available, including gel, cream, pledgets, and solution. Nevertheless, the concentration may not exceed 0.1% at any time. According to the results of a research, the efficacy of adapalene gel with a concentration of 0.3% is significantly improved. (Thiboutot et al., 2006)

Figure 12 Adapalene gel 0.3% used by the patient shown over a period of 12 weeks, starting from baseline. (Thiboutot et al., 2006)

It was found that adapalene gel 0.3% was significantly better at treating inflammatory lesions of acne than adapalene gel 0.1%. The ability of adapalene to lower comedones and inflammation is dependent upon the dosage used. (Thiboutot et al., 2006) The higher concentration of adapalene gel used showed effects faster than the 0.1% concentration. Even though both concentrations of the gel did not show any adverse effects, such as irritation of the skin, patients with skin sensitivity to topical retinoids would be better off using the 0.1% adapalene gel at first, and increasing to 0.3% if necessary. (Thiboutot et al., 2006)

Chapter 2

Structure-activity relationships

It is important to study SAR of compounds, as it provides information that is imperative to the formulation of novel drugs. SAR of existing drug molecules are researched so investigate where the drug is lacking and how effect of the drug can be optimized.

Drugs that are currently available only affect the pathological factors that cause acne. However, the main source for the generation of acne has been found to be androgen production by 5α-reductase enzyme. Therefore, by examining binding pockets of this enzyme, and optimizing the effects so that other biological functions are not hampered, novel drugs can be synthesized that work better than existing drugs.

2.1 SAR of 5-α reductase inhibitors

Both isoenzymes of S5αR result convert testosterone to dihydroxytestosterone (DHT). It is important to block both of the isozymes in order to prevent this decline from occurring. While both of the isozymes have the same effect on testosterone, they are not distributed in the same tissues, and they have different amino acid sequences as well. Despite this, their actions on testosterone are the same. A powerful enzyme that plays a role in the manufacture of steroids is known as adrenal 3β-hydroxy-Δ^5-steroid dehydrogenase/3-keto-Δ^5-steroid isomerase (3BHSD). In addition to potency against both S5αR isozymes, selectivity against human adrenal 3BHSD has been discovered. This is significant because an important steroidal pathway is blocked if this enzyme is inhibited. This is an important selectivity criterion for the majority of S5αR inhibitors since it is caused by the similarities in the conversions that are catalysed by S5αR and 3BHSD. (Kurup, Garg, & Hansch, 2000)

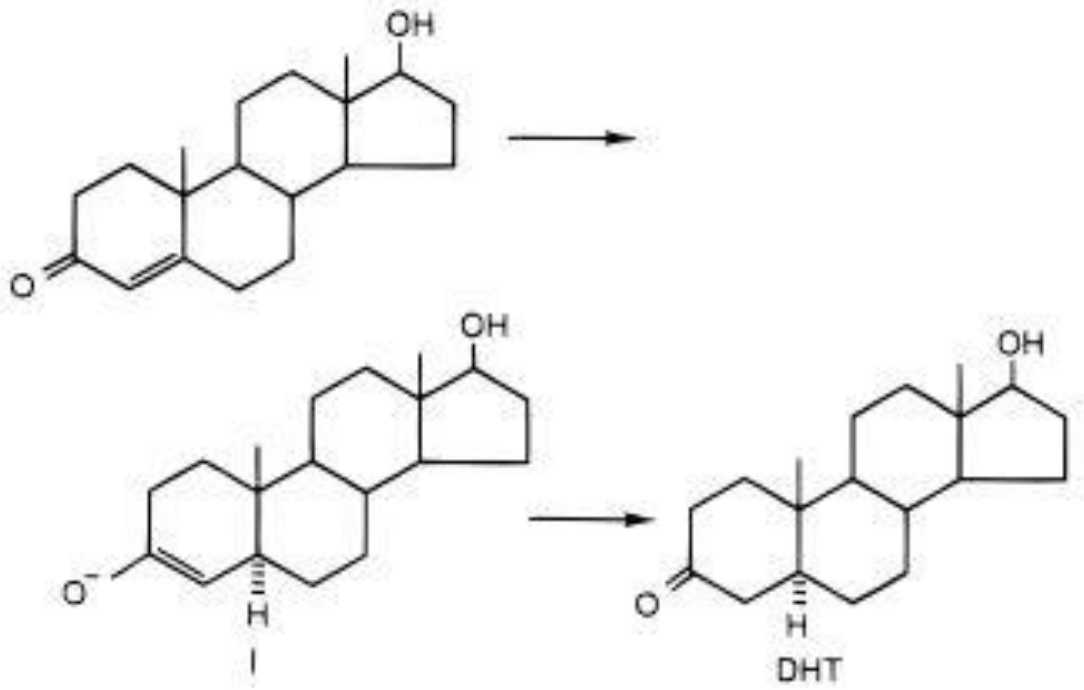
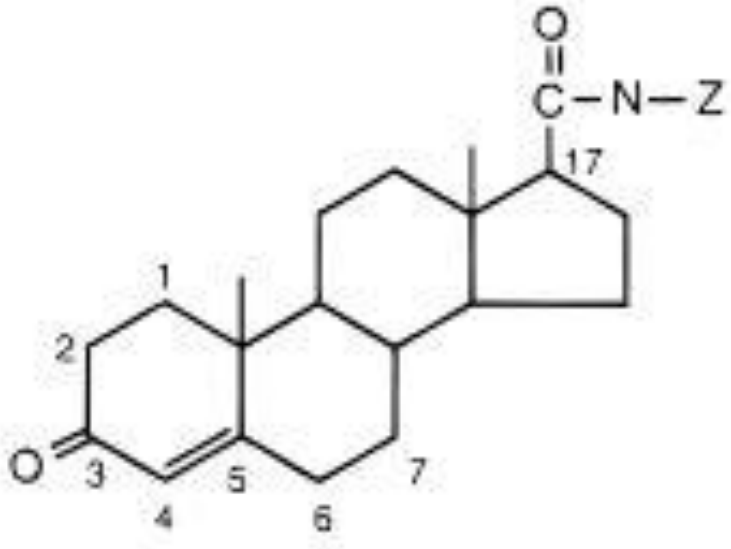


Figure 13 Conversion of testosterone to DHT by enzyme 5*α*-reductase, using NADPH as a cofactor. (Kurup, Garg, & Hansch, 2000)

Because all of the molecules, with the exception of estrien-3-carboxylic acids, are azasteroids, displaying inhibitory action, four or six azasteroids are ideal molecules to inhibit all three different types of enzymes.



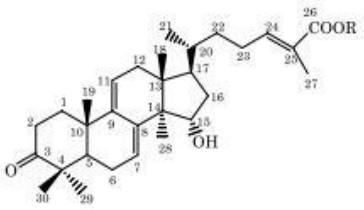
It would appear that there is a hydrophobic region where C-17 substituents engage, whereas the interaction at the other sites is predominately steric in nature. This provides more evidence

that the presence of a lipophilic group at carbon 17 is extremely advantageous. Unsaturated ketone, particularly those with the ketonic group at C-3 and the unsaturation at C-4, contribute positively to the inhibition of human S5αR types 1 and 2, and a conjugated system at C-3, -4, and -5 performs well. It appears that having a double bond at C-1 in addition to the one at C-4 has a very negative impact on the activity. Further, it is important to point out that substances containing a double bond at the C-1 position are said to be irreversible inhibitors of the enzyme. Because of its length, any substituent at position C-4 seems to cause steric hindrance in the process of binding to the receptor. On the other hand, groups with smaller bulk appear to be well tolerated. Compounds with substituents in the fourth position, such as Me, chlorine, or bromine, have high levels of activity. The presence of substituents at position C-6 results in steric hindrance for type 1 enzymes. The use of substitutes in this position has been shown to have a negative impact on the activities. It appears that groups at position C-7 are also experiencing linear steric hindrance while trying to bind to the receptor. When the volume and length are increased, the substituents have a negative effect on the activity. It would appear that there is a slight electrical impact caused by the groups at the 4-N-X and 7-Y positions. Electron-donating groups appear to be beneficial to activity through the use of inductive effects. In the case of type 2, the activity is diminished by any substituent at position C-6. It would be beneficial for the -CON group to be present at C-17. Additionally, when an aryl group is linked to a CON- group, the action is amplified. The aryl ring most likely binds in the enzyme's hydrophobic site, and the presence of substituents at the ortho position of the aryl ring improves this binding. (Kurup, Garg, & Hansch, 2000)

Rat S5αR differs from human S5αR in that, unlike S5αR, the presence of the -CON group reduces S5αR activity. However, the presence of the CONH group is extremely important for the development of the activity. The hydrophobicity is also very significant, with a value of 5.10 being optimal. Even in this case, the existence of a double bond at carbon-1 seems to have a negative impact on the activity. In the case of 3BHSD, the most notable distinction can be seen in terms of the substituents that are located at carbon number 17. Steric obstruction to the binding can be caused by bulky groups at position C-17. In contrast to S5αR, the activity is inhibited by substituents that are located in the ortho position of the phenyl ring that is linked to –CONH at carbon 17. On the other hand, similar to S5αR, less bulky groups such as Me and Cl at the 4 position appear to be well tolerated, whereas the presence of a double bond at the C-1 position is detrimental to the activity. (Kurup, Garg, & Hansch, 2000)

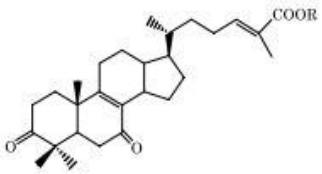
2.2 5α-reductase inhibition by triterpenoids

Compounds derived from *G. lucidum* were investigated, and after being separated using silica gel column chromatography, a distinctive fraction including triterpenoids was shown to exhibit considerable S5αR inhibitory activity. It was claimed that *G. lucidum* was used to isolate oxygenated lanostane-type triterpenoids with S5αR inhibition, as well as ganoderic acid TR, ganoderic acid DM, and 5alanosta-7,9(11),24-triene-15a,26-dihydroxy-3-one, all of which had inhibitory effects on S5αR. (Liu, Kurashiki, Shimizu, & Kondo, 2006)



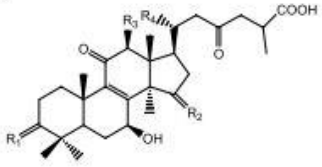
ganoderic acid TR (**1**)

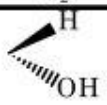
methyl ester of **1** (**14**)

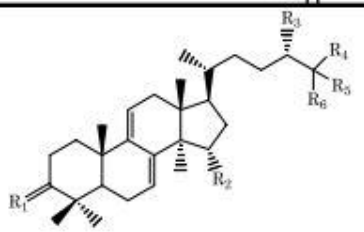


ganoderic acid DM (**2**)

methyl ester of **2** (**15**)



constituent	R_1	R_2	R_3	R_4
ganoderic acid A (**3**)	O		H	H
ganoderic acid B (**4**)		O	H	H
ganoderic acid C2 (**5**)			H	H
ganoderic acid D (**6**)	O	O	H	H
ganoderic acid I (**7**)		O	H	OH



constituent	R_1	R_2	R_3	R_4	R_5	R_6
5α-lanosta- 7,9(11),24- triene-15α,26- dihydroxy-3-one (**8**)	O	OH	$\Delta^{24(25)}$		CH_2OH	CH_3
ganoderol B (**9**)		H	$\Delta^{24(25)}$		CH_2OH	CH_3
ganodermatriol (**10**)		H	$\Delta^{24(25)}$		CH_2OH	CH_2OH
ganodermanontriol (**11**)	O	H	OH	OH	CH_2OH	CH_3
ganoderiol A (**12**)		H	OH	OH	CH_2OH	CH_3
ganoderiol F (**13**)	O	H	$\Delta^{24(25)}$		CH_2OH	CH_2OH

Figure 14 Structures of compounds 1-13 that were extracted from ethanol extracts of *G. lucidum*. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

Ganoderic acid TR (1), ganoderic acid DM (2), and 5a-lanosta-7,9(11),24-triene-15a,26-dihydroxy-3-one (8) were more effective inhibitors of type 1 S5αR than other compounds. Even though the quantities of 1, 2, and 8 were raised, the remaining enzyme activity continued to show a rapid decline without being totally inhibited. Despite the fact that structure 8 is structurally similar to structure 1, 1 had an IC50 value that was higher than (8). The only distinction between these two compounds may be found at carbon number 26. Here, 1 has 26-carboxy, while 8 has 26-hydroxy at the same position. Based on these findings, it appears that the 17β-side chain of 1 must have a carboxyl group in order to successfully elicit the inhibitory effect. The same tendency was seen when comparing the methyl ester of 1 and 14 with that of 2 and 15. In comparison to 1 and 2, the methyl ester of this compound (14 and 15) exhibited S5αR inhibition that was significantly less. Using this assay, it was determined that the more powerful inhibitory chemicals are related with counterparts that are unsaturated at C-24 and C-25. For instance, three of the most powerful inhibitors (1, 2, and 8) have sp2 hybridization from carbon 24 to carbon 25, although fully saturated triterpenoids are far less powerful. In addition, the C-26-hydroxy compound (12) is not as effective at inhibiting enzyme activity as the C-26-carboxy compound (1), indicating that an acidic functionality must be present at the 26-carbon in order for this series of compounds to be effective at inhibiting enzyme activity. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

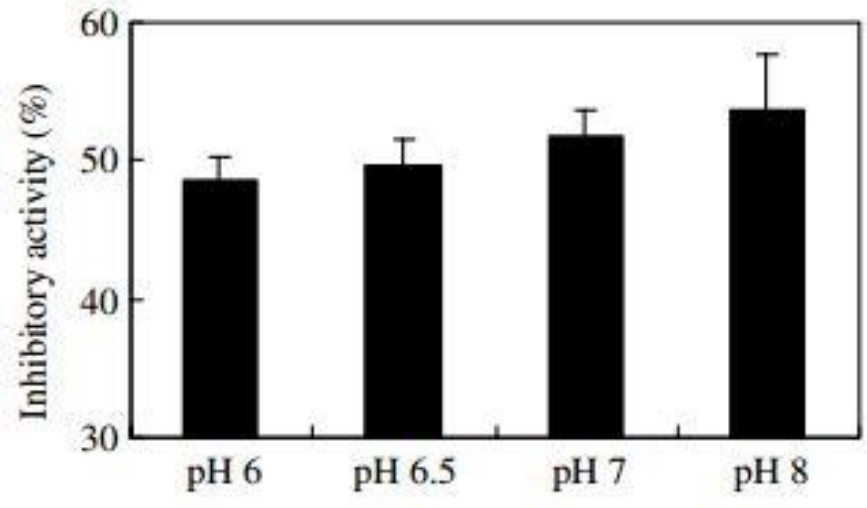


Figure 15 Inhibitory activity of compound 2 in differing pH conditions, where increase in pH from 6 to 8 increases inhibition of the enzyme. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

Inhibition of S5αR requires a significant increase in pH. In the S5αR experiment, the level of conversion from T to DHT by the S5αR remained constant across the pH range of 6–8.

According to these findings, the ability of 26-carboxy triterpenoids to inhibit S5αR was dependent on the carboxylate anion of the triterpenoid inhibitor. The positively charged, electrophilic version of this compound might perhaps coordinate with the carboxylate anion of the triterpenoid inhibitor, which would then result in the enzyme-bound complex becoming more stable. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

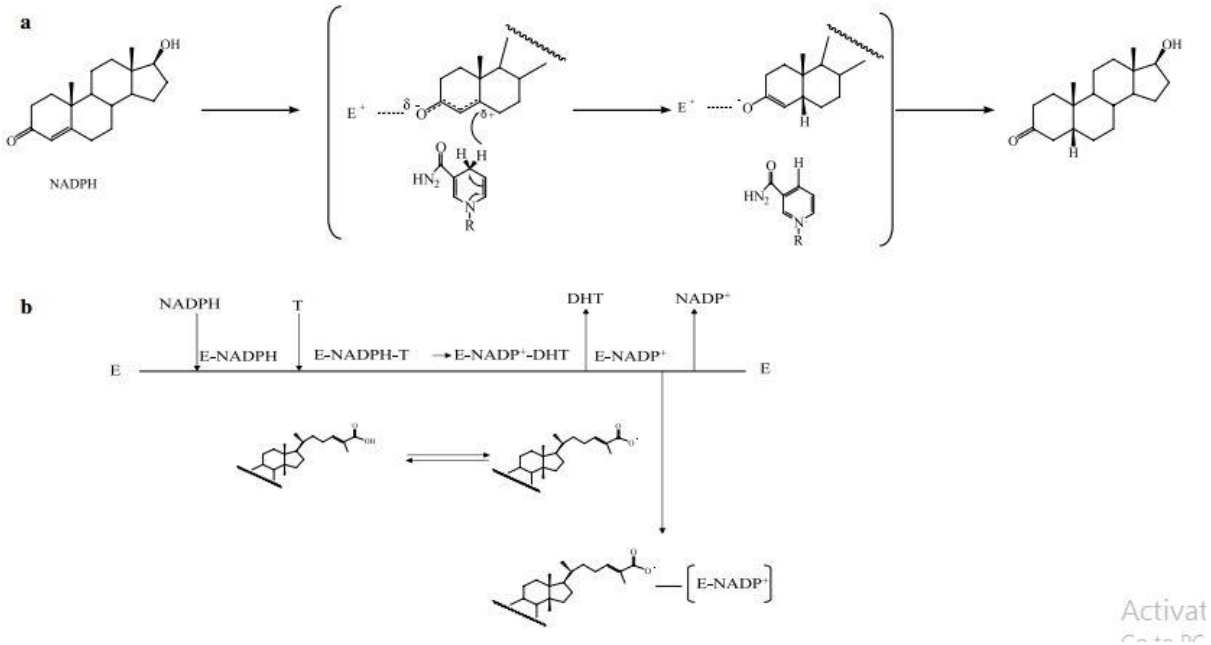


Figure 16 a) Transfer of hydride from NADPH to C-5 position of T, forming 2,4-enolate. b) Enzyme-bound reaction intermediates showing interaction between binding site of enzyme and α,β-unsaturated 17β-side chain. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

It has been hypothesised that the molecular mechanism of S5αR involves the direct transfer of hydride from NADPH to the C-5 position of T. This would allow an enzyme-associated electrophile to stabilise the 3,4-enolate that would be produced as a result of the reaction. The NADPH molecule is the first to become attached to the enzyme, and the NADP+ ion is released from the surface of the enzyme at the very end. As a consequence of this, NADPH and NADP+ are able to interact with free enzymes in their own right, so steroidal inhibitors can bind to these binary enzyme complexes. It would be easier to complete the hydride transfer if, during the first activation of the enone substrate, coordination to this electrophilic centre was used to form a triterpenoid species with cationic character at C-26. DHT would be produced after the subsequent protonation of the C-4 position of the enolate intermediate. High enzyme affinity should be demonstrated by structures that have chemical stability and they have electronic similarity between the enzyme-bound intermediates formed in the reaction. This should be

accomplished by exploiting the specific interactions that are involved in the stabilisation of the active site-associated transition or intermediate states. Because of this, it is important to take into account the a,b-unsaturated 17β-side chain in order to achieve the best possible interaction with the binding site of S5αR. Because of this, the unsaturated 26-carboxyl triterpenoids should be formulated as molecules that are bound to enzymes. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

The analysis of a number of different 17β substituents revealed that less polar functions were more successful at interfering with the enzyme. However, polarity was not the only element that contributed to the potency of the interference. There was a low probability that ganoderma alcohol would be most effective on these triterpenoid frameworks; however, increasing the lipophilicity of the 17β (9 and 10) substituents led to maximum inhibitory efficacy. It was shown that inhibitory activity decreased with increasing levels of hydroxyl. The inhibition of S5αR was unaffected by the presence of the hydrophilic chemicals. Because S5αR is an enzyme that is hydrophobic, the hydrophobic bond that exists between S5αR and the chemicals may cause the bond to become more stable. It was observed that the C-3 carbonyl group compound had a tendency to have a significant inhibitory action between the numbers 11 and 12, as well as between the numbers 9 and 12. The C-3 anionic functionality is important in increasing efficacy of inhibiting the steroid S5αR-NADP+ complex. (Liu, Kurashiki, Shimizu, & Kondo, 2006)

Chapter 3

Synthesis of 5α-reductase inhibitors

3.1 Synthesis of unsaturated carboxysteroids

The drugs used for BPH target the enzyme 5α-reductase and inhibit its function. Acne is caused by the production of androgens from this enzyme; therefore Finasteride can be used as a start for a novel strategy of treatment of acne.

The drug used for the treatment of benign prostatic hyperplasia (BPH) is Finasteride (Figure 17). It is a strong inhibitor of S5αR type II but has limited in vitro activity for S5αR type I. Plasma DHT levels were reduced by 65–80% when it was administered at a therapeutic dose of 5 mg per day. Another similar medication, Dutasteride (4), was used for the symptomatic treatment of benign prostatic hyperplasia (BPH). In contrast to Finasteride (3), Dutasteride (4) is a competitive inhibitor of both type I and type II isozymes of the S5αR enzyme, and it lowers levels of DHT by more than 90 percent after oral dosing for a period of one year. It is a time-dependent inhibitor, just like Finasteride (3), and it creates a stable complex that has a slow rate of dissociation. However, it does not bind to the androgen receptor. A new S5αR inhibitor known as Epristeride (5) is classified as a member of the carboxysteroid group. It has a significant inhibitory effect on S5αR II but only a moderate effect on S5αR I. It has been demonstrated that it is an inhibitor that does not compete with T or NADPH in any way. The acrylate preferentially binds in a ternary complex with the enzyme and NADP+, which leads to the uncompetitive kinetic process. This is likely the result of electrostatic contact between the carboxylate and cofactor, which has a positive charge. (Aggarwal et al., 2012)

Another family of chemicals that have demonstrated 5α-reductase inhibitory activity is known as 17a-aza-D-homosteroids. Several metabolising enzymes contain active sites that are capable of accommodating steroid binding in either of two possible orientations. According to findings from studies on inhibitors of 5a-reductase, steroids without side chains are able to attach to enzymes in a manner that involves the A-ring of the substance imitating the D-ring of the substrate, while the D-ring imitates the A-ring. It is possible that, as a result of this, these molecules will exhibit the same mechanism of action as 4-azasteroids. (Aggarwal et al., 2012)

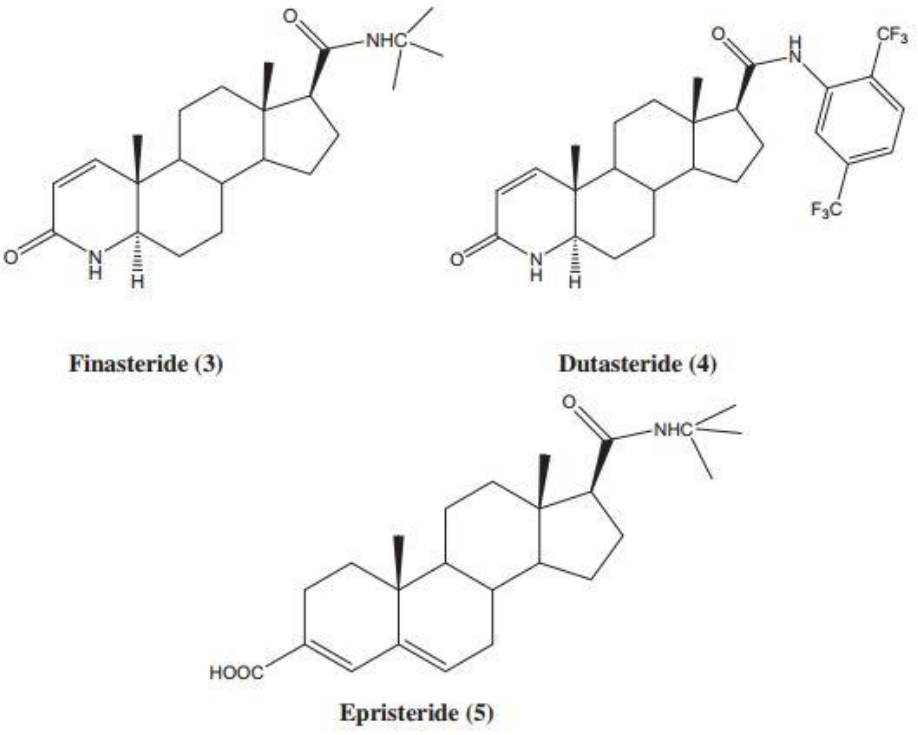


Figure 17 Structures of active molecules that inhibit 5α-reductase enzyme. (Aggarwal et al., 2012)

3.1.1 Synthesizing of 17a-aza-D-homo-4-androstene-3,17-dione

The initial step in the synthesis of the proposed compounds was the synthesis of 17a-aza-D-homo-4-androstene-3,17-dione (10), which was accomplished by following normal protocols and beginning with 17-oxo-5-androsten-3b-yl acetate (dehydroepiandrosteroneacetate) (6) as the starting material. Refluxing it in ethanol along with hydroxylamine hydrochloride and sodium acetate trihydrate caused it to be transformed into its oxime (7). Beckmann's rearrangement with thionyl chloride resulted in the formation of 17-oxo-17a-aza-D-homo-5-androsten-3b-yl acetate from 17-oximino-5-androsten-3b-yl acetate (7). (8). In order to obtain (9), the lactam (8) was subjected to hydrolysis by refluxing with methanolic potassium hydroxide. An initial oxidation of compound 9 was performed in a solvent mixture consisting of cyclohexanone and toluene, which resulted in the production of 17a-azaD-homo-4-androstene-3,17-dione (10). (Aggarwal et al., 2012)

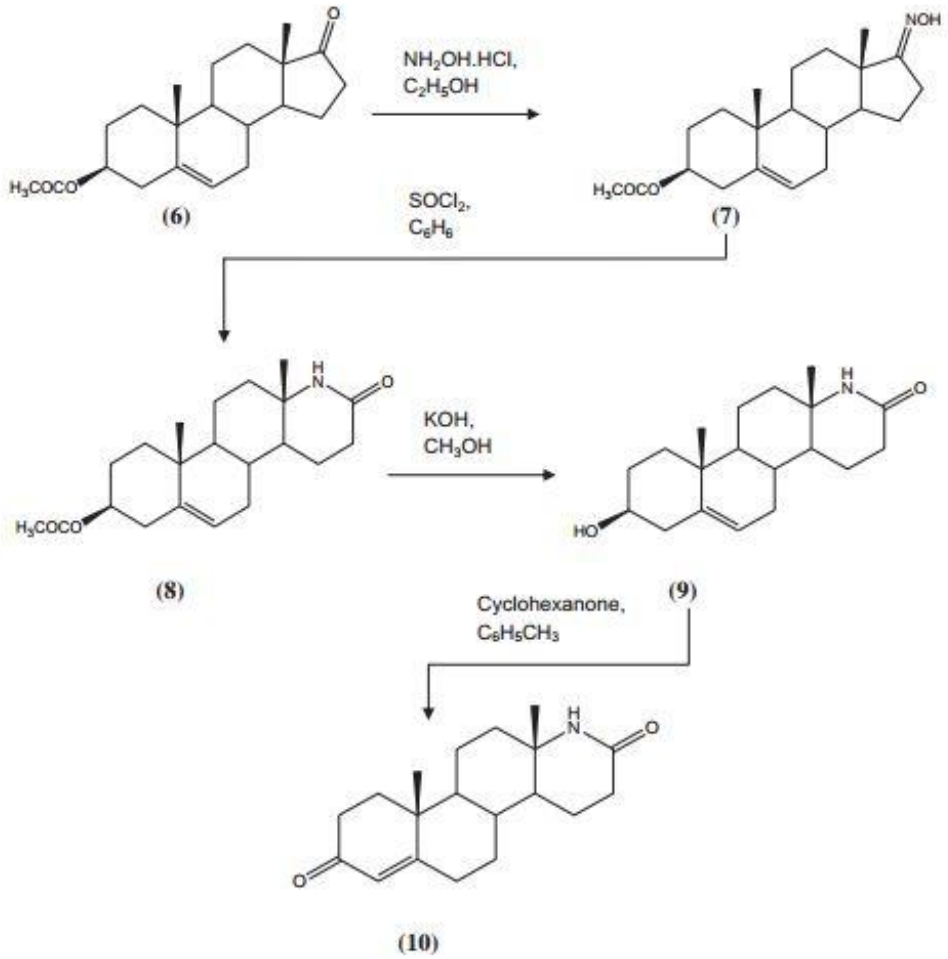


Figure 18 Synthesis of 17a-aza-D-homo-4-androstene-3,17-dione (10). (Aggarwal et al., 2012)

3.1.2 Synthesizing 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androsta-dien-17-ones

After being treated with phosphorus tribromide in glacial acetic acid, (10) produced (11) with a yield of 85.0%. The bromo compound (11) was subjected to a refluxing process with copper cyanide in dimethylformamide, which resulted in the production of (12). By using sodium hydride, (12) was subjected to treatment with methyl iodide, which produced (13). The compound (12) was also subjected to treatment with ethyl bromide, allyl bromide, benzyl chloride, acrylonitrile, and acetyl chloride, respectively, in order to obtain (14), (15), (16), (17) and (18), (19). (Aggarwal et al., 2012)

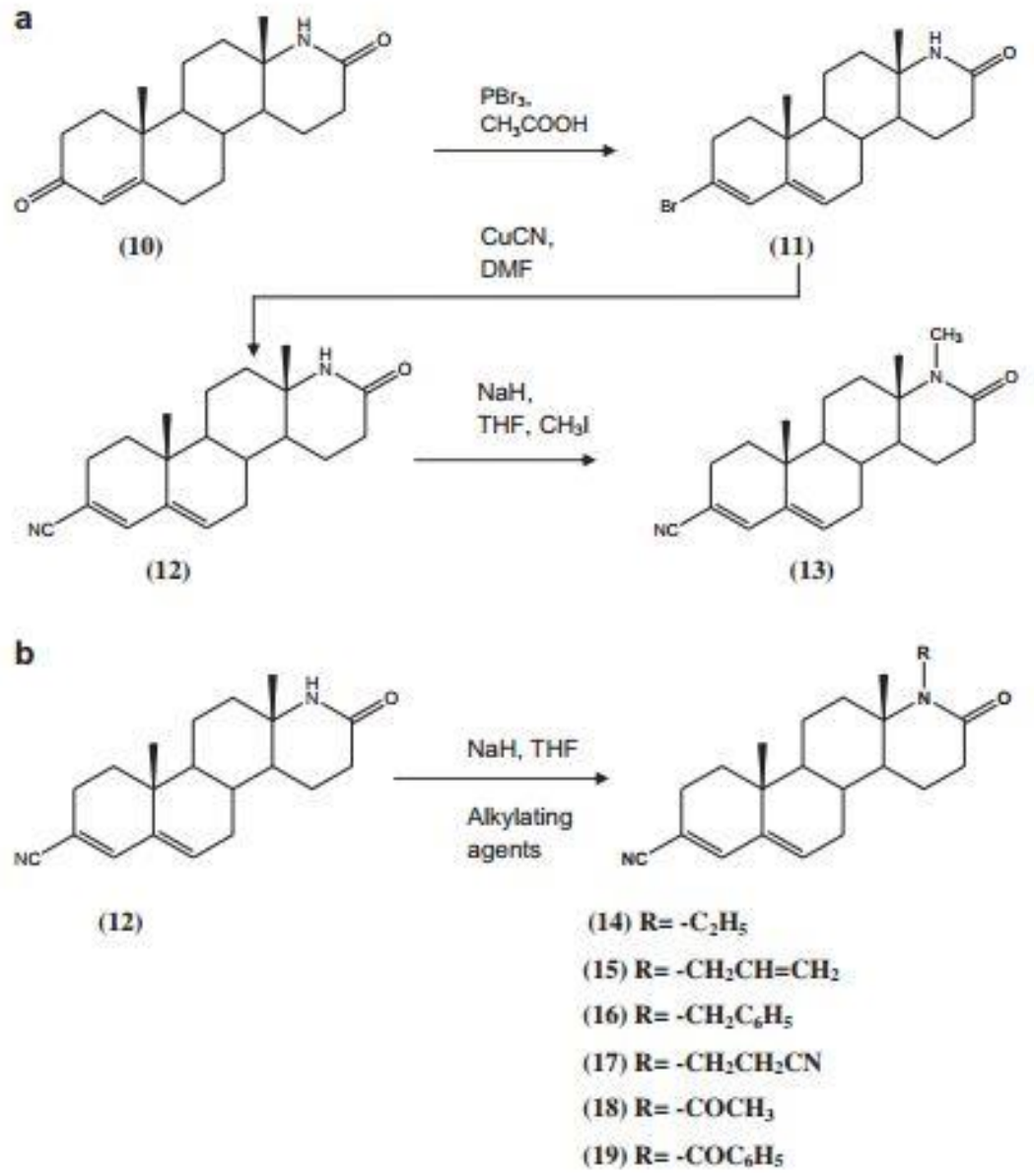


Figure 19 Synthesis of 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androsta-dien-17-ones. (Aggarwal et al., 2012)

3.1.3 Synthesizing 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids

In order to get (20), (12) was hydrolysed using ethanolic sodium hydroxide. In addition, compounds 14–18 were subjected to hydrolysis, which resulted in the respective production of N–methyl (21), N–ethyl (22), N–allyl (23), N–benzyl (24), and N-17a–(2-propionoxyethyl) (25). (26) was obtained by reacting (20) directly with acetic anhydride in pyridine. On the other hand, 19 was unable to undergo hydrolysis as the N-benzoyl side chain breaks down when a strong base was used. (Aggarwal et al., 2012)

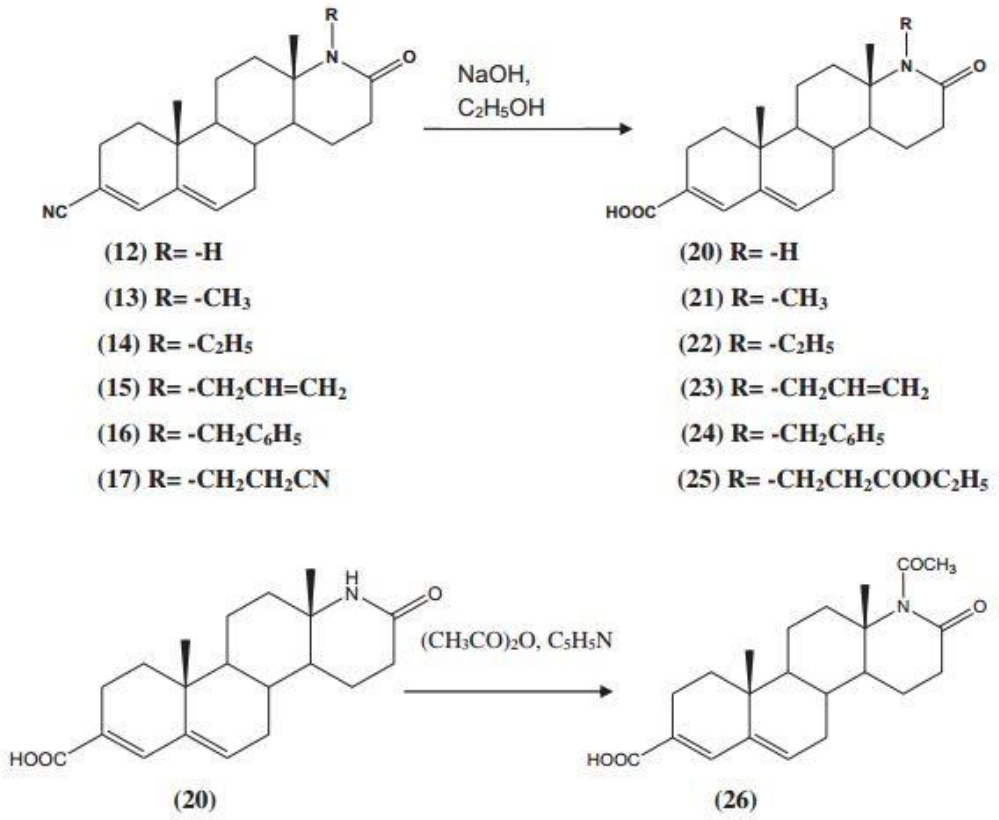


Figure 20 Synthesis of 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids. (Aggarwal et al., 2012)

3.1.4 Synthesizing 17-oxo-19-nor-3,5-androstadien-3-oic acid

By treating 4-androstene-19-nor-dione (27) with phosphorus tribromide in glacial acetic acid, the compound was able to be transformed into (28). In order to obtain 3-cyano-19-nor-3,5-androstadien-17-one, the bromo molecule was subjected to a refluxing process with copper cyanide in dimethylformamide (29). Obtaining (30) required hydrolysis of the cyano molecule (29), which was carried out with the addition of ethanolic sodium hydroxide. (Aggarwal et al., 2012)

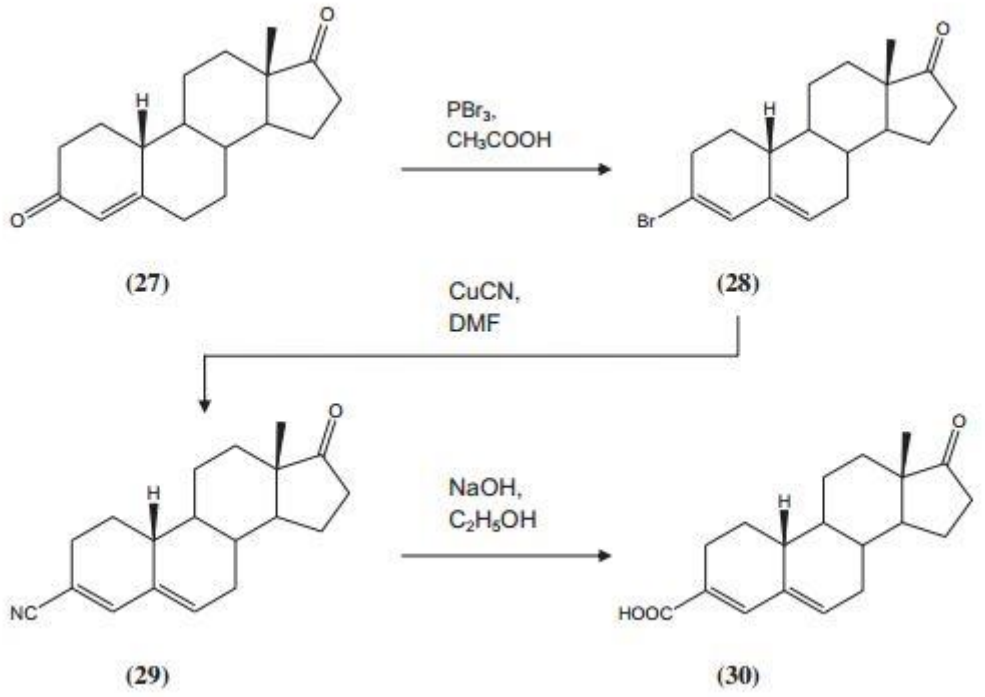


Figure 21 Synthesis of 17-oxo-19-nor-3,5-androstadien-3-oic acid (30). (Aggarwal et al., 2012)

In vitro biological testing on HEK cells demonstrated that the majority of the compounds that were produced showed more inhibitory action in comparison to the conventional medication finasteride (3). The most powerful compounds, 21–23, and 25, demonstrated 5α-reductase II inhibition with IC50 values of 54.1, 22.1, and 72.8 nM respectively. This is in comparison to Finasteride, which had an IC50 value of 30.3 nM. Additionally, there was a substantial reduction in the prostate weights of rats. Compound 13 was also discovered to be a powerful inhibitor of the enzyme 5α-reductase I. It is indicated that the D-homo ring can be tolerated properly at the enzyme site, and that the activity can be improved by replacing the alkyl groups in the 17a-aza position. (Aggarwal et al., 2012)

Chapter 4

Conclusion

Acne originates mainly during puberty but can remain a chronic condition for many sufferers. The start of puberty triggers androgen production, subsequently causing increased sebum production and enlargement of sebaceous glands. This, combined with the growth of *P. acnes*, leads to inflammatory responses in the skin, and formation of papules and pustules. Therefore, acne is formed in the skin of the face, back, and various other regions of the body.

Current drugs can be optimized to suit each patient's skin. As the skin of different patients react differently to each medication and its dosages. Therefore, it is important to find medication that would be suitable for most, if not all, skin types.

In the future, enzymes and hormones can be directly targeted which have been proven to be the leading cause of acne. The SAR study of 5α-reductase is a novel strategy, as it can help to reduce the production of androgens in those with hormonal imbalance. There are drugs already available which target the enzyme; however they have not been tested for acne. The drug, Finasteride, which is used to treat BPH, can be used as a lead compound to synthesize compounds that would only target the type 1 isoenzyme and reduce androgen production, and further reduce the excess growth sebaceous glands.

Impact

The purpose of the review article was to show the medications available for the treatment of acne vulgaris, and the areas where treatment can be improved upon. For several acne patients, current medications are not enough to fully alleviate their skin problems. Therefore, other methods and compounds were looked into as potential treatment options in the future.

The review article signifies the structure-activity relationship of existing compounds with 5α-reductase, an important enzyme in the development of acne vulgaris. These compounds were seen as starting compounds for the synthesis of inhibitors of the enzyme.

Finasteride is an existing drug, which inhibits S5αR. It has been used as the basis of this review article to study synthetic methods that can be used to formulate more potent inhibitors. The synthesis of carboxysteroids from this drug, as well as the SAR of S5αR and triterpenoids acts as important starting points for better inhibition of excess androgen production, and subsequently acne generation.

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