REPUBLIC OF TURKEY
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SYNTHESIS of BENZIMIDAZOLE SUBTITUTED TRI-HYDROXY STEROIDS from *trans*-DEHYDROEPIANDROSTERONE

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Master Thesis
Department of Chemistry, Organic Chemistry
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JUNE-2016

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MASTER THESIS

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JUNE-2016

DEDICATION

This work is dedicated to the memory of my father late B. Kolo Geidam without his courage and support it would not have been possible, and to my mother Hadiza Dala Kolo and entire Kolo Geidam`s family.

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ABSTRACT

SYNTHESIS of BENZIMIDAZOLE SUBTITUTED TRI-HYDROXY STEROIDS from *trans*-DEHYDROEPIANDROSTERONE

Azolyl steroids and oxysterols are important class of steroids which were reported to have possess variety of biological activities; anti-cancer, anti-apoptotic and some androgen-dependent diseases. 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene was synthesised by using $POCl_3$ and DMF with Vilsmeier-Haack reaction of 3β-hydroxyandrost-5-en-17-one. This reagent was used as starting materials for C-17-azolyl steroids (**4a, 4b)** and C-16 azolyl steroid (**8**) synthesis. The formyl group on **4a** was then reduced with $NaBH_4$ to the corresponding hydroxy compound **5** and *m*-chloroperoxybenzoic acid and was used to convert the double bond in B ring of **5** into *cis*-diols (3β-Acetoxy-17-(1H-benzimidazol-1-yl)-5,6-dihydroxy-16-(hydroxymethyl)-16-ene, **6)**. Different α,β-unsaturated tri-hydroxy aza-steroids (**11a-c)** were also synthesized from the reaction of α,β-unsaturated aza-steroids (**10a-c)** with *m*-chloroperoxybenzoic acid in dichloromethane. Synthesis of 3β,5α,6β-trihydroxy androstanone **(12)** was carried out from reaction of DHEA (1) and *m*-CPBA in dichloro methane. The hydroxyl groups on **12** were converted to acetoxy groups by acylation reaction to obtained 3β, 5α,6β-triacetoxyandrost-17-one **(13).**

Keywords: Dehydroepiandrosterone (DHEA), benzimidazole, Vilsmeier-Haack reaction, *m*-CPBA, azolyl steroids

ÖZET

trans-DEHİDROEPİANDROSTERON'dan BENZİMİDAZOL SÜBSTİTÜE TRİHİDROKSİ STEROİDLERİN SENTEZİ

Azolil ve oksi-steroidler, steroidlerin önemli bir sınıfı olup anti-kanser, anti-apototik ve bazı androjene bağlı hastalıklar gibi farklı biyolojik aktiviteleri sahip oldukları rapor edilmektedir. 3β-asetoksi-17-kloro-16-formilandrosta-5,16–dien, 3β-hidroksiandrost-5-en-17-on, $POCl_3$ ve DMF kullanılarak Vilsmeier- Haack reaksiyonu ile sentezlendi. Bu reaktif C-17 azolil ve C-16 azolil sterodlerin (**4a,b** and **8)** sentezi için başlangıç maddesi olarak kullanıldı. **4a'**nın üzerindeki formil grubu $NaBH_4$ ile indirgenerek karşılık gelen hidroksi bileşiği **5** elde edildi. **5** bileşiğin B halkasındaki çift bağ, *m*-kloroperoksibenzoik asit ile *cis*-diol (3β-asetoksi-17-(1*H*-benzimidazol-1-il)-5,6-dihidroksi-16-(hidroksimetil)-16–en **6**) dönüştürüldü. Farklı α,β-doymamış tri-hidroksi'de (**11a-c),** diklormetan içeresinde *m*-kloroperoksibenzoik asitle α,β-doymamış aza-steroidlerin (**10a-c**) reaksiyondan sentezlendi.

3β,5α,6β-trihidroksiandrostan-17-on'un **(12)** sentezi diklormetan içerisinde DHEA (**1**) ve *m*-CPBA' in reaksiyonundan sentezlendi. 3β,5α,6β-triacetoxyandrost-17-on'u **(13)** elde etmek için 12' nin hidroksil grupları açilizasyon reaksiyonu ile asetoksi grubuna dönüştürüldü.

Anahtar kelimeler: Dehidroepiandrosteron (DHEA) , benzimidazol, Vilsmeier – Haack reaksiyonu, *m*-CPBA, azolil steroidler.

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SYMBOLS AND ABBREVIATION

Aq. : Aqueous

AÜ : Adiyaman University

$CDCl_3$: Chloroform-D

d : doublet

DHEA : Dehydroepiandrosterone

DMF : *N`N*-Dimethylformamide

EtOAc : Ethyl Acetate

FÜ : Firat University

g : Gram

g/mol : Gram per mole

h : Hour

HDL : High-Density Lipids

HIV : Human Immunodeficiency Virus

IR : Infrared

J *:* Coupling Constant

KCN : Potassium Cyanide

KOH : Potassium Hydroxide

L : Litre

LDL : Low-density Lipids

M : Molar

m : Multiplet

***m*-CPBA** : *meta*-Chlorperoxybenzoic acid

$MgSO_4$: Magnesium Sulphate

MHz : Mega Hertz

mL : Millilitre

mmol : Millimoles

MMPP : Magnesium Monoperoxyphthalate

$NaBH_4$: Sodium Borohydride

NaOH : Sodium Hydroxide

NBS : *N*-Bromosuccinimide

NMR : Nuclear Magnetic Resonance

ppm : Part per million

RNA : Ribonucleic Acid

s : Singlet

SAR : Structure-Activity Relationship

t : Triplet

TLC : Thin Layer Chromatography

δ : Chemical Shift

1 INTRODUCTION

Chemistry of naturally occurring compounds such as steroids have got much more attention of organic and medicinal chemist because of their large number of biological activities. Many researches carried out on steroids has outlined this biological functions. Modification on a different ring of steroid (A, B, C, or D) explores different pharmacological roles of the natural compound.

Steroid modified by the fused of heterocyclic compounds or linkage of heterocyclic compounds like benzimidazole, imidazole, pyrazole, indole, imidazoline etc. have shown potential increase in their biological functions.

Epoxysteroids and Oxysterols are another class of steroids modified with epoxy or hydroxyl groups on either side of the steroids, this class of compounds also contain very important biological and pharmaceutical functions.

1.1 Steroids

Steroids are group of organic compounds with a tetracyclic ring system fused together, three six-membered rings and a five-membered ring. They are simply characterized as lipids due their inability to undergo hydrolysis like oils, fats or waxes. *Cholesterol,* sex hormones, *Bile acid*, *Cortisones*, and anabolic steroids are good examples of steroids [1]. Steroids are compounds derived from perhydrocyclopentanophenanthrene [2]. Their basic structure is always based on the tetracyclic ring system.

The four rings are labelled with letters A, B, C and D starting from the left cyclohexane ring to the cyclopentane ring which is designated as D. The carbon atoms are numbered starting from ring A as shown in **Figure 1.1** [2].

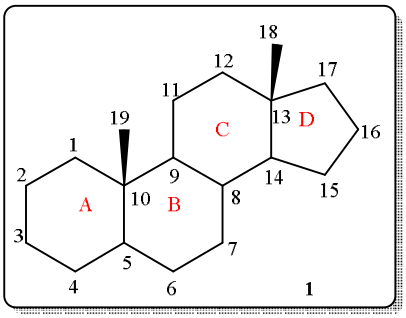


Figure 1.1. General structure of steroid

The A-B ring intersection of steroids can either be *cis* or *trans* while the B-C and C-D ring intersection are generally *trans,* the possibility of A-B rings intersection to be either *cis* or *trans* categorize steroids into two general groups; those with A-B rings intersection as *cis* are called 5α series while steroids with A-B ring junction connected as *trans* are 5β series as in **Figure 1.3** [2].

Geometric isomerism of decalin can be used as an example to explain stereochemistry of fused ring systems such as steroids, for example, *androstane* can contain either *trans* or *cis* stereoisomer at each ring intersection, this resembles that of decalin. In *trans*-decalin **2**, the two hydrogens are on opposite side one on the top and the other at the bottom of the plane while in *cis*-decalin **3** both of the hydrogens are on the same side making one of the rings to fall down below the other. *trans* and *cis*-decalin have different physical properties [1,2].

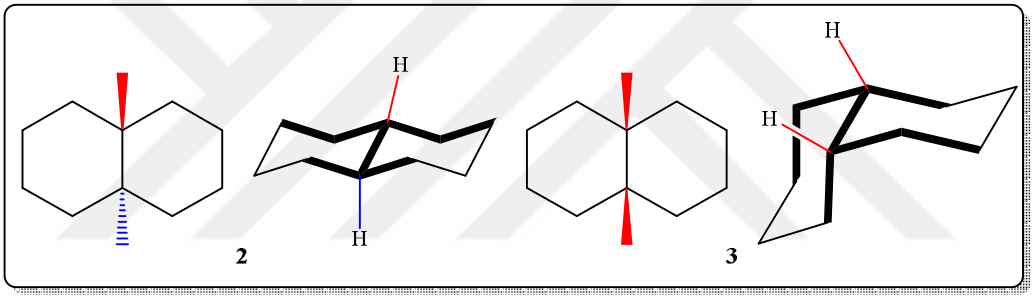


Figure 1.2. Structure of *trans* and *cis*-decalin

The above illustration is also applicable to natural products such as steroids. *Trans* isomers of steroids are more likely rigid and plane than the *cis* isomers which are relatively flexible. The two methyl group in *trans* A-B ring intersection steroids **5** (C_{18} and C_{19}) and the two hydrogens at C_5 and C_{14} are in *trans* position to each other. In *cis* A-B steroids **6**, C_{19} methyl is in *cis* position with hydrogen at C_5, this makes the A ring to fall down the other rings. **Figure 1.3** illustrate the *trans* and *cis* A-B ring junction steroids [2].

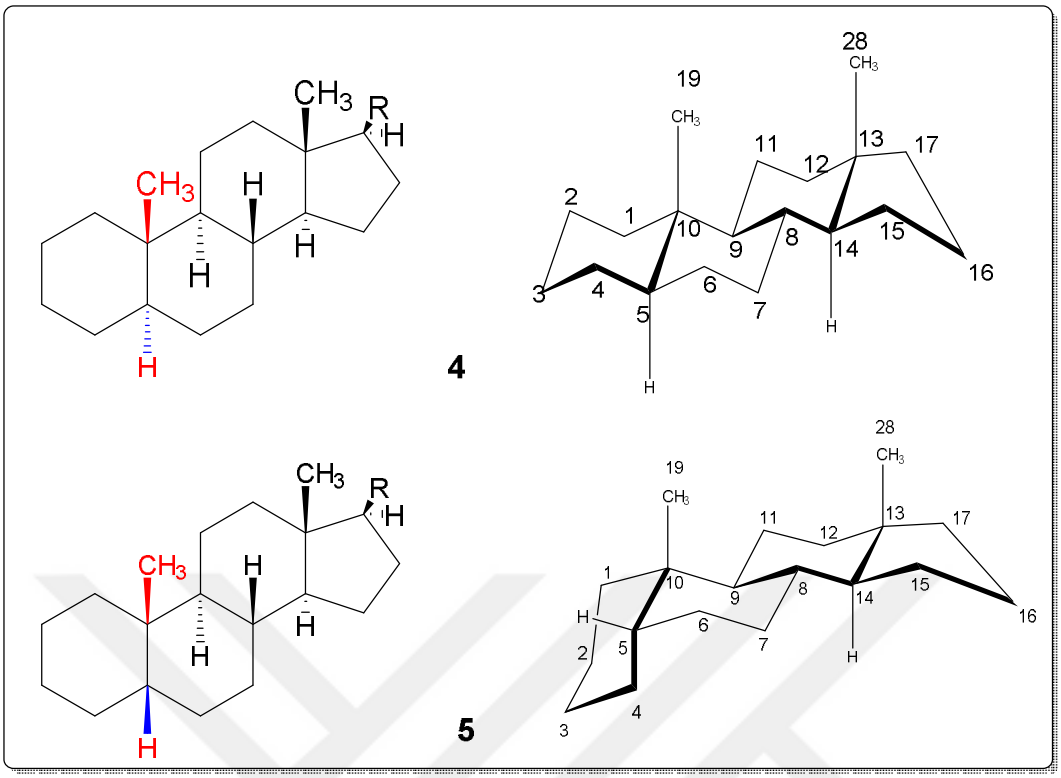


Figure 1.3. *trans* and *cis* A-B ring junction steroids

Nomenclature of steroids can easily be made base on describing the modifications to one of these five possible backbone structures; *Cholestane* **6**, *Pregnane* **7**, *Gonanes* **8**, *Androstane* **9**, and *Estrane* **10** [3].

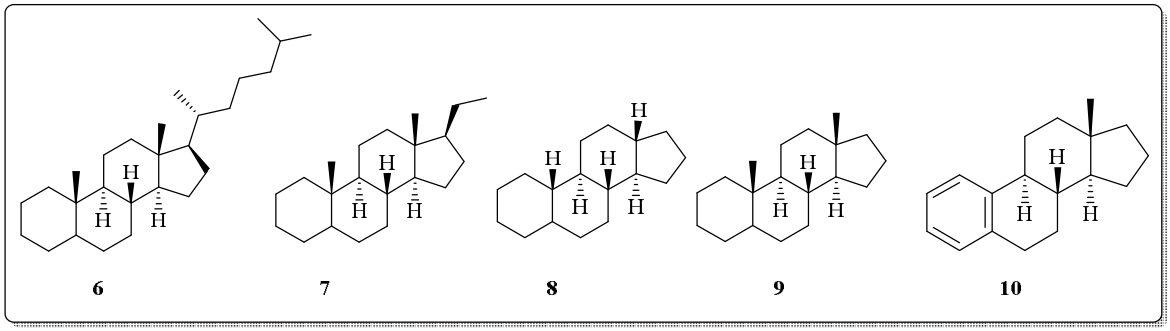


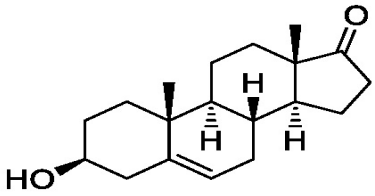
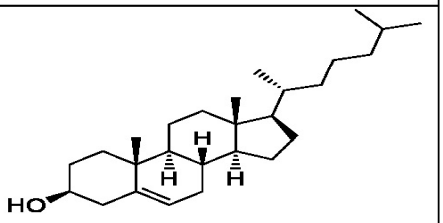
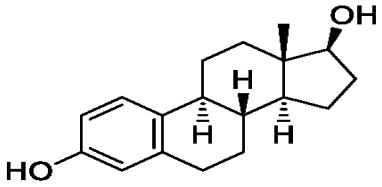
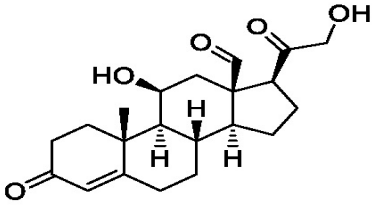
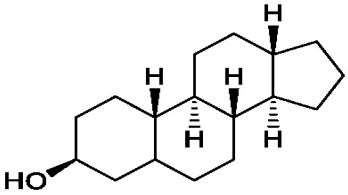
Figure 1.4. Backbone structure of steroids

Steroids structure as in **Figure 1.4** depends on these five backbone structures, all the backbone is the same only that the side chain differs from one another. *Cholestane* and *Pregnane* are somehow same only that the side chain on *Cholestane* is longer than in *Preganane*. The difference between *Gonane* and *Androstane* is a lack of the two methyl

groups on the *Gonane* structure, aromaticity of the *Estrane* backbone make it differ from *Androstane.*

The **Table 1.1** below summarized the examples of the above naming system and structure of some common steroids.

Table 1.1. Naming system and structure of some common steroids.

Backbone	Trivial name	Chemical name	Structure
Androstane	DHEA	5-androstene-3β-ol-17-one	
Cholestane	Cholesterol	5-choestane-3β-ol	
Estrane	Estradiol	1,3,5(10)-estratrien-3-17β-diol	
Preganane	Aldosterone	4-pregnene-11β-21-diol-3,18,20-trione	
Gonane		Gonan-3β-ol	

1.2 Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) **11** is steroid hormone produces from adrenal glands of a body. It is also known as 3β-hydroxyandrost-5-en-17-one or 5-androsten-3β-ol-17-one it has a molecular formula of $C_{19}H_{28}O_2$ and Molar mass: 288.424 g/mol. The structure of DHEA is shown in **Figure 1.5** below;

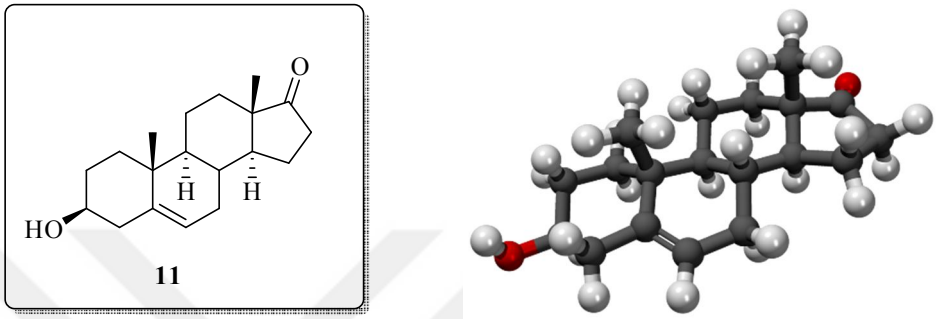


Figure 1.5. Structure and 3D view of DHEA

Dehydroepiandrosterone is a starting material for the synthesis of many steroid hormones with various biological functions. It was reported that modifications carry out on the rings of DHEA produce a new compound with a huge number of potential biological effects such as neuroprotective, anti-apoptotic anti-prostate cancer [4] etc.

1.2.1 Some Chemical Reactions of DHEA

Reacting dehydroepiandrosterone with PCl_5 replaces the hydroxyl group at position 3 to chloride ion **12**, also, Oppenauer reaction of DHEA with aluminum isopropoxide in the presence of a ketone oxidized the hydroxyl group at position 3 into ketone with product androst-4-ene-3,17-dione **13**. DHEA also serves as starting material for preparation of *estranes* **14**, conversion began with the Oppenauer oxidation of DHEA followed by several steps. Another interesting reaction is the preparation of 17-methyl *testosterone* **15** from dehydroepiandrosterone with an excess CH_3-MgBr the same method can be used to prepare 17-ethynyl derivative **16** by replacing the Grignard reagent with lithium acetylide. The reaction of DHEA acetate with KCN in acetic acid leads to the cyanohydrin dehydration of this compound followed by addition of methyl magnesium bromide to the product and hydrolaxation of the imine compound produces *16-dehydropregnenolone* **17**. Several heterocyclic containing DHEA have synthesized and their biological activities have been elucidated for example galenterone **18** with heteroaryl at the C_{17} also heteroaryl

at C_{16} via methine bridge **19** has been described in the literature [3,5]. Schematic summary of this synthesis is shown in **Figure 1.6**.

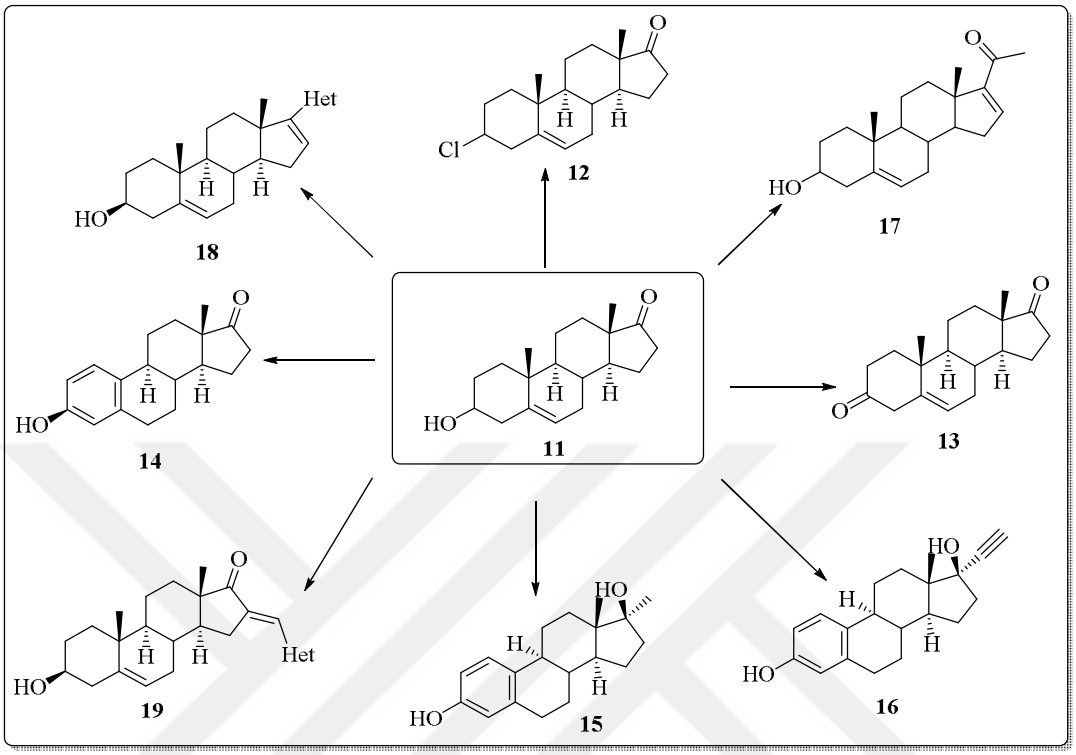


Figure 1.6. Different product synthesised from DHEA

1.3 Cholesterol

One of the most naturally and abundantly occurring steroids exists in almost all animals tissues is cholesterol **20** [2]. *Cholesterol* serves as starting materials for biosynthesis of other steroids found in mammals systems [3]. Stereoisomerism rule of 2^n suggested that *cholesterol* should have 2^8 or 256 basic structure forms due to the presence of eight chirality centres in *cholesterol*, but only one form is said to be cholesterol [2].

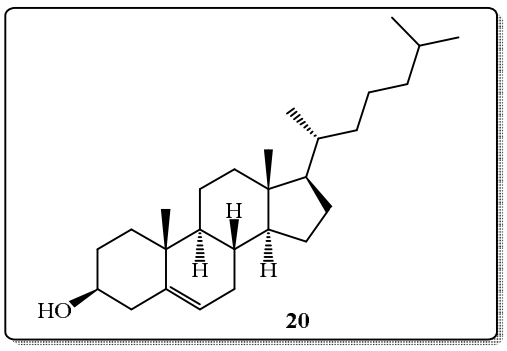


Figure 1.7. Structure of cholesterol

Cholesterols are found in large quantities in human body particularly in gallstones (which may consists of about 90% of neutral cholesterol) [3]. Our body system can synthesize all we need of *cholesterol*, so it's essential to life. Sex hormones, bile acids, vitamin D and adrenocorticoid hormones are all synthesized from cholesterol steroids [2].

High level of *cholesterol* in the blood can cause hardening of arteries and heart attacks, *cholesterol* is not soluble in water they are transported from the liver by LDLs and return by HDLs. LDLs are said to be bad *cholesterol*s it carries biosynthesized lipids from liver to the tissues while HDLs are known as good cholesterols for their responsibility of transporting lipids from tissues back to the liver for degradation [2].

1.4 Sex Steroids (Hormones)

Chemical substances responsible for reproductive functions in mammals were particularly known as sex hormones, these substances are categorized into three different classes: *Androgens, Estrogens*, and *Progestins*. The biological activity and structure of these compounds differ from each other [3].

1.4.1 Estrogens

Estrogen or female sex hormones is a first sex hormone to be isolated in 1929 by Adolf Butenandt and Edward Doisy, they used pregnant woman's urine to isolate *Estrone* **14**, later Doisy used sow ovaries to isolate *estradiol* the true female sex hormones were he extract 4 tons of sow ovaries and obtain only 12 mg of *Estradiol* **21** [2]. *Estradiol* is an important hormone that controls the menstrual cycle and reproductive process likewise female secondary sex characteristics are developed by this hormone [6].

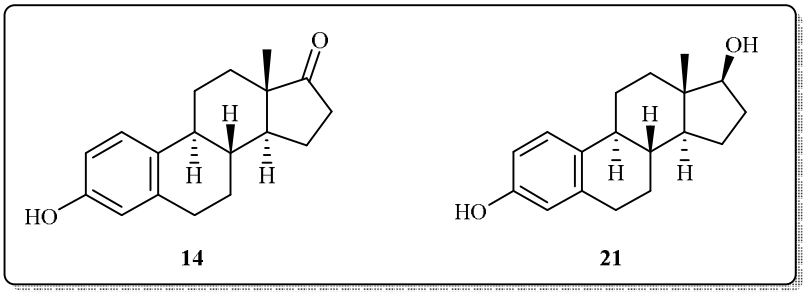


Figure 1.8. Structures of *Estrone* (**14**) and *Estradiol* (**21**)

1.4.2 Progestin

Progestin in another name pregnancy hormone is another female sex hormone which is developed during the menstrual cycle. *Progesterone* **22** being the most important pregnancy hormone process the lining of the uterus for implantation of the fertilized ovum, continued release of *Progesterone* is also important for completion of pregnancy [2].

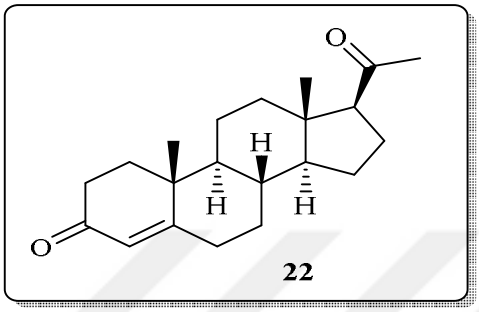


Figure 1.9. Structure of *Progesterone*

1.4.3 Androgens

The male sex hormone which was isolated in 1931 by Butenandt and Kurt Tscherning, they extract about 15000 L of male urine and obtain 12 mg of *Androsterone* **23**. In 1935 Ernest Laqueur discovered another male sex hormone from bull testes, Ernest's work clearly shows that the true male sex hormone is *Testosteron*e **24** and the **23** excreted in the urine is metabolized form of **24** [2].

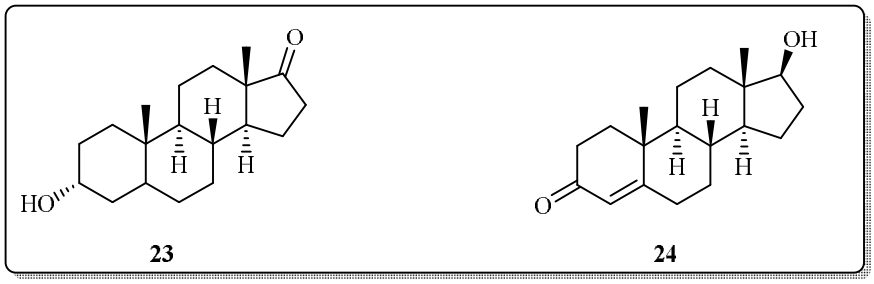


Figure 1.10. Structure of *Androsterone* and *Testosterone*

Development of secondary male characteristics are stimulated by testosterone hormone, this includes; voice deepening, facial evolution, growth of body hair, muscular buildout, male sex organs' and other male characteristics [2].

Testosterone **24** "maleness" and *Estradiol* **21** "femaleness"; these two compounds slightly differ in their structural formula. There is additional CH_3 at C_6 and carbonyl functional group at C_{17} on testosterone while A ring of estradiol is aromatic, has no methyl at C_6, there is hydroxyl group at C_3 and C_{17} respectively [2].

1.5 Adrenocortical Hormones

Adrenocortical hormones are physiologically important steroids synthesized by the adrenal cortex [1]. *Cortisol* **25** and *Cortisone* **26** are some of these steroids, they possess likely structural formula only that keto group on cortisone at C_{11} and a hydroxyl group at C_{11} on cortisol structure differentiate them. A huge number of biological activities such as metabolism of proteins, carbohydrate and lipids are regulated by adrenocortical hormones. *Cortisol* is also used in the treatment of psoriasis (inflammatory diseases of skin), rheumatoid and asthma diseases [2].

1.6 Vitamin D

D vitamins are other groups of steroids derived from 7-dehydrocholesterol found in the tissue of the skin. 7-dehydrocholesterol is transformed to vitamin D_3 during the photochemical reaction [6]. There are two steps for this reaction, firstly, 7-dehydrocholesterol is converted to pre-vitamin D_3 from sunlight followed by spontaneous isomerization of pre-vitamin D_3 produces vitamin D_3 [2].

Proper bone growth is achieved as a result of absorption of calcium Ca^{2+} by vitamin D_3 from the intestine. Insufficiency of vitamin D_3 in body courses bone disease known as *rickets* [6].

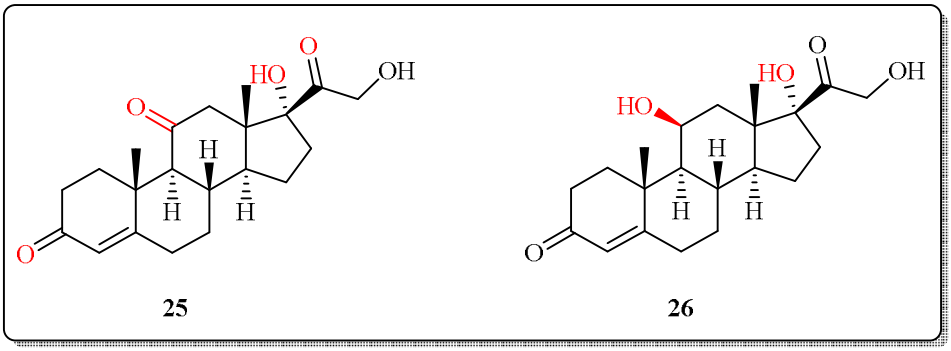


Figure 1.11. Structure of *Cortisone* and *Cortisol*

1.7 Chemical Reactions of Some Steroids

The structure of steroid shows that it can contain a double bond, a hydroxyl group, a keto group, aromatic etc. All of the reactions in the above-mentioned functional group undergoes is applicable to steroid molecules [2]. These reactions may be oxidation, nitration, carbon-carbon bond formation and so on [3]. There are several chemical reactions and modification of steroids molecules available, because of a huge number of the reactions only some of them will be summarized in this work.

A solution of **14** in acetic acid can undergo nitration reaction with nitric acid to form 2-nitroestrane **28** and 4-nitroestrane **27**. This two compounds can later be converted to nitroestradiol **29** and **30** by treating them with sodium borohydride $NaBH_4$ [3].

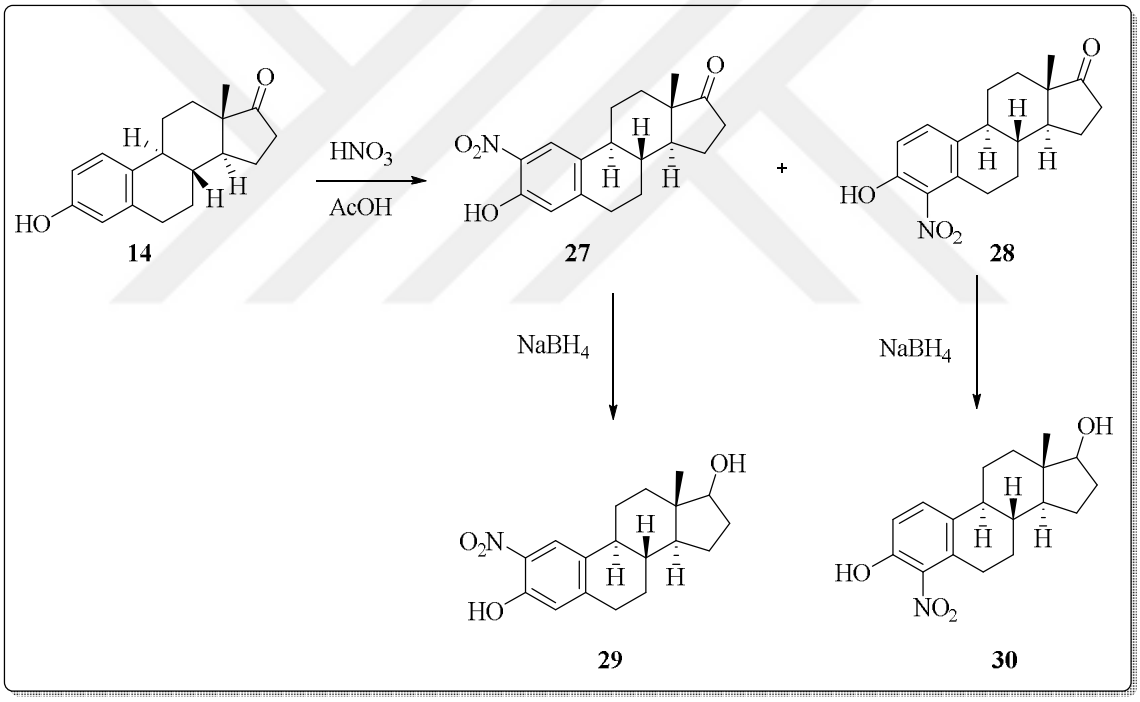


Figure 1.12. Nitration reaction of *Estrone*

The reaction of **14** with isopropylidene acetate will convert the hydroxyl group at position 3 to phenolic ester **31** and also the carbonyl at position 17 of **14** to acetate. Treating this product with perbenzoic acid gives a α-oxirane product **32**. Acetolysis of α-oxirane intermediate product with $LiAlH_4$ reduces the carbonyl and also the phenolic ester to yield *trans*-diol-16α-hydroxyl-17β-estradiol **34**. The same product can be obtained directly reducing the α-oxirane intermediate product with $LiAlH_4$ **34** [3].

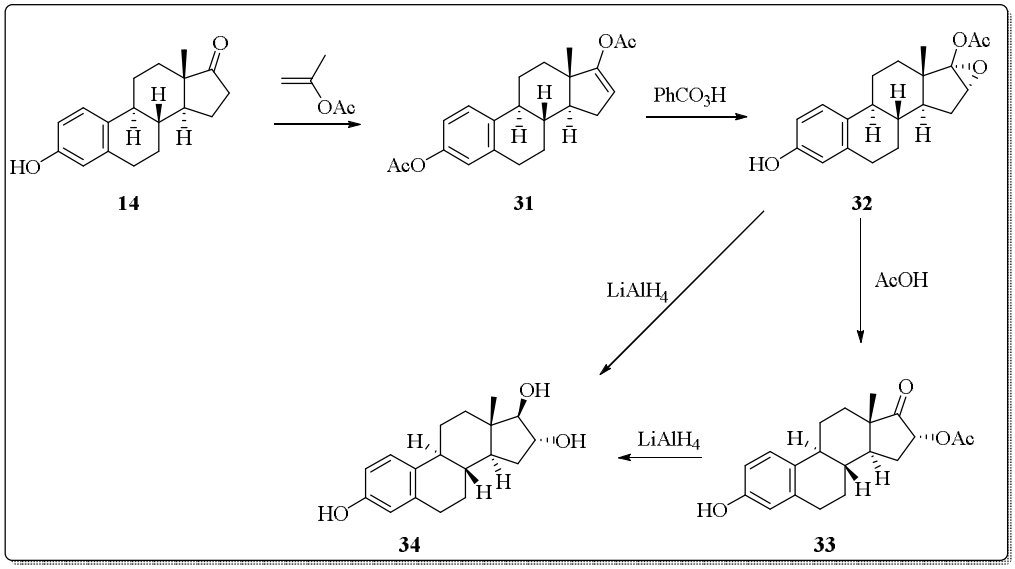


Figure 1.13.Synthesis of *trans*-diol-16α-hydroxyl-17β-estradiol

Mannich reaction is also applicable to steroids compounds, an example of this is the reaction of dihydrotestosterone **35** with dimethylamine and formaldehyde resulting to the 2-dimethylaminomethyl derivative **36** of a steroid compound. Catalytic hydrogenation of **36** derivative steroid at high temperature substitute the dimethylamino group with a hydrogen **37**. Bromination of **37** compound provides 2-bromo derivative **38**. Treatment of **38** with Li_2CO_3 and DMF eliminate hydrobromic acid and produced a new compound which is anabolic androgen *Stenbolone* **39** a 2-en-3-one system [3].

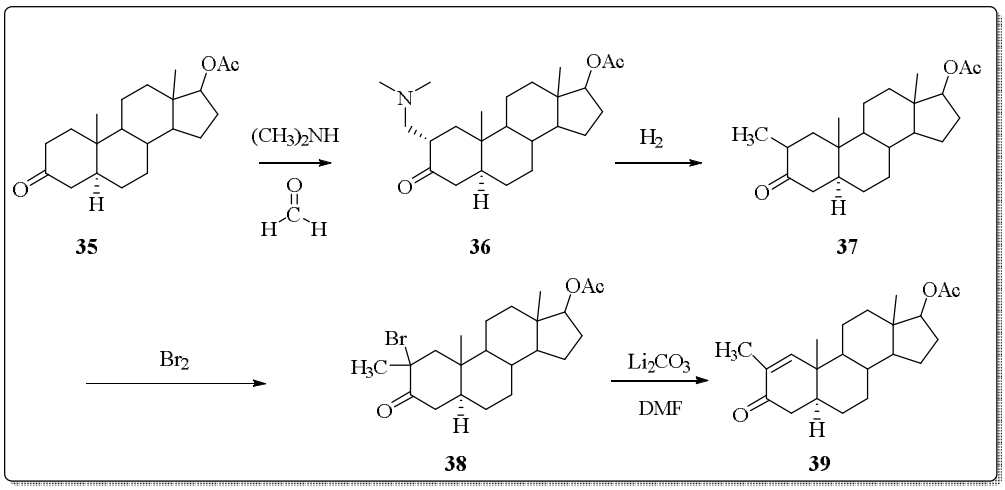


Figure 1.14. Synthesis of *Stenbolone* starting from dihydrotestosterone

Another reaction is adding cyanide to the androst-4-one-3,17-dione **40** to form cyanohydrin derivative with 17β-nitrile **41**. Treatment of this cyanohydrin compound with

methanolic hydrogen chloride transform the nitrile group to imino ether **42**. Closing the ring with methyl chloroformate produces spirooxazoline ring **43**. Acidic hydrolysis of this intermediate leads to the formation of oxazolidine-2,4-dione **44** [3].

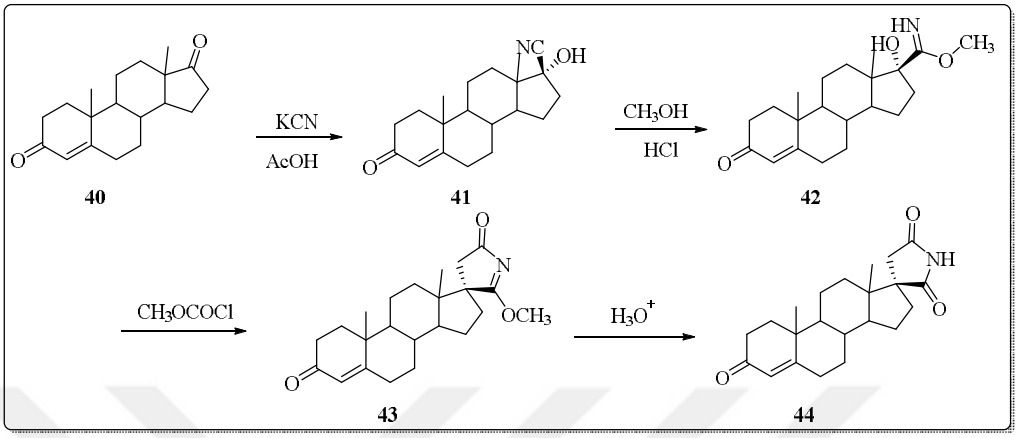


Figure 1.15. Synthesis of oxazolidine-2,4-dione from androst-4-on-3,17-dione

Alkaline hydrogen peroxide is used to epoxidized double bonds that are in a conjugational position with a carbonyl. An example of this in steroids is the reaction of *Pregnenolone* **17** with H_2O_2 in the presence of NaOH to produce an epoxidized *Pregnenolone* **45** [3]..

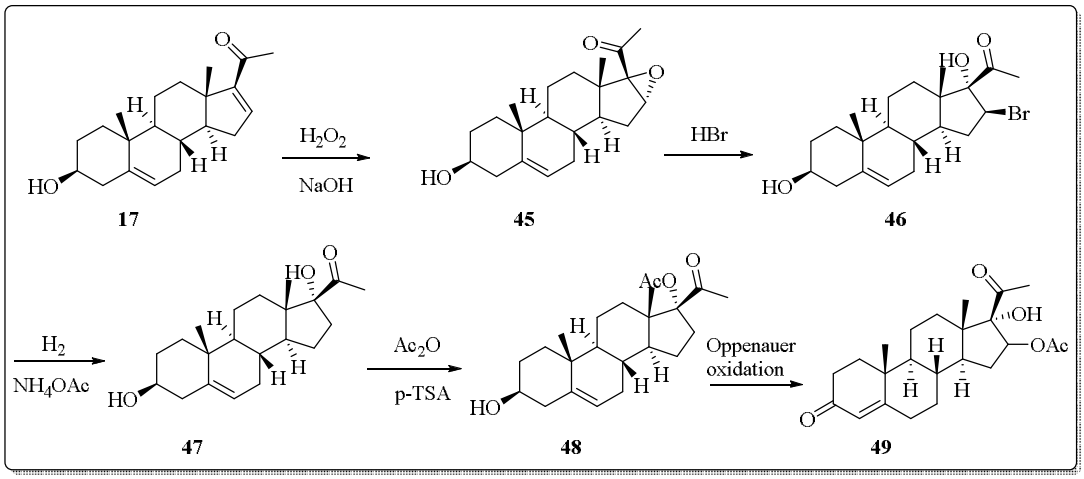


Figure 1.16. Synthesis of 17α-hydroxy *Progesterone* from *Pregnenolone*

The reaction of epoxidized *Pregnenolone* **45** with HBr lead to the diaxial opening of the epoxide ring **46.** The halogen attached on this intermediate can be eliminated by catalytic hydrogenation NH_4OAc as a buffer to form a 17α-hydroxy compound **47**, *p*-

toluenesulfonic acid catalyzed the formation of a 17α-acetate compound **48** from the **47** intermediate with acetic anhydride. Oppenauer reaction is then used to oxidize the hydroxyl group and shifts the double bond to conjugation position and yield the 17α-hydroxyprogesterone **49** [3].

The reaction of *16-pregnenolone* **50** with Grignard reagent CH_3-MgBr add a methyl group to C_{16} during this process acyl group is hydrolysed **51**. Oppenauer oxidation reaction then converts the intermediate compound to 16α-methyl progesterone **52** [3].

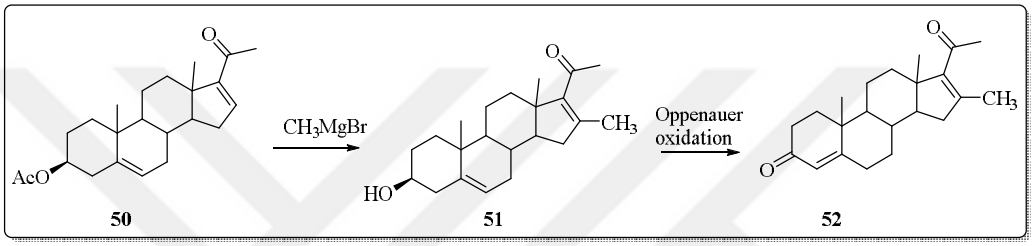


Figure 1.17. Grignard reaction of *16-Pregnenolone*

Dehydrogenation reaction of *testosterone* **24** with 2,3-dichloro-5,6-dicyano benzoquinone (DDQ) gives 1,4-dien-3-one compound **53**. Oxidizing the hydroxyl group at C_{17} of this compound with any available oxidation method **54** followed by the Bayer-Villiger oxidation reaction with a peracid result to the formation of testolactone **55** [3].

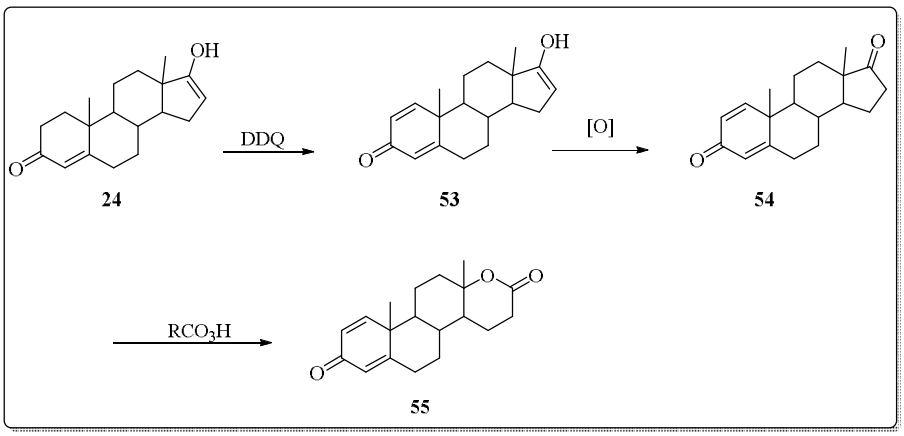


Figure 1.18. Testolactone from *Testosterone*

Catalytic hydrogenation of **20** results in the formation of *5α-cholestan-3β-ol* **56** with 85-95% yield. Also, *meta*-chloroperoxybenzoic acid (*m*-CPBA) epoxidized the double bond in the B ring of *Cholesterol* and form 5α,6α-epoxycholestan-3β-ol **57** as the only product [2].

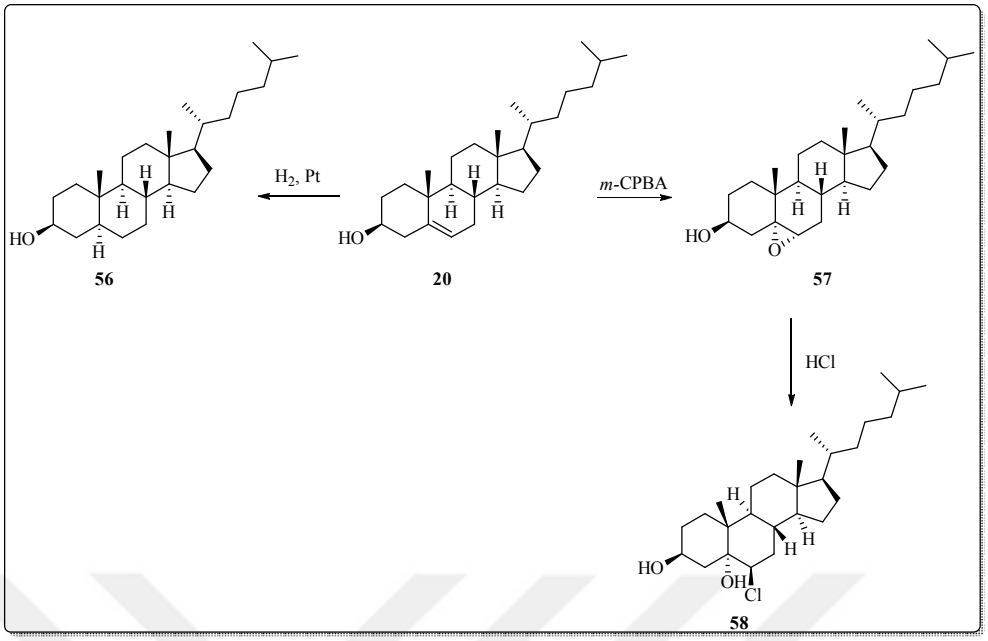


Figure 1.19. Catalytic hydrogenation of *Cholesterol*

The acidic opening of the epoxy ring of this compound with HCl is done by chloride ion attacking the β face forming a diaxial product **58** [2].

1.8 Vilsmeier-Haack Reaction

Vilsmeier-Haack reaction is a type of reaction used to add a formyl group to an aromatic compounds or alkenes, electron rich aromatic compound reacts with dimethylformamide to yield aromatic aldehyde. Also, alkenes undergo Vilsmeier-Haack reaction. The reaction starts with the formation of the formylation reagent simply known as Vilsmeier-Haack reagent or Vilsmeier complex from *N'N*-dimethylformamide and phosphorus oxychloride which is formed in *situ*, this chloromethyl iminium salt act as an electrophile [7]

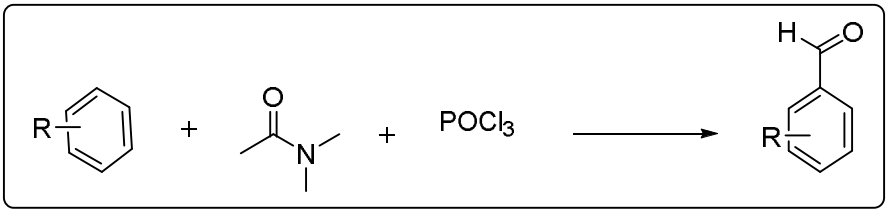


Figure 1.20. Vilsmeier-Haack reaction

However, heterocyclic compounds such as pyrrole, furan indoles, and other five-membered ring heterocyclic compounds also undergo Vilsmeier-Haack reaction [8]. Vilsmeier formylation can also be applied to some of the biologically important molecules, for example, steroids, proteins, amino acid etc [9]. *trans*-Dehydroepiandrosterone can also be formylated with Vilsmeier-Haack reaction at the D ring with the conversion of the carbonyl group to Cl and aldehyde at the C_{17}.

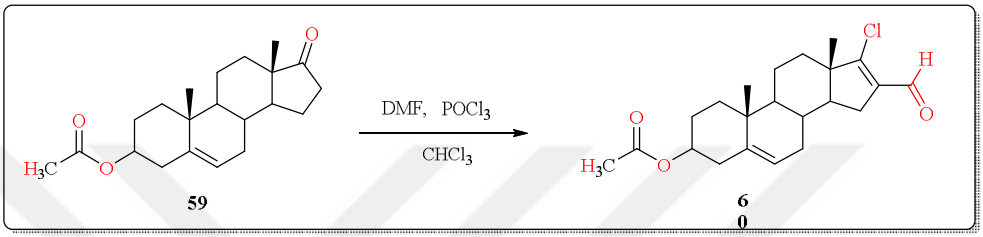


Figure 1.21. Vilsmeier-Haack reaction of DHEA

1.9 Epoxidation Reaction

Reactions of alkenes with an organic peroxy acid or in another name peracid such as *meta*-chloroperoxybenzoic acid or MMPP, this process is known as epoxidation [2,10].

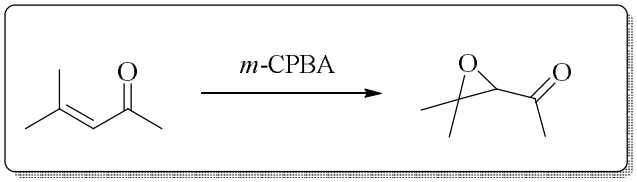


Figure 1.22 Epoxidation reaction α-β carbonyl compounds

The electrophilic oxygen in the peroxy acid is transferred to the double bond of the alkene to form oxycyclopropane ring with two carbons bond to single oxygen [10].

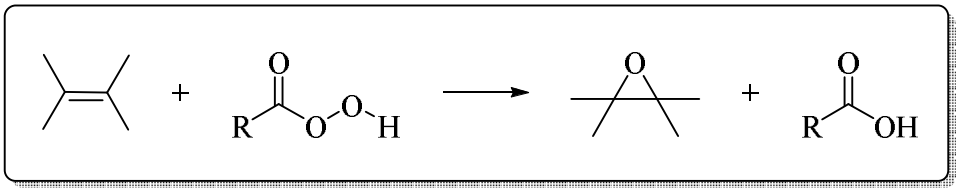


Figure 1.23 Epoxidation reaction of alkene compounds

Epoxidation of alkenes with peracid involve *syn* addition and its stereospecific [10], for example, the epoxidation of *trans*-2-butene produces *trans*-2,3-dimethyloxacyclopropane with the retention of the stereochemistry of the *trans*-2-butene, also, *cis-2*-butene have the same condition [10].

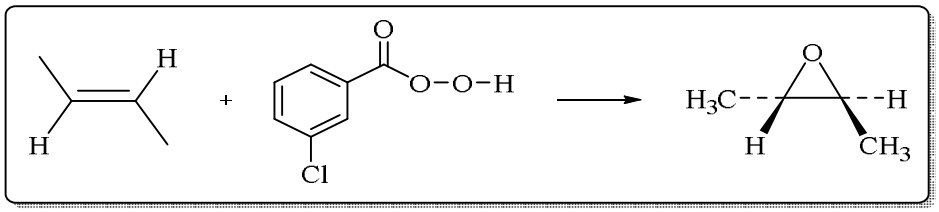


Figure 1.24. Epoxidation reaction of *trans* alkenes

1.10 Benzimidazole

Benzimidazole is an aromatic heterocyclic compound consists of C, H and N with a molecular formula $C_7H_6N_2$. Benzimidazoles are such organic heterocyclic compound formed by the fusion of benzene to five-membered imidazole ring as shown below the benzene is fused to 4^{th} and 5^{th} position carbons of imidazole [11]. Benzimidazoles were also known as benzoglyoxalines and benzimidazoles [12].

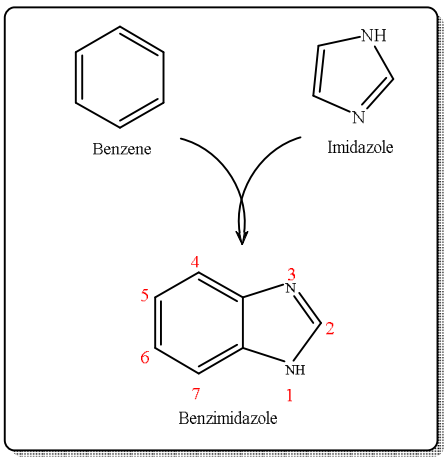


Figure 1.25. Structure of benzimidazole

Benzimidazole is an amphoteric organic compound i.e. it shows both acidic and basic properties, the amine group on the benzimidazole shows strong acidic properties and also weak base properties. Moreover, the H at the N-1 position of benzimidazole tautomerizes to the N-3 position of the benzimidazole as shown in the scheme below [12].

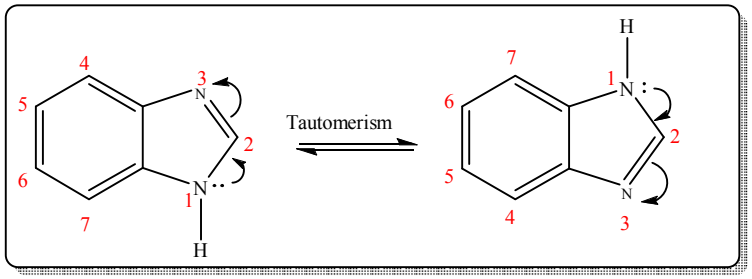


Figure 1.26. Tautomerism of benzimidazole

1.10.1 Synthesis of Benzimidazole

The first synthesis of benzimidazole was carried out by Hoebrecker in 1872. He synthesized 2,5-dimethylbenzimidazole or 2,6–dimethylbenzimidazole by the reduction of 2-nitro-4- methylacetanilide. The same compound was obtained by Ladenburg by refluxing 3,4-diamino toluene in the presences of acetic acid [12].

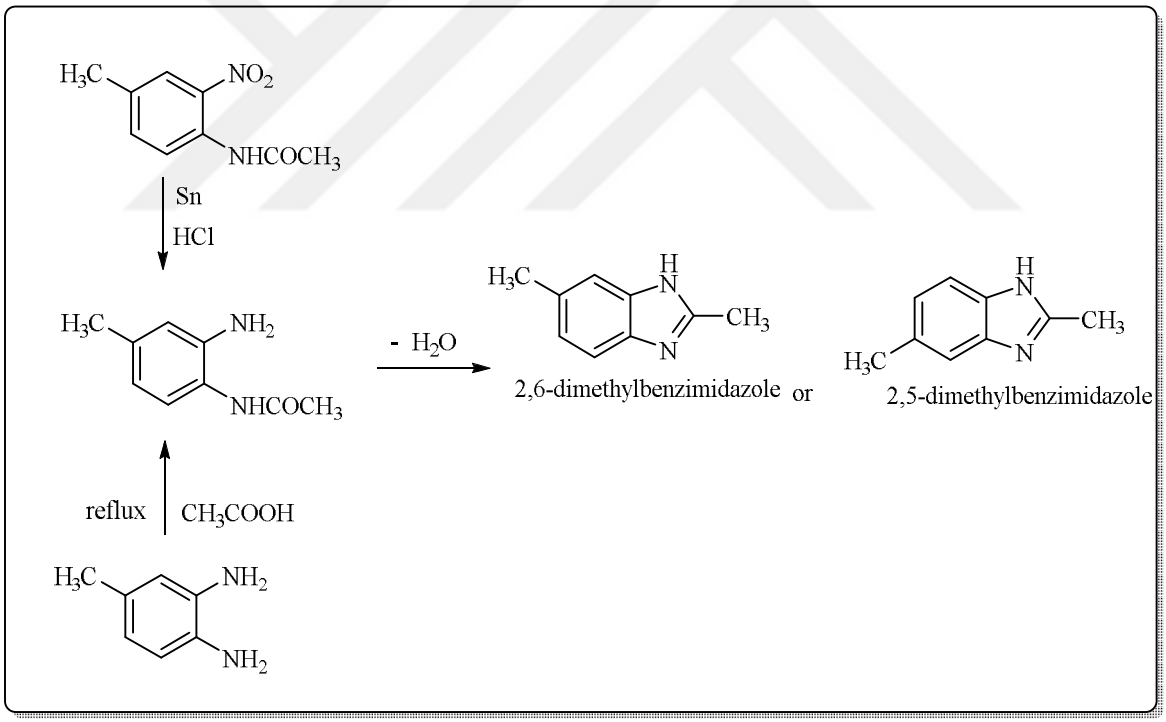


Figure 1.27. First synthesis of benzimidazole

Several synthetic approaches were used for the synthesis of benzimidazoles, a summary of some of this synthetic procedure have shown in the scheme [13].

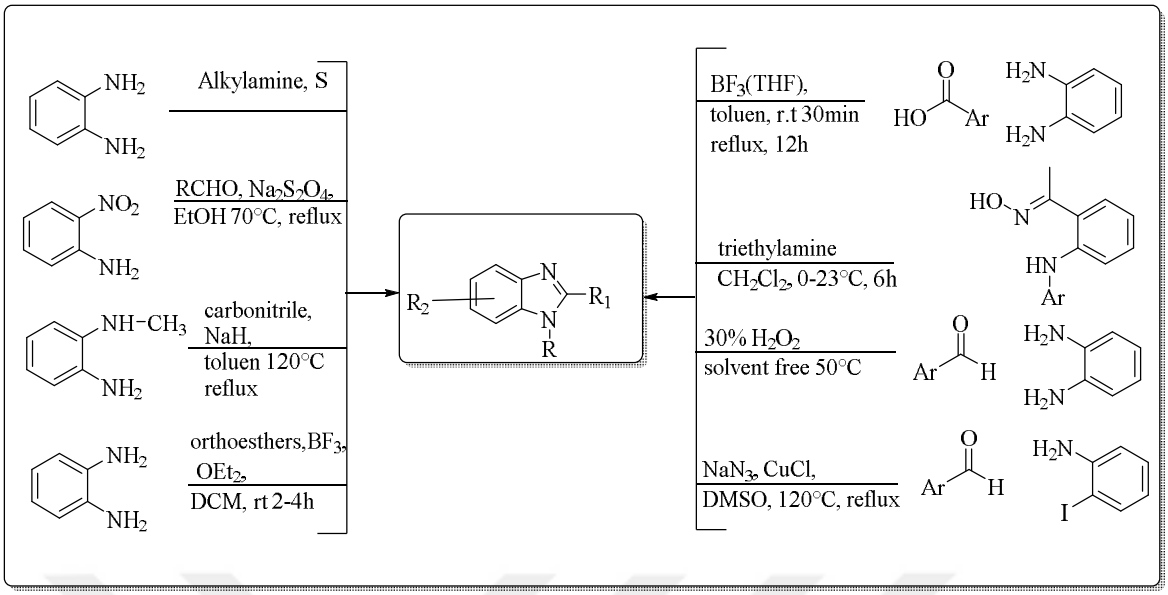


Figure 1.28. Synthesis of Benzimidazole derivatives

1.10.2 Biological Functions of Some Derivatives of Benzimidazole

There are a huge number of biological functions of benzimidazoles which include many mechanisms such as enzymatic actions, oxidative stress, etc. Biological researches conducted on benzimidazoles elucidate that modifications or substitution made on benzimidazole produces different types of biological activities. These different types of activities and their related drugs are summarised in **Figure 1.29** [12]. Derivatives of benzimidazole have shown potential activities on viruses like HIV [14] RNA [15] and influenza virus [16].

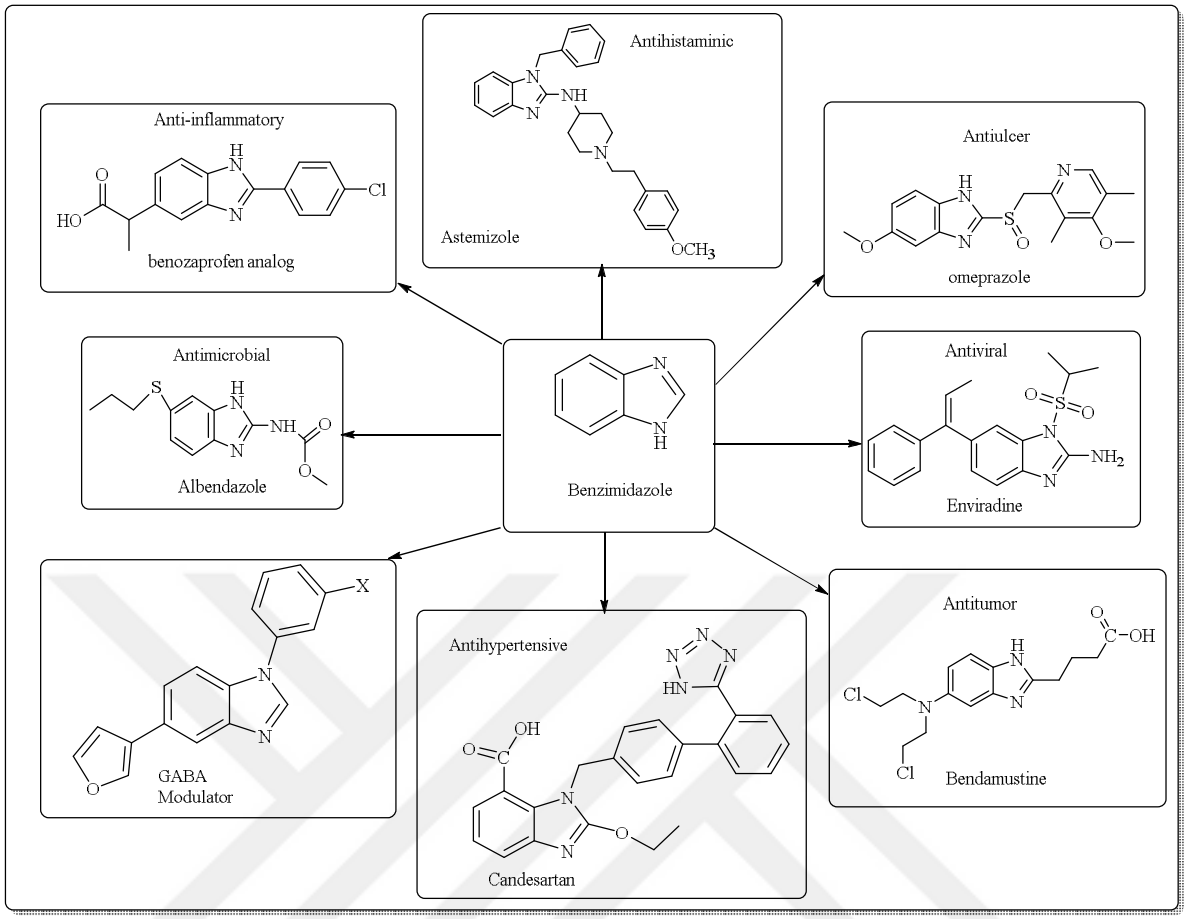


Figure 1.29. Drugs base on benzimidazole and their functions

The **Figure 1.29** show some of the biological activity and their related drugs of benzimidazole, also, benzimidazole derived compounds have potential pharmacological properties like; anti-cancer, anti-oxidant, proton pump inhibitors, anti-diabetic, anti-protozoal, analgesic, anti-convulsant, anti-mycobacterial, anthelmintic, and anti-viral activity [13].

2 LITERATURE REVIEW

Modification of naturally occurring steroids and biological activities of this steroid have attracted the attention of synthetic organic chemist and pharmaceutical researchers. Modified steroidal derivatives constitute a rich source of potential pharmaceutically applicable drug candidates. Many researchers have shown that, if different functional groups are introduced to the tetracyclic skeleton of steroids shows different types of biological activities. Most of the steroids use as medicines and different types of biological activities are such steroids compounds that basically have undergone important modifications [17].

Androgens are responsible for many androgen-dependent diseases such as prostate cancer, due to this reason this enzymes inhibitors are quite useful in the treatment of such diseases. Steroids with azole groups have very strong impacts on 5α-reductase inhibitors, with these impact steroids modified azole groups provide different biological activities and various types of drugs. Synthesis of appropriate types of aza-steroids for the medical applications has attracted the attention of many synthetic and pharmaceutical chemist [18-21].

Specific biological activity can be obtained when an azole group is placed on some biologically active compounds. Theoretically, the hydrophilic and basicity of an azole group can change the biological function of a steroid. Various synthetic steroids contained anti-fungal, anti-lipemic, neuromuscular blocking agents, local anaesthetics, antimicrobial, antioxidant, 5α- reductase inhibitors and anti-cancer activity. In addition, as part of an entire drug azole functional group can interact with some enzymes such as cytochrome P450. Azoles steroid are found to be powerful inhibitors of the enzyme (17α-hydrolase-C17, 20-Lyase) that catalyse the transformation of *Progesterone* and *Pregnenolone* to *Androgens* [22].

2.1 Modification with Heterocyclic Compound at D Ring

A new synthetic method for introducing heterocyclic compounds containing nitrogen to the C_{17} of *trans*-dehydroepiandrosterone steroids has been developed in 1996 and 1997 by V. C. O Njar *et al* [23] where the successfully displaced the chlorine atom at C_{17} of formylated DHEA with heterocyclic compound such as imidazole, pyrazole, tetrazole etc.

Reacting 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene **60** with heteroaryl compound like imidazole or pyrazole in DMF at 80°C in the presence of K_2CO_3 afforded the corresponding aza-steroids **61** in the C_{17} position via the nitrogen of the azole compounds in a high yield (73-96%) [23]. The aldehyde functional group is later removed by refluxing the azole group in benzonitrile in the presence of 10% Pd/C, likewise, the acyl group also is hydrolyzed to hydroxyl functional group **62** [23].

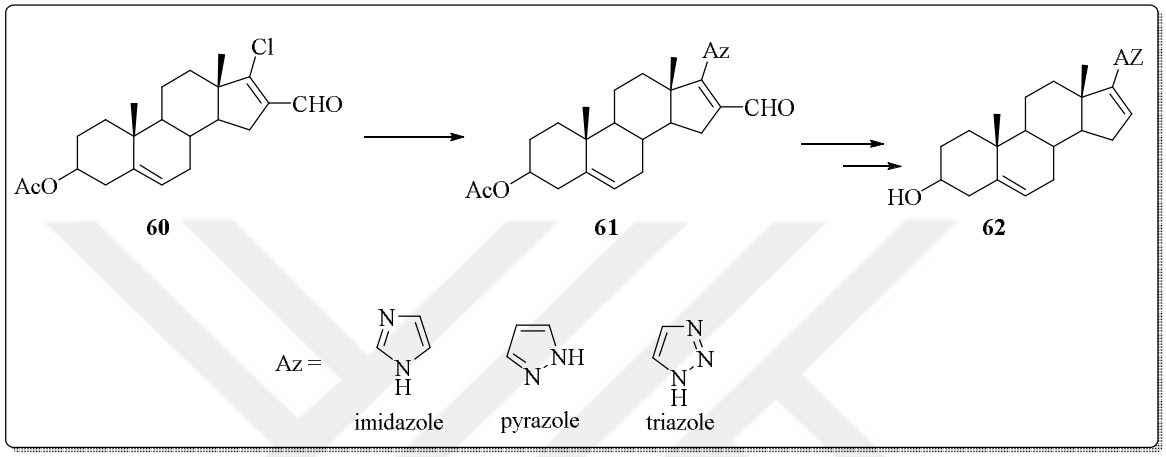


Figure 2.1. Synthesis of C-17 azasteroids

In the previous work, synthetic route of attaching azole to the D-ring of steroid has been described which is based on substitutions reaction of **60** via nucleophilic vinylic "addition-elimination" with azolyl nucleophile replacing the chlorine atom. Moreover, in 1997, these compounds were reported to have possessed very important biological functions which involve inhibition of human and rat testicular $P450_{17\alpha}$, inhibition of steroid 5α reductase and anti-androgenic activity [22].

Additional synthesis of new steroid with benzazoles and pyrazine has been reported in the *Journal of Medicinal Chemistry* by Handratta *et al.* [24]. Synthesis route for new 17-benzazoles and 17-diazines has been fully elucidated in Handratta *et al* research in 2004, where heterocyclic compounds like benzimidazole pyrazine and pyrimidine are attached to the D ring of steroid at C-17. The important intermediate compound for this synthesis is still **60**. Benzimidazole reacts with **60** in dimethylformamide with potassium carbonate at nearly 80°C and gave the C-17 benzimidazole steroids **63** in quantitative yield. Also, benzotriazoles react in the same condition to give another benzotriazole derivatives **69**. As in the previous work the product is deformylated by refluxing it in benzonitrile in the

presence of 10% Pd/C, hydrolysis of the acetoxy group in basic condition followed by Oppenuenur reaction to yield ketone group at the A ring of the products **64** [24].

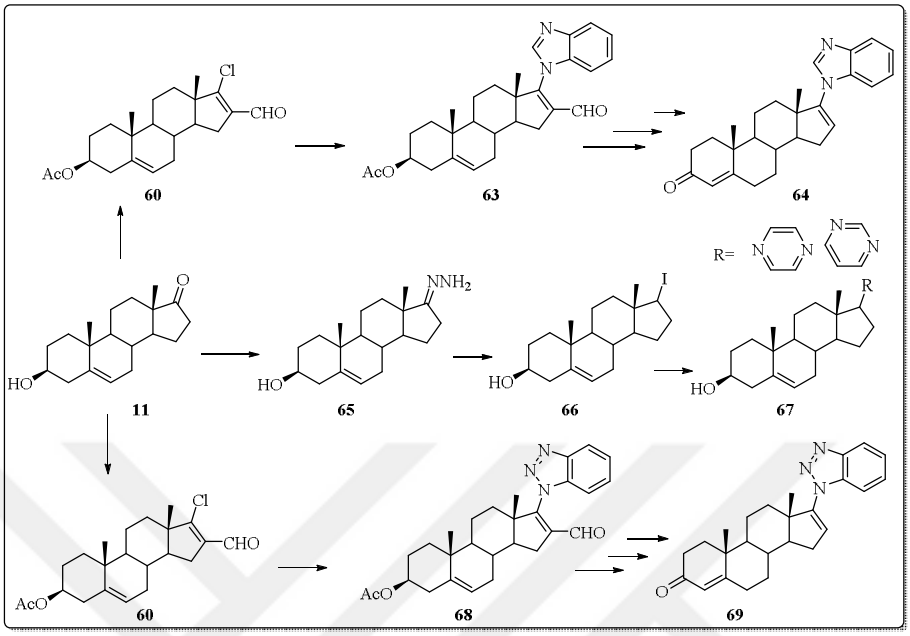


Figure 2.2. Synthesis of steroid with benzazoles and pyrazine

The biological activity of these compounds has also been reported in the paper which shows that 3β-hydroxy-17-benzimidazole has a potential effect on inhibition of CYP17, anti-androgenic anti-tumor, antagonism, and also inhibition of prostate cancer cell [24].

Discovery and development of 3β-(hydroxy)-17-(1H-benzimidazol-1-yl) androsta-5,16-diene currently name as *Galeterone* or TOK-001 or VN/124-1 for the treatment of prostate cancer have been describe by Vincent C. O. Njar and Angela M. H. Brodie in 2015 [25]. This drug candidate has been successfully undergoes phase II clinical test and currently on phase III clinical development by Tokai pharmaceuticals [25].

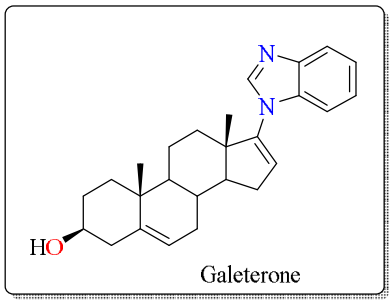


Figure 2.3. *Galeterone* or TOK-001

In another attempt to modified C-17 benzimidazole steroid formally known as VN/124-1 or TOK-001 by R.D. Bruno *et al*. [26] in 2011 to obtained more efficient compound for treatment of prostate cancer, several modifications have been made on the 3β-hydroxy-17-(1H-benzimidazol-1-yl)-5α-androsta-16-ene such as replacement of the 3β-hydroxyl group with another functional groups like fluoro (F) **70** or N_3 (azido) **71** group which are metabolically stable. Another modification made are reduction of Δ^{16}- double bond, reduction of Δ^5-double bond, reduction of both Δ^5- and Δ^{16}- double bond and oxidation of 3β-hydroxyl to 3-oxo compound [26].

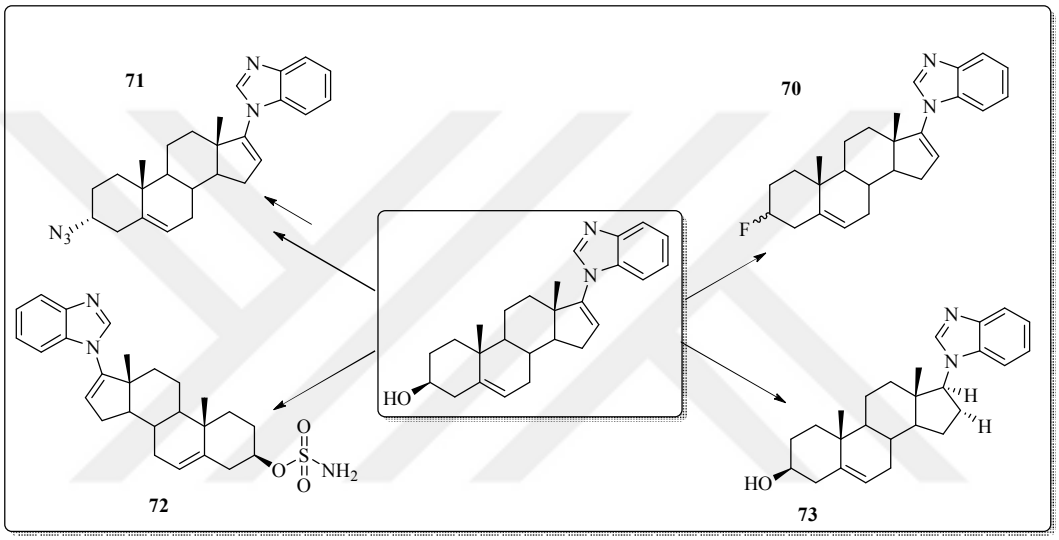


Figure 2.4 Modification on C-17 benzimidazole (Galenterone)

However, in the synthesis of C-17 benzimidazole with saturation in the B ring, *trans-androsterone* was used instead of DHEA. The method used for attaching benzimidazole is the same as in DHEA. Saturation carry out on the D ring followed by hydrolysis of this compound yielded 3β-hydroxy-$\Delta^{5,16}$-tetrahydro-17-benzimidazole oxidation of the hydroxyl group afforded 3-oxo-$\Delta^{5,16}$-tetrahydro-17-benzimidazole (compounds **74-79) Figure 2.5** [26].

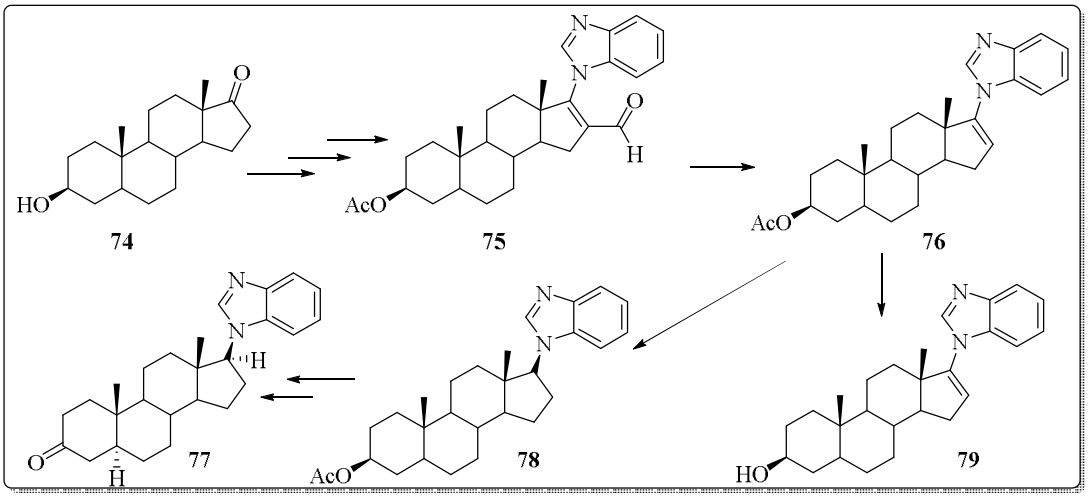


Figure 2.5. C-17 Benzimidazole from *trans*-androsterone

The above reported compounds are found to have potential bioactive properties which include inhibition of CYP17, androgen receptor, prostate cancer, and AR down-regulation but they are not as effective as 3β-hydroxy-17-(1H-benzimidazol-1-yl) androsta-5,16-dien. Structure activity relationship (SAR) conducted on this compounds has proven that saturation in the B or D ring has no positive change. Also, replacing 3β-OH with F or N_3 has no potential change in the biological activity the scheme below shows the SAR C-17 benzimidazole [26].

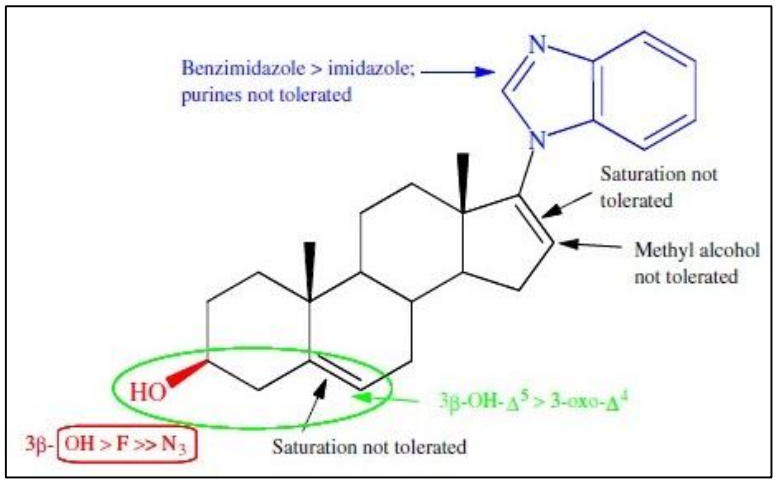


Figure 2.6 SAR C-17 benzimidazole

In another work by Purushottamachar *et al* 2013 [27] which focus on modification of their previously synthesised compound 3β-(hydroxy)-17-(1H-benzimidazol-1-yl) androsta-5,16-diene (Galenterone) also drug candidates for treatment prostate cancer, they described route for synthesis of C-17 benzimidazole from DHEA and other heteroaryl compounds,

they have reported about 26 novel compounds obtained from the intermediate compound 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene, some of the heteroaryl compound used are benzimidazole, indole-3-carbaldehyde, 5,6-dimethylbenzimidazole, 5(6)-cyanobenzimi dazole or 5(6)-methoxybenzimidazole, naphthoimidazole, 2-chlorobenzimida zole, the reaction was carried out in DMF in the presence of K_2CO_3 (compounds **80-85**) [27].

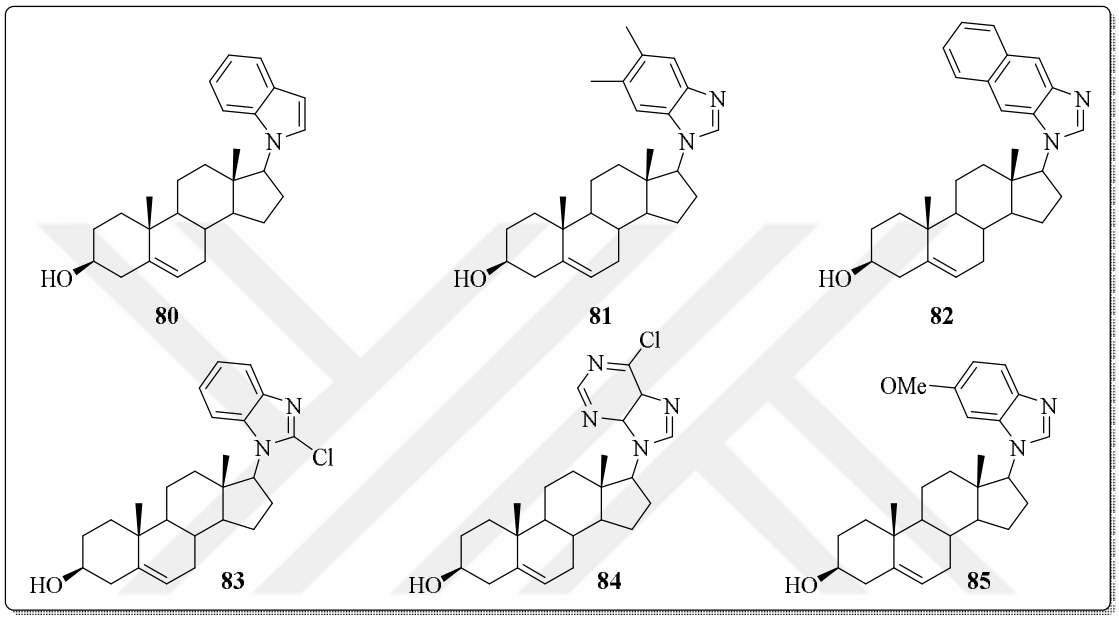


Figure 2.7. Different C-17 heteroaryl compounds from DHEA

Research made by V.M. Moreira *et al* [5] differs from the above-mentioned researches were the heterocyclic structure is attached to the C_{16} core of the steroid via methine carbon bridge, they synthesized *E/Z*-16-azolylmethylene-17-oxoandrostanes compound from the intermediate compound 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene in the presence of K_2CO_3 and DMF [5].

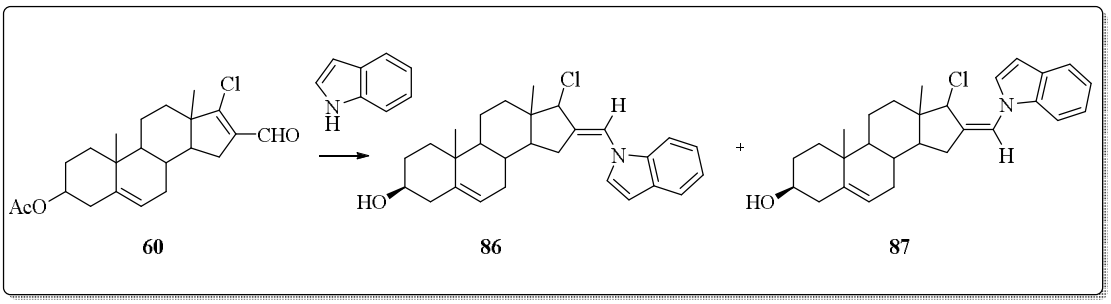


Figure 2.8. *E/Z*-16-azolylmethylene-17oxoandrostanes compounds.

2.2 Epoxysteroids and Oxysterols

Synthesis of epoxysteroids has been fully described by J.F.S Carvalho et al 2009. Different types of steroids **88** were used as starting material, the double bond of the B ring was epoxidised by using magnesium bis(monoperoxyphthalate) hexahydrate (MMPP) in acetonitrile **89**, the process which is stereoselective and with a quantitative yield. The epoxysteroids were reported to have important biological activities like cell proliferation, *Cholesterol homeostasis*, cytoxicity and mutagenic agents. Moreover, chemical and enzymatic method were combined to the developed library of epoxysterols [28, 29].

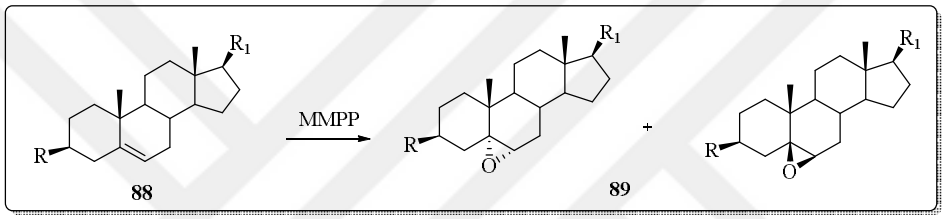


Figure 2.9. Formation of epoxy ring on B ring of steroid with MMPP

Synthesis of 3β,5α,6β-trihydroxysteroids **91** with different functional groups at D ring have been described, in this research the double bond in the B ring of the steroid is epoxidized with MMPP followed by ring-opening with $Bi(OTf)_3$ in situ reaction and yield hydroxyl groups at 5 and 6 position of the steroids, some of the starting materials are cholesterol, DHEA, sitosterol stigmasterol **90** etc. [30]. Oxysterols possess many biological functions as polyhydroxy steroids with hydroxyl groups at 3β, 5α, 6β are found in the marine organism and some of this are cytotoxic anticancer agents among other activities [31].

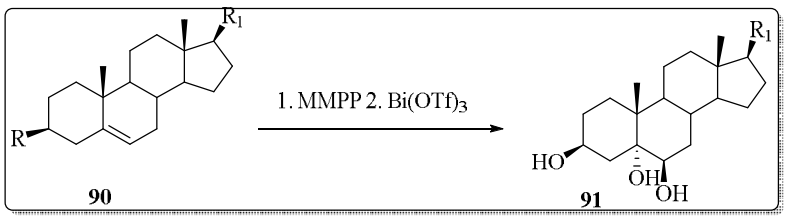


Figure 2.10. *trans*-Diol from MMPP and $Bi(OTF)_3$

Alain Rahier and Maryse Taton [32] synthesized 5α-cholest-7-ene-3β,5α,6α-triol **93**, they first obtain cholest-7-ene-3β,5α-diol-6α-yl *m*-chlorobenzoate using *m*-CPBA and dichloromethane followed by work up process with methanolic potassium hydroxide to obtain pure 5α-cholest-7-ene-3β,5α,6α-triol [32].

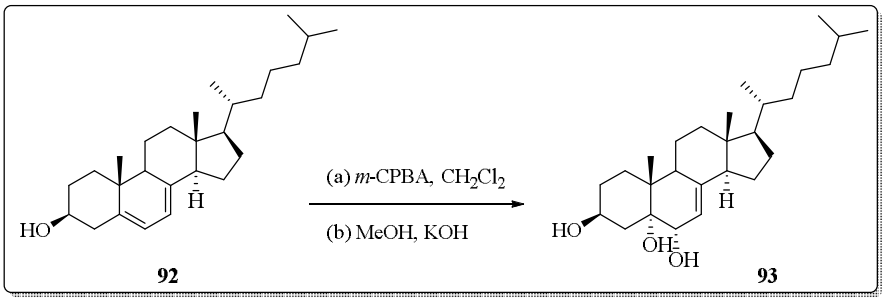


Figure 2.11 *cis*- Diol from *m*-CPBA

Tao Li and Chunbao Li in 2013 [33] reported the method for transforming the double bond at the B ring of steroid into 5α,6β-dihydroxylated steroid with KI as a catalyst in the presence sulfuric acid, and 30% hydrogen peroxide with an excellent yield [33].

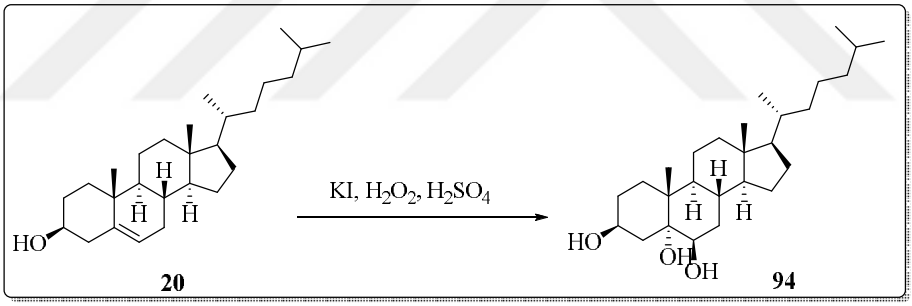


Figure 2.12 *trans*-Diol from KI catalyzed reaction

Another interesting dihydroxylation of steroid was the reaction of cholesterol, stigmasterol, and DHEA with *N*-bromosuccinimide (NBS) in the presence of acetic acid and acetone. The Δ^5 bond of these compound was transformed to 5α and 6β diol this result to the formation of triol steroids (**Figure 2.13)** [34,35].

The above summarise dihydroxylation of steroid with different types of specific reactive has shown that the MMPP, KI, and NBS method have afforded compounds with same stereochemistry that is the hydroxyl groups are in *trans* positions compounds **91, 94,** and **95.** However, the *m*-CPBA method of hydrolyzation gives a product with a hydroxyl group at the *cis* position **93**.

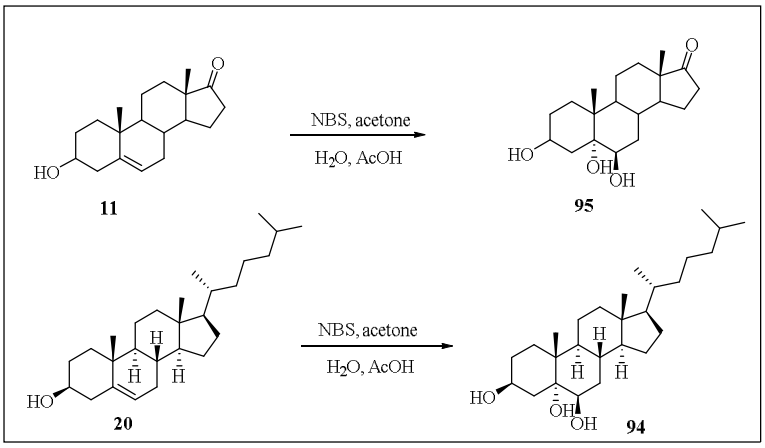


Figure 2.13 *trans*-Diol from NBS

2.3 Aims of Study

Steroids, heterocyclic compounds, aza-steroids and oxysterols have recently attracted much more attention of synthetic and medicinal chemist. It was reported that these compounds and their various derivatives have shown important biologically significant activities. Therefore, our study focuses on combining aza-steroids and oxysterols as one compound.

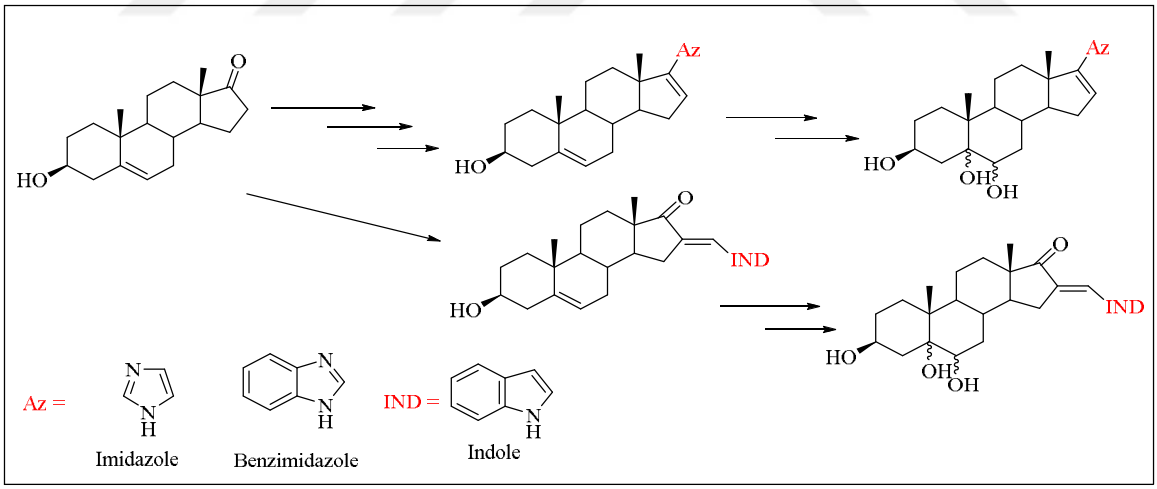


Figure 2.14. Aims of the study

The first part of this research will modify C-16 and C-17 carbon atoms of *trans*-dehydroepiandrosterone with heterocyclic compound (benzimidazole or imidazole) followed by converting the Δ^5 bond to epoxy ring by two different methods. The last part of this study will be the formation of trihydroxy steroids by opening the epoxy ring with different methods.

3 MATERIAL AND METHODS

Melting points were determined with an Electrothermal IA 9100 and Stuart apparatus and are uncorrected, DSC was taking using DSC-50 SHIMADZU differential scanning calorimeter machine, TLC was carried out on precoated silica gel plates, Column chromatography was done with silica gel 60 (0.063-0.200). Denver APX-200 digital scale was used for weighing, Heidolp 4001 Rotary Evaporator was used for evaporating solvents at reduced pressure. Some of the apparatus used are beaker, boiling flask, conical flask, dropper, measuring cylinder, condenser, funnel, filter paper, magnetic hot plate, thermometer, separation funnel, column, erlenmeyer flask, vacuum oven, etc.

Infrared spectra were recorded on Perkin Elmer Spectrum Two ATR-FTIR and spectrums were recorded on BRUKER 400 MHz ^{1}H-NMR and 100 MHz ^{13}C- NMR spectrometer with TMS as internal standard, otherwise stated all spectra were obtained in $CDCl_3$ and are reported in ppm. Some of the reagent and solvent and their providing company are summarised in the table below;

Table 3.1 Reagent and solvent sources

Reagents (%)	Source
DHEA 99%	Sigma-Aldrich
Benzimidazole > 99%	Alfa Aesar
Indole 99%	Merck
Imidazole 99%	Merck
NBS 99%	ABCR
m-CPBA	Acros Organic
$POCl_3$ 99%	Acros Organic
K_2CO_3 99%-101	Sigma-Aldrich
DMF 99%	Sigma-Aldrich
DCM 99%.5	Scharlau
KOH 85%	Merck
n-Hexane 95%	Emplura
Chloroform 99-99.6%	Scharlau
Ethyl acetate 99.96%	Scharlau
Ethanol 99%	Merck
Petroleum Ether 75%	Sigma-Aldrich
NH_3 solution 25%	Carlo Ebra
Pyridine 99%	Carlo Ebra
Acetic anhydride	Sigma-Aldrich

3.1 Experimental

All the reagents were used as received in this thesis. All the solvents used were purified and dried according to the standard purification method available. Some α-β unsaturated compounds **10a-c** previously synthesised in the laboratory in another thesis were also used in this thesis for the synthesis of α-β unsaturated *cis*-diol product, similar to the above compounds the double bond in the B ring was transformed to *cis*-diol [36].

3.2 Summarized Synthetic Route for Synthesis of Azole-Steroids

Compound **2** was prepared from acylation reaction of DHEA **1** with acetic anhydride, formylation reaction of **2** with Vilsmeier-Haack reagents afforded the key intermediate product, compound **3. 4a, 4b,** and **8** were obtained from the reaction of **3** with aza-compounds like benzimidazole, imidazole, and indole.

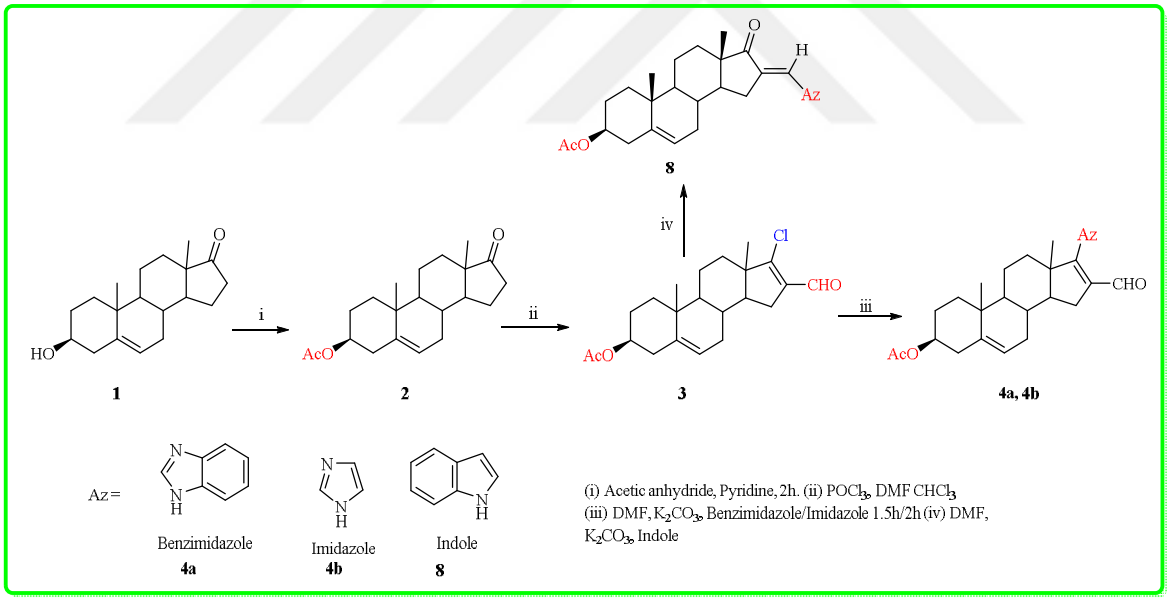


Figure 3.1 Summarised synthetic route for Synthesis of Azole Steroid

3.2.1 Synthesis of 3β-acetoxyandrost-5-en-17-one (2)

Acetic anhydride (16.9 mL, 99%, 1.08 g/mL) and pyridine (35 mL, 0.978 g/m 99.5%) were added to the DHEA **1** (5.0 g, 17.16 mmol) in round bottom flask. The mixture was heated on a steam bath for 2 hours. The mixture was allowed to attain room temperature and poured onto ice water, NH_3 solution was used to neutralize the pH of the solution. The

white precipitate obtained were filtered washed and dried to give a white solid (99%, mp 169-171°C).

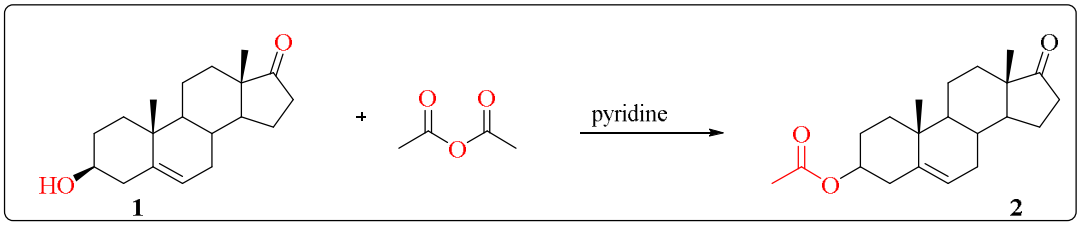


Figure 3.2 Synthesis of 3β-acetoxyandrost-5-en-17-one **(2)**

3.2.2 Synthesis of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (3)

A solution of **2** (5.0 g, 17.16 mmol) in $CHCl_3$ (100 mL) was added dropwise to the cold mixture of $POCl_3$ (25 mL) and DMF (25 mL) and stirred. The pink coloured mixture was allowed to attain room temperature and refluxed at 80ºC for 5 hours under argon gas. The brown coloured solution was concentrated and poured onto ice-water and extract with mixture of ether and ethyl acetate. The organic layer was dried over $MgSO_4$ and filtered, after evaporating the solvent, the solid was crystallized with a mixture of methanol-acetone and then recrystallized with the mixture of petroleum ether and ethyl acetate. (77.7%, mp 157-161°C).

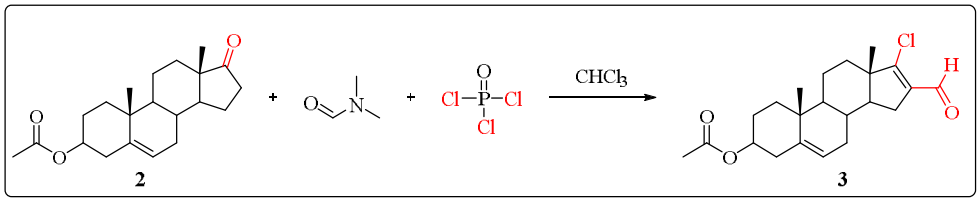


Figure 3.3 Synthesis of 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene.

3.2.3 Synthesis of 3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (4a).

Compound **3** (2.5 g, 6.65 mmol), benzimidazole (2.35 g, 19.9 mmol) and K_2CO_3 (2.75 g, 19.9 mmol) were added to DMF (30 mL) at 80°C and stirred on a hot magnetic plate for 1 hour 30 min. The mixture was left to attend room temperature and poured dropwise onto ice water. The precipitate was filtered, washed with water till pH is 7 and dried, white dirty solid was obtained. The crude product was purified by using column chromatography (petroleum ether, EtOAc). (78%, mp 222-227°C).

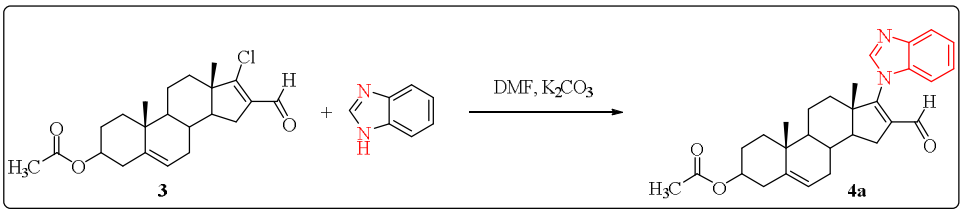


Figure 3.4 Synthesis of 3β-acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene **(4a)**.

3.2.4 Synthesis of 3β-Acetoxy-17-(1*H*-imidazol-1-yl)-16-formylandrosta-5,16-diene (4b)

Compound **3** (2 g, 5.31 mmol), imidazole (0.544 g, 7.99 mmol) and K_2CO_3 (2.20 g, 15.92 mmol) were added to the DMF (40 mL), respectively. The mixture was heated at 80°C and stirred under Argon for 2 hours. The mixture was allowed to attain room temperature and poured dropwise onto ice water. The white precipitate was filtered wash with water and dried. The product was crystallized with the mixture of *n*-hexane and ethyl acetate white shining crystals are obtained. (92%, mp 220-222°C).

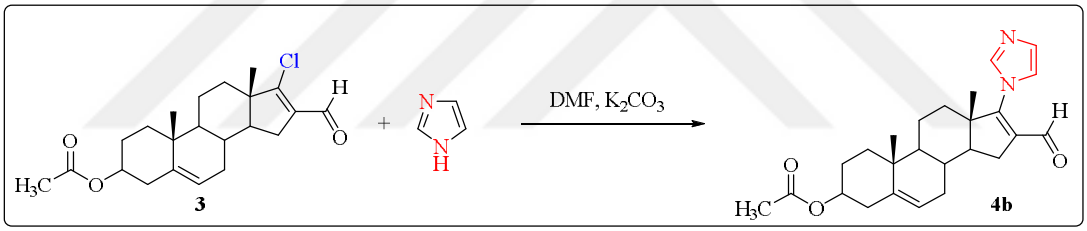


Figure 3.5 Synthesis of 3β-acetoxy-17-(1*H*-imidazol-1-yl)-16-formylandrosta-5,16-diene **(4b)**

3.2.5 Synthesis of 16-(1*H*-indol-1-yl) methylen-17-oxoandrost-5-en-3β-yl acetate (8)

Compound **3** (0.6 g, 1.6 mmol), indole (0.28 g, 2.24 mmol) and K_2CO_3 (0.440 g, 3.2 mmol) were added to DFM (15 mL), respectively. The solution was heated at 80°C for 22 h under argon gas. After 22 h additional K_2CO_3 (0.16 g, 1.2 mmol) was added and continued heating for another 5h. The mixture after attaining room temperature was poured dropwise onto ice water, precipitated and then filtered. The product was washed with water, dried and crystallized with ethyl acetate. (75%, mp 247-249°C).

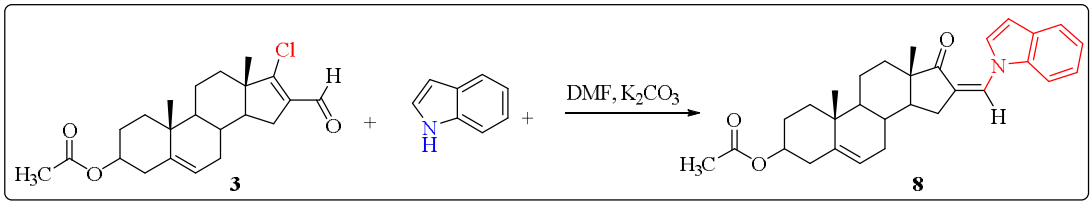


Figure 3.6 Synthesis of 16-(1*H*-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate **(8)**

3.3 Summarized Synthetic route for reduction of 16-formyl group on Azole-Steroid

The compound **5** was prepared from the reaction of **4a** with $NaBH_4$, while compound **6** was synthesized from reaction of compound **5** with *m*-CPBA in dichloromethane.

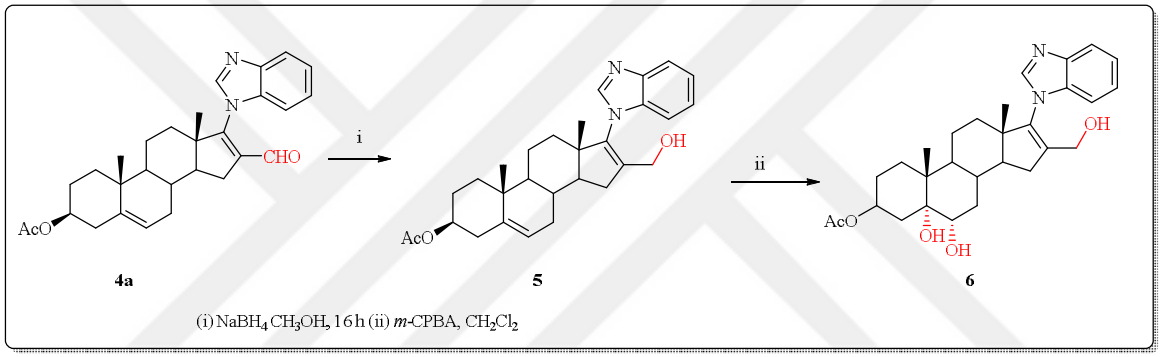


Figure 3.7. Reduction of 16-formyl group

3.3.1 Synthesis of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-(hydroxymethyl) androsta-5,16-diene (5)

A solution of **4a** (0.7 g, 1.53 mmol) in methanol (20 mL) in round bottom flask at 0°C was added $NaBH_4$ (0.174 g, 4.58 mmol), the reaction mixture was stirred at room temperature for 16 h. Then, it was diluted with saturated aq. $NaHCO_3$ (15 mL), the phases formed were separated and the aqueous layer was extracted with ethyl acetate. Organic phases were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was crystallized with chloroform then with petroleum ether and ethyl acetate. (m.p Decomposed after 120-130°C)

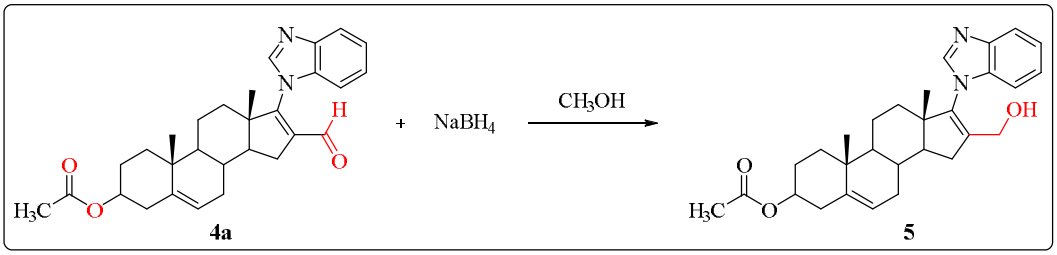


Figure 3.8 Synthesis of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-(hydroxymethyl) androsta-5,16-diene **(5)**

3.3.2 Synthesis of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-5,6-dihydroxy-16-(hydroxymethyl)-16-ene (6)

To solution of **5a** (0.250 g, 0.543 mmol) in dichloromethane (25 mL) was added *m*-CPBA (0.148 g, 0.651 mmol) and stirred for 18 h. The reaction mixture was washed twice with a saturated solution of sodium thiosulfate in a separation funnel and twice with aqueous sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the intermediated solid product which was then dissolved in 5% methanolic KOH and stirred for 24 hours. The product was extracted with ethyl acetate and dried with $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was crystallized with ethanol.

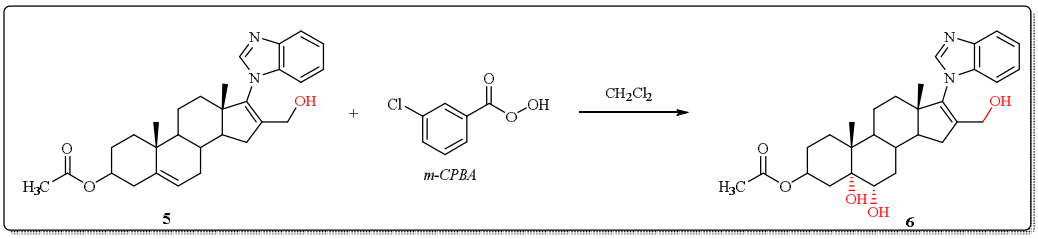


Figure 3.9. Synthesis of 3β-acetoxy-17-(1H-imidazol-1-yl)-5,6-dihydroxy-16-(hydroxymethyl)-16-ene **(6)**

3.4 Summarized Synthetic Route for Synthesis of α,β-Unsaturated *cis*-Diol Steroids

Different α,β-unsaturated trihydroxy aza-steroid **11a-c** were synthesized from the reaction of α,β-unsaturated aza-steroids **(10a-c)** with *m*-chloroperbenzoic acid in dichloromethane.

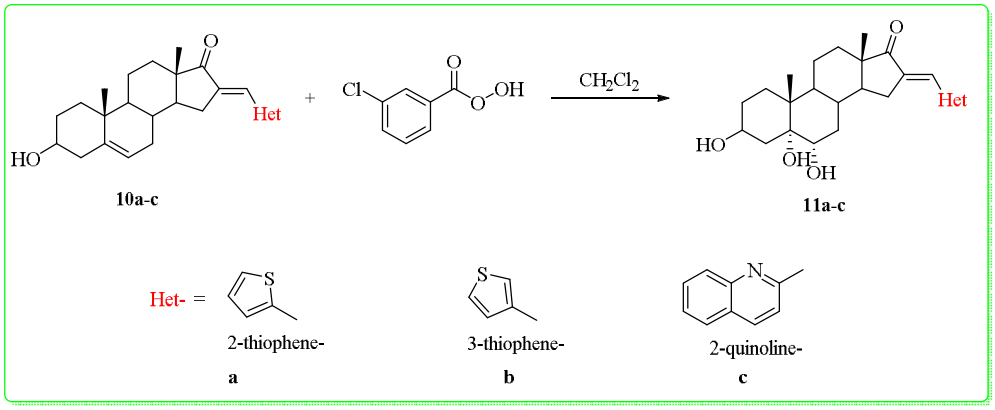


Figure 3.10 Summarized synthetic route for synthesis of α,β-unsaturated *cis*-diol steroids

3.4.1 Synthesis of (16*E*) -(2-Thiophen)-3β,5α,6β-trihydroxyandrostanone (11a)

To a solution of **10a (**0.25 g 0.653 mmol) in dichloromethane (20 mL) was added *m*-CPBA (0.150 g 0.653 mmol) and stirred for 15 hours. The reaction mixture was washed twice with a saturated solution of sodium thiosulfate in a separation funnel and twice with aqueous sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the intermediated solid product which was then dissolved in 5% methanolic KOH and stirred for 24 hours. The product was extracted with ethyl acetate and dried with $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was crystallized with ethanol. (m.p 230-235°C)

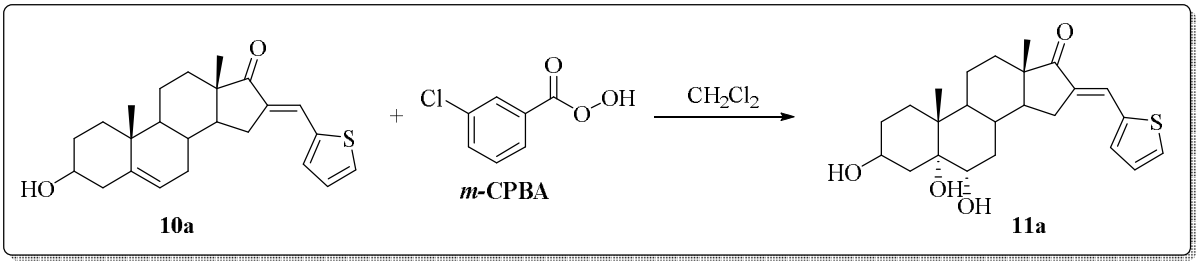


Figure 3.11 Synthesis of (16*E*) -(2-Thiophen)-3β,5α,6β-trihydroxyandrostanone **(11a)**

3.4.2 Synthesis of (16*E*) -(3-Thiophen)-3β,5α,6β-trihydroxyandrostanone (11b)

To a solution of **10b (**0.2 g 0.523 mmol) in dichloromethane (20 mL) *m*-CPBA (0.119 g 0.523 mmol) was added and stirred for 15 hours. The reaction mixture was washed twice with a saturated solution of sodium thiosulfate in a separation funnel and twice with aqueous sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$

and the solvent was removed under reduced pressure to afford the intermediated solid product which was then dissolved in 5% methanolic KOH and stirred for 24 hours. The product was extracted with ethyl acetate and dried with $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was crystallized with ethanol. (m.p 218-221°C)

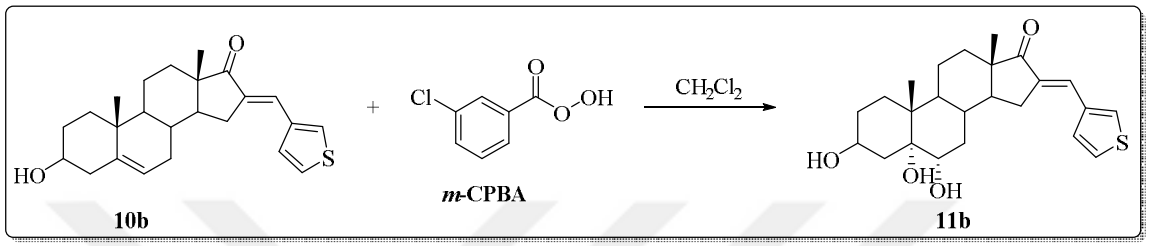


Figure 3.12 Synthesis of (16*E*)-(3-Thiophen)-3β,5α,6β-trihydroxyandrostanone **(11b)**

3.4.3 Synthesis of (16*E*) -(2-Quinolin)-3β,5α,6β-trihydroxyandrostanone (11c)

To a solution **10c (**0.150 g 0.350 mmol) in dichloromethane (20 mL) was added *m*-CPBA (0.079 g 0.350 mmol) stirred for 15 hours. The reaction mixture was washed twice with a saturated solution of sodium thiosulfate in a separation funnel and twice with aqueous sodium hydrogen carbonate. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the intermediated solid product which was then dissolved in 5% methanolic KOH and stirred for 24 hours. The product was extracted with ethyl acetate and dried with $MgSO_4$. The solvent was evaporated under reduced pressure. The crude product was crystallized with ethanol.

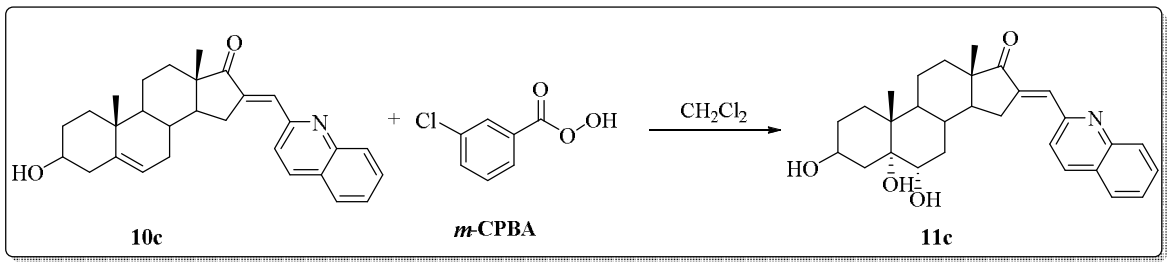


Figure 3.13 Synthesis of (16*E*)-(2-Quinolin)-3β,5α,6β-trihydroxyandrostanone **(11c)**

3.5 Synthesis of 3β,5α,6β-trihydroxyandrostanone (12)

A solution of DHEA **1** (0.5 g, 1.92 mmol) in acetone (30 mL) and H_2O (3.12 mL) stirring in a 50 mL round bottom flask on magnetic stirrer was added NBS (0.365 g, 2.05 mmol) and acetic acid (0.312 mL, 1.05 g/ml, 100%). The reaction mixture was stirred for 24 hours. After 1 hour the colourless solution turns to yellowish then orange colour and turn back to colourless again. After 24h Aq. NaOH (2.0 mL, 2 M) was added, the mixture was extracted with dichloromethane and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was crystallised with petroleum ether and ethyl acetate.

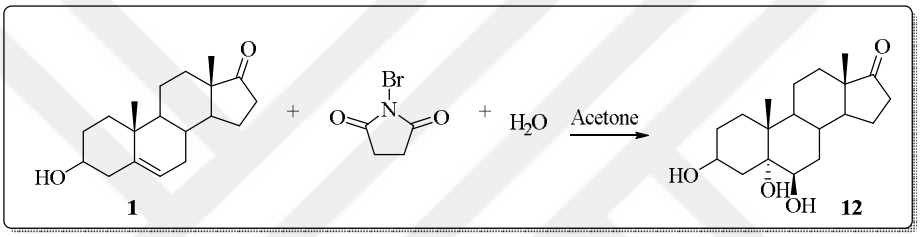


Figure 3.14. Synthesis of 3β,5α,6β-trihydroxyandrostanone **(12)**

3.6 Synthesis of 3β, 5α,6β-triacetoxyandrost-5-en-17-one (13)

Acetic anhydride (3.4 mL 1.08 g/mL, 99%) and pyridine (6.9 mL 0.98 g/mL, 99.5%) were added to the **12 (**0.3 g, 0.930 mmol) in round bottom flask. The mixture was heated on a steam bath for 2 hours. The mixture was allowed to attain room temperature and poured onto ice water, NH_3 solution was used to neutralize the pH of the solution. The white precipitated obtained were filtered washed and dried to give a white solid. m.p 205-207°C

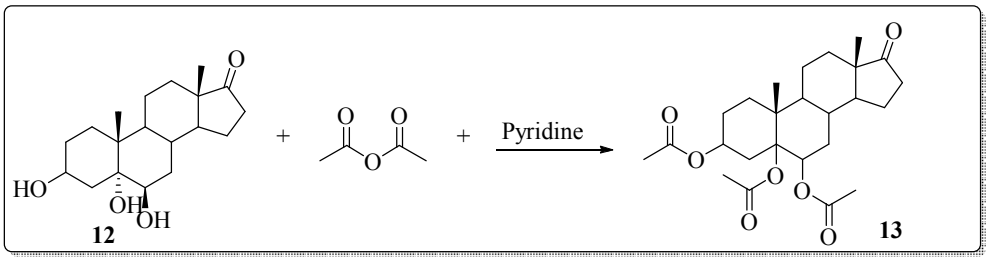


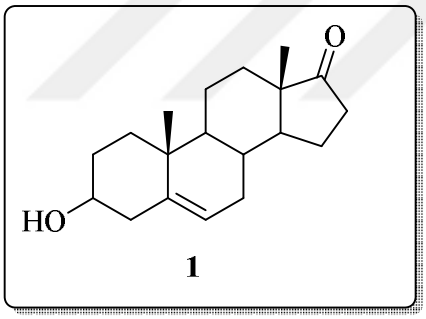
Figure 3.15 Synthesis of 3β, 5α,6β-triacetoxyandrost-5-en-17-one **(13)**

4 RESULT

As mentioned above all the ^{1}H-NMR and ^{13}C-NMR spectrum otherwise specified were recorded in $CDCl_3$. IR spectrum were taking with PERKIN ELMER Spectrum Two on ATR unit. The characterization of the infrared spectrum, NMR spectrum, melting point and percentage yields of all the compound synthesised in this thesis are given below in this section. The spectral data were given in the appendices section of this thesis.

4.1 Characterization of 3β-hydroxyandrost-5-en-17-one (1).

3β-Hydroxyandrost-5-en-17-one (DHEA) was starting material for all of our compounds synthesised in this thesis, IR, ^{1}H-NMR and ^{13}C-NMR spectrum of this compound is given in **Figure (4.1-4.3)**. Looking into ^{1}H-NMR and ^{13}C-NMR of **1,** it is difficult to analyse all the peaks in the spectra due to the complexity, only those that are clearly seen will be taking into consideration here.



1

Melting point: 148.5°C

Chemical Formula: $C_{19}H_{28}O_2$

Molecular Weight: 288.43

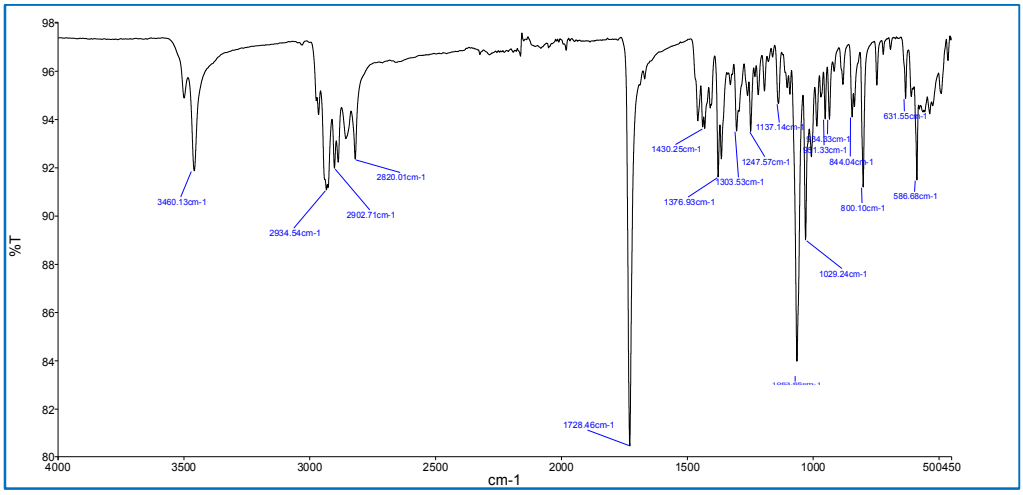


Figure 4.1 Infrared spectrum of 3β-hydroxyandrost-5-en-17-one

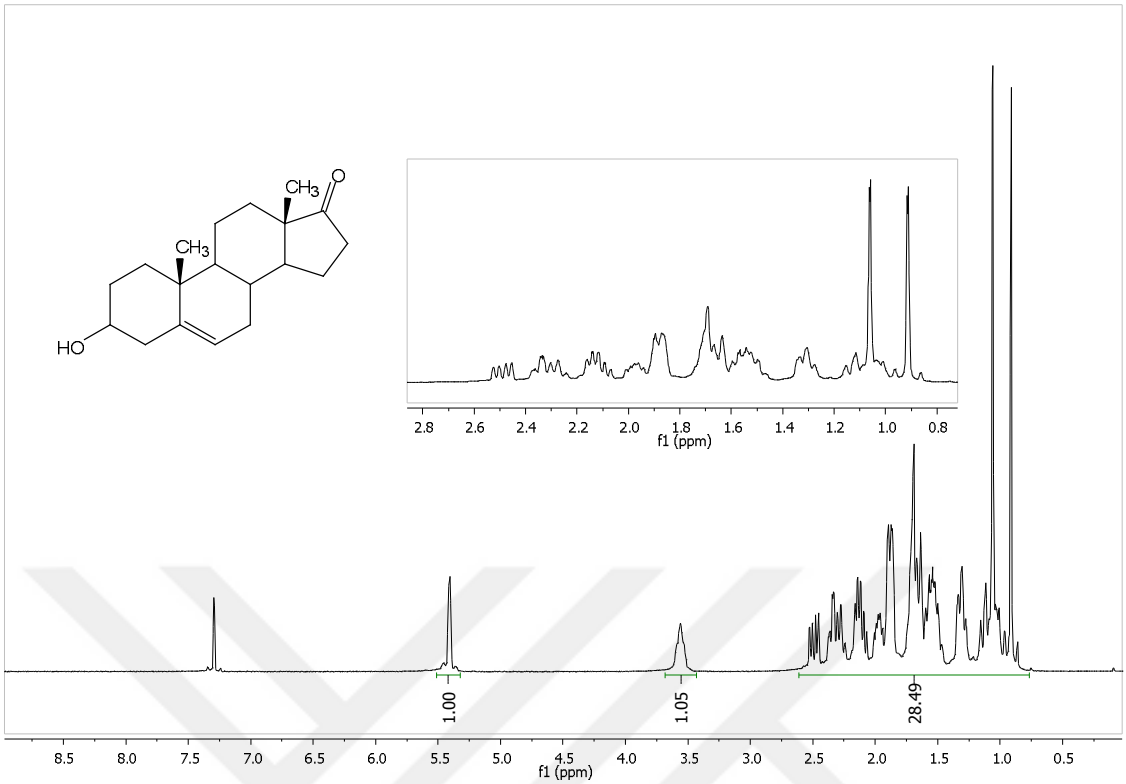


Figure 4.2 ^{1}H-NMR spectrum of 3β-hydroxyandrost-5-en-17-one

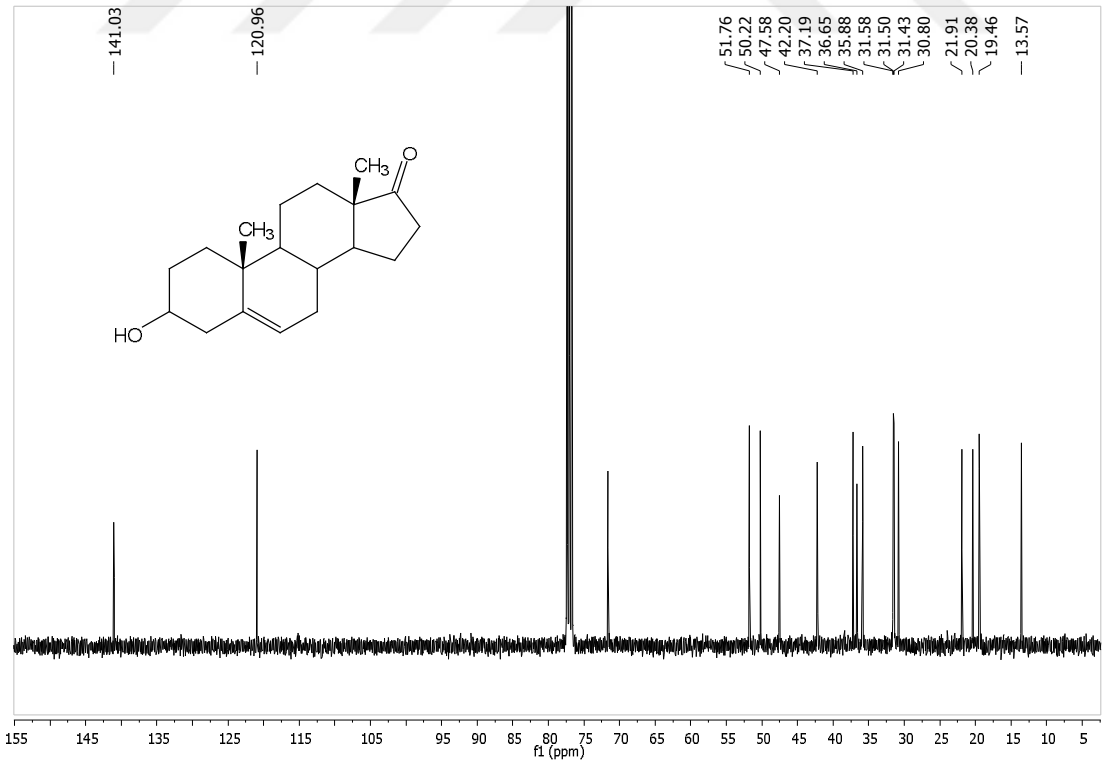
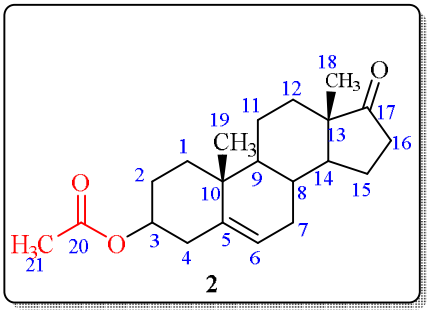


Figure 4.3 ^{13}C-NMR spectrum of 3β-hydroxyandrost-5-en-17-one (1)

4.2 Characterization of 3β-acetoxyandrost-5-en-17-one (2)



2

Melting point: 169-171°C

%Yield: 99%

Chemical Formula: $C_{21}H_{30}O_3$

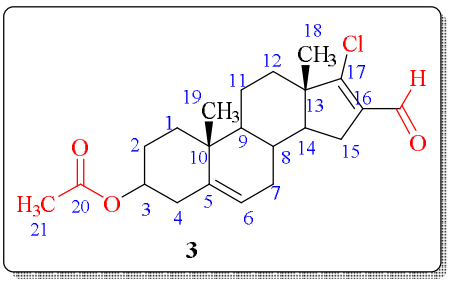
Molecular Weight: 330.47

IR (ATR, cm^{-1}): 2947, 2899 (aliphatic C-H stretching), 1738 (ester stretching, $CH_3\underline{CO}OC$ C=O), 1724 (D-ring ketone C=O stretching,) 1234 (C-O-C stretching).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum revealed the presence of following peaks at the corresponding chemical shifts. δ (ppm)= 0.91 (s, 3H 18-CH_3), 1.07 (s, 3H, 19-CH_3), 2.07 (s, 3H, 3β-OAc), 4.59-4.67 (m, 1H, 3α-H, HC-3), 5.44 (doublet, 1H *J* = 4Hz), Solvent peak is also visible at 7.37 ppm.

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum revealed the presence of the following peaks δ (ppm)= 19.15 (CH_3, CH_3-18), 20.33 (CH_3, $\underline{CH_3}COOC$), 21.90 (CH_3 CH_3-19), 73.74 (C-O, C_3), 121.92 (C-6), 139.90 (C-5), 170.61 (C=O, 3*β*-OAc).

4.3 Characterization of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (3)



3

Melting point: 157-161°C

%Yield: 77.7%

Chemical Formula: $C_{22}H_{29}ClO_3$

Molecular Weight: 376.92

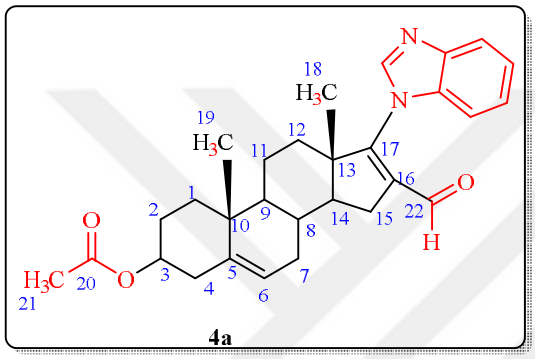
IR (ATR, cm^{-1}): 2978-2840 (aliphatic C-H stretching) 1732 (C=O ester stretching, CH_3COOC-), 1675 (C=O, aldehyde stretching), 1593 (C=C, B ring) 1237 (C-O-C stretching).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum revealed the presence of following peaks at the corresponding chemical shifts. δ (ppm)= 1.02 (s, 1H, 18-CH_3), 1.09 (s, 3H 19-

CH_3), 2.07 (s, 3H, 3β-OAc), 4.59-4.67 (m, 1H, 3α-H C-3), 5.43 (d, 1H *J*= 4Hz), 10.02 (singlet 1H, 16-CHO aldehyde proton).

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum revealed the presence of the following peaks δ (ppm)= 19.25 (CH_3, $\underline{C}H_3COOC$), 20.31 (CH_3 CH_3-19), 21.47 (CH_3, CH_3-18), 73.68 (C-O, C_3), 139.9 (C-5), 162.40 ($\underline{C}$-Cl, C-17), 170.59 (C=O, 3*β*-OAc), 188.19 (C=O, $\underline{C}$HO).

4.4 Characterization of 3β-Acetoxy-17-(1*H*-benzimidazol-1-yl)-16-formylandrosta-5,16-diene (4a)



4a

Melting point 222-227°C

%Yield 78%

Chemical Formula: $C_{29}H_{34}N_2O_3$

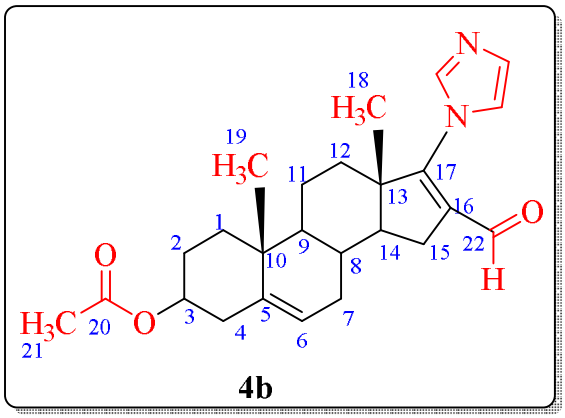
Molecular Weight: 458.60

IR (ATR, cm^{-1}): 3129, 3058 (aromatic C-H stretching), 2978-2843 (aliphatic C-H stretching), 2823 (aldehyde C-H stretching), 1724 (C=O, ester CH_3COOC stretching), 1669 (C=O aldehyde stretching), 1633 (B ring C=C stretching), 1613 (aromatic C=C stretching) 1248 (C-O-C stretching).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum revealed the presence of singlet peak at chemical shift δ (ppm)= 1.08 (singlet, 3H, 18-CH_3), 1.09 (s 3H 19-CH_3), 2.05 (s, 3H, CH_3 3*β*-OAc), 4.60-4.68 (m, 3α-H C-3, 1H), 5.43 (doublet (d) 1H *J* =4Hz,) δ 7.31 (s, 2H, aromatic hydrogens in the benzimidazole ring), 7.87 (1H aromatic hydrogen), 7.88 (1H, aromatic-proton), 8.01 (singlet, 1H, H-C-N, benzimidazole ring), 9.61 (singlet 1H, aldehyde 16-CHO).

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum revealed the presence of the following peaks, δ (ppm)= 21.26 (CH_3, C-18), 21.40 (CH_3, C-19), 21.45 (CH_3, $\underline{C}H_3COOC$), 73.68 ($\underline{C}$-O, C-3), 120, 124.78, 123, ($\underline{C}$H benzimidazole ring), 121.76 ($\underline{C}$-6, CH), 136 (C-16), 140.62 (C-5), 141.46, 144.62 (C, benzimidazole ring), 159.40 (C-benzimidazole, C-17), 170.59 (C=O, 3β-OAc), 188.19 (aldehyde, $\underline{C}$HO).

4.5 Characterization of 3β-Acetoxy-17-(1H-imidazol-1-yl)-16-formylandrosta-5,16-diene (4b).



4b

Melting point 221-222°C

%Yield 92.62%

Chemical Formula: $C_{25}H_{32}N_2O_3$

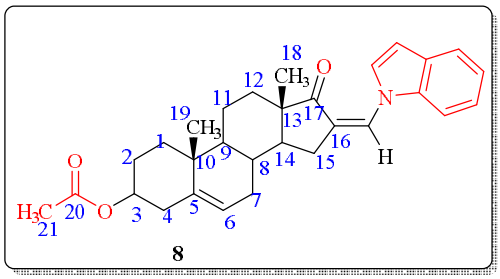
Molecular Weight: 408.54

IR (ATR, cm^{-1}): 3146, 3119 (aromatic C-H stretching), 2967-2850 (aliphatic C-H stretching), 2823 (aldehyde C-H stretching), 1732 (ester C=O stretching, CH_3COOC), 1660 (C=O aldehyde stretching), 1616 (C=C stretching, B ring), 1236 (C-O-C stretching).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum revealed the presence of peaks at corresponding chemical shift; δ (ppm)= 1.10 ppm (s, 18-CH_3 and 19-CH_3 6H), 2.07 (s 3H 3*β*-OAc), 4.64 (m, 3α-H C-3, 1H, *J*=4Hz), 5.45 (doublet (d) 1H *J*=4Hz), 7.15 (CH ,1H, imidazole ring), 7.29 (CH, 1H imidazole ring), 7.71 (CH, 1H, imidazole ring), 9.77 (singlet 1H, 16-CHO aldehyde proton).

^{13}C-NMR: The neat signal on the 13C-NMR spectrum revealed the presence of the following peaks, δ (ppm)= 19.37 (CH_3 C-18), 21.90 (CH_3 C-19), 20.41 (CH_3 $\underline{C}H_3COOC$), 73.61 (C-O C_{-3}), δ 120.05 (CH, imidazole ring), δ 121.97 (CH, C-6), 124.63 (CH, imidazole ring), 134.11 (C-16), 134.11 (CH, imidazole ring), 139.98 (C-5), 160.46 (C-17). 170.59 (C=O, $CH_3\underline{C}OOC$), 187.69 (C=O, $\underline{C}HO$).

4.6 Characterization of 16-(1*H*-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate (8)



8

Melting point 247-249°C

%Yield 78%

Chemical Formula: $C_{30}H_{35}NO_3$

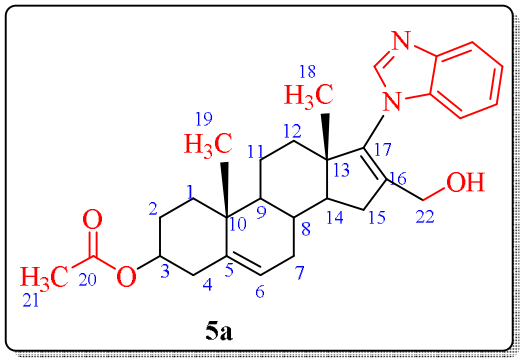
Molecular Weight: 457.61

IR (ATR cm^{-1}): 3156, 3115, 3038 (aromatic C-H stretching), 2964-2834 (aliphatic C-H stretching), 1722.79 (ester C=O stretching), 1628.7 (ketone C=O stretching), 1582.92 (C=C stretching), 1522.89 (C=C stretching), 1241 (C-O stretching).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum revealed the presence of the following peaks at the corresponding chemical shift; δ (ppm) 1.03 (s, 3H, 18-CH_3), 1.13 (s, 3H, CH_3, 19-CH_3), 2.07 (s, 3H, 3*β*-OAc), 4.65 (m, 3α-H C-3, 1H), 5.47 (doublet (d) 1H *J*= 4Hz), 6.77 (d, 1H indole, 3-CH), 7.29 and 7.36 (m, 1H, indole 4-CH and m, indole ring 5-CH), 7.62 (m, indole ring), 8.18 (singlet, methine-H, 1H).

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum revealed the presence of the following peaks, δ (ppm) 19.38 (CH_3, C-19), 20.38 (CH_3, C-18), 21.41 (CH_3, CH3COOC), 73.72 (C-O C-3), 108.08, 110.30 (2 CH of indole ring), 117.82, 121.27, 121.69 (C-7), 122.44, 123.65, 124.99 127.56, 128.95, 137.08, (C-5), 140.19 C-5, 170.51 (CH_3COOC), 209.42 (C=O, C-17).

4.7 Characterization of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16- (hydroxymethyl) androsta-5,16-diene (5a)



5a

Chemical Formula: $C_{29}H_{36}N_2O_3$

%Yield 75%

Melting point. Decomposed (105-120)

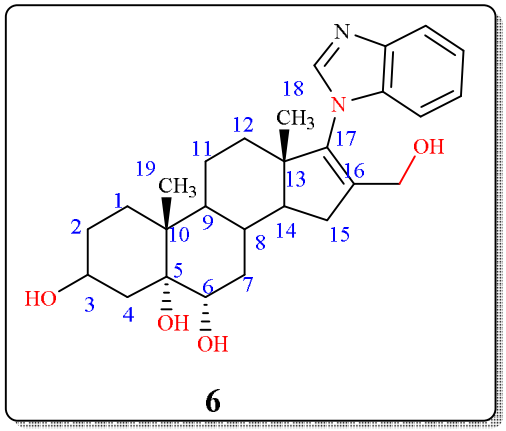
Molecular Weight: 460.62

IR (ATR cm^{-1}): 3593-3156 (OH broad band), 3085, 3041 (aromatic C-H stretching), 2934, 2902, 2846 (aliphatic C-H stretching), 1729 (ester C=O stretching for CH_3COOC), 1612.16 (C=C stretching), 1237 (C-O, stretching vibration).

^{1}H-NMR: The neat signal on the ^{1}H-NMR spectrum of **4b** revealed the presence of singlet peak at chemical shift δ (ppm)= 0.78 (1H, OH, CH_2OH), 1.05 (singlet, 3H, 18-CH_3), 1.14 (s 3H 19-CH_3), 2.06 (s, 3H, CH_3 3β-OAc), 4.01 (2H, $\underline{CH_2}OH$) 4.61-4.67 (m, 3α-H C-3, 1H), 5.47 (doublet (d) 1H *J*= 4Hz,) δ 7.23 (s, 2H, aromatic hydrogens in the benzimidazole ring), 7.30 (1H, aromatic hydrogen), 7.73 (1H, aromatic-proton), 7.80 (singlet, 1H, HCN benzimidazole ring).

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum of **4b** revealed the presence of the following peaks, δ (ppm)= 21.49 (CH_3, C-18), 20.49 (CH_3, C-19), 19.24 (CH_3, $\underline{C}H_3COOC$), 53.79 (CH_2, $\underline{CH_2}OH$) 73.79 ($\underline{C}$-O, C-3), 111.07, 120, ($\underline{C}$H benzimidazole ring), 122.06 ($\underline{C}$-6, CH), 122.56, 123.75 (benzimidazole ring CH) 124.63 (C-16), 128.82 (CH benzimidazole), 130.92 (C-17), 134.85 (C-5), 140.10, (CH benzimidazole), 142.36 (CH, benzimidazole ring, HCN), 170.68 (C=O, 3β-OAc).

4.8 Characterization of 3β-hydroxy-17-(1H-benzimidazol-1-yl) -5,6-dihydroxy-16-(hydroxymethyl)-16-ene (6)



6

Chemical Formula: $C_{27}H_{36}N_2O_4$

%Yield 80%

Melting point: 120-130

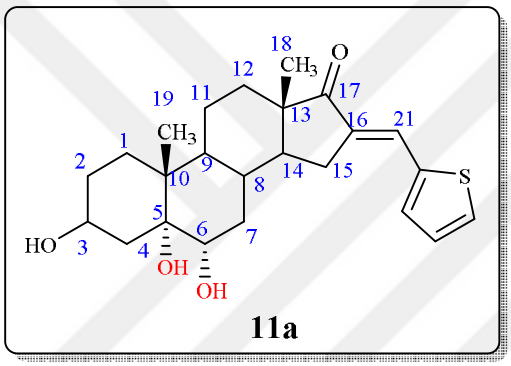
Molecular Weight: 452.60

IR (ATR cm^{-1}): 3546-3146 (OH stretching), 3082 (aromatic C-H stretching), 2927-2854 (aliphatic CH stretching), 1032 (C-OH stretching).

^{1}H-NMR: δ (ppm)= 0.84-2.74 (multiplet peaks, equivalent to 25 protons which are CH_3, CH_2 and CH of the DHEA ring), 3.37 (m, 1 H, 3α-H, HO-C-H), 3.98 (CH, H-C_6-OH). 7.77-7.21 (aromatic CH, equivalent to 5 protons of benzimidazole ring).

^{13}C-NMR: The neat signal on the ^{13}C-NMR spectrum of **6** revealed the presence of the following peaks, δ (ppm)= 21.49 (CH_3, C-18), 16.96 (CH_3, C-19), 54.56 (CH_2, $\underline{CH_2}OH$) 68.43 (C-OH, C-3), 63.27 (C-OH, C-5), 59.06 (C-OH, C-6), 110.98 110.47, (CH benzimidazole ring), 122.85, 123.76 (benzimidazole ring CH) 130.82 (C-16), 128.82 (CH benzimidazole), 134.19 (C-17), 142.27, 140.24 (CH, benzimidazole ring).

4.9 Characterization of (16*E*) -(2-thiophen)-3β,5α,6β-trihydroxyandrostanone (11a)



11a

Melting point; 230-235°C

%Yield % 75

Chemical Formula: $C_{24}H_{32}O_4S$

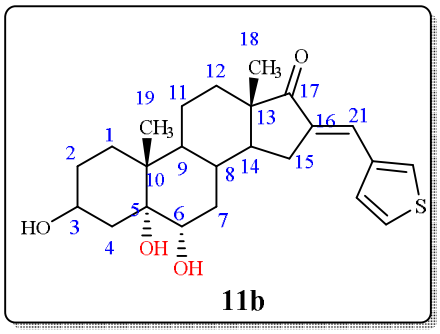
Molecular Weight: 416.58

IR (ATR cm^{-1}): 3411, 3176 (OH stretching), 2941-2863 (aliphatic CH stretching), 1707 (C=O), 1615 (C=C, C_{16}=C_{21}).

^{1}H-NMR: δ (ppm)= 0.91 (s, 3H, CH_3-19), 1.15 (s, 3H, CH_3-18), 1.61 (s, OH), 1.71 (s, OH), 2.08 (s, OH) 3.94 (m, 1H, C_3H), 4.16 (m, 1H, C_7H), 7.54 (s, 1H, CH), 7.16, 7.36 and 7.64 (aromatic CH, thiophene ring).

^{13}C-NMR: δ (ppm)= 15.99 (CH_3-18), 20.96 (CH_3-19), 58.76 (C_3, C-OH), 65.90 (C_6, C-OH), 68.59 (C_7, C-OH), 127.96 (C-20, C=C), 126, 129.78 and 132.50 (aromatic C=C), 133.27 (C_{21}), 139.87 (C-16, C=C), 209,03 (C=O, ketone).

4.10 Characterization of (16*E*) -(3-thiophen)-3β,5α,6β-trihydroxyandrostanone (11b)



11b

Chemical Formula: $C_{24}H_{32}O_4S$

%Yield % 80

Melting point: 180-185°C

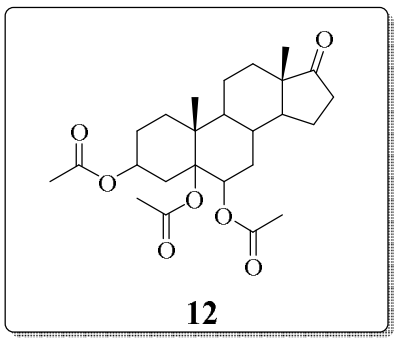
Molecular Weight: 416.58

IR (ATR cm^{-1}) 3411, 3246, 3115 (OH stretching), 2941-2853 (aliphatic CH stretching), 1707 (C=O stretching), 1615 (C=C stretching, C_{16}=C_{21}).

^{1}H-NMR: δ (ppm)= 0.93 (s, 3H, CH_3-19), 1.16 (s, 3H, CH_3-18), 1.28 (s, OH), 1.28 (s, OH), 2.90 (s, OH) 3.75 (m, 1H, C_3H), 3.97 (m, 1H, C_7H), 7.57 (s, 1H, CH), 7.33, 7.40 and 7.46 (aromatic CH, thiophene ring).

^{13}C-NMR: δ (ppm)= 14.36 (CH_3-18), 16.00 (CH_3-19), 58.75 (C_3, C-OH), 66.51 (C_6, C-OH), 68.61 (C_7, C-OH), 126.79 (C-20, C=C), 126.33, 128.39 and 128.73 (aromatic C=C stretching), 134.22 (C_{21}), 137.73 (C-16, C=C).

4.11 Characterization of 3β, 5α,6β-triacetoxyandrost-5-en-17-one (13)



12

Melting point: 205-207

%Yield % 90

Chemical Formula: $C_{25}H_{36}O_7$

Molecular Weight: 448.56

IR (ATR cm^{-1}) 2949-2823 (aliphatic CH streching), 1731 (C=O, H_3COOC), 1720 (C=O stretching, D ring ketone), 1247-1227 (C-O-O, stretching).

^{1}H-NMR: δ (ppm)= 0.90 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 2.04 (s, 6H, CH_3), 2.12 (s, 6H, CH_3), 4.77 (t, 1H), 5.17 (m, 1H *J*= 8 Hz)

^{13}C-NMR: δ (ppm)= 13.95 (CH_3-18), 16.32 (CH_3-19), 70.61 (C_3), 74.72 (C_6), 75.85 (C_5), 170.32, 170.88 (C=O, CH_3COOC), 221.12 (C=O, ketone).

5 DISCUSSION

In the first part of this studies, C-17 aza-steroids and C-16 aza-steroids were synthesised from the available methods in the literature. We started with acylation reaction of DHEA to protect the hydroxyl group at C-3 from given a side reaction. The Vilsmeier Haack reaction of 3β-acetoxyandrost-5-en-17-one which afforded our intermediate 3β-acetoxy-17-chloro-16-formylandrosta-5,16-diene product is also successful with high yield. Benzimidazole and imidazole were used to synthesised C-17 aza-steroid while indole to synthesised C-16 aza-steroid via methine bridge.

Hydrolysis of the acetoxy group in basic condition on the aza-steroid with the aldehyde functional group present was unsuccessful. Different aza-steroids were tried but its difficult to isolate the desired compounds, here we conclude that the aldehyde group may give different reaction such as Cannizzaro reaction due to lack of alpha hydrogen on our product. Converting the aldehyde functional group into acetal group with ethylene glycol was not successful, possible due to steric hindrance.

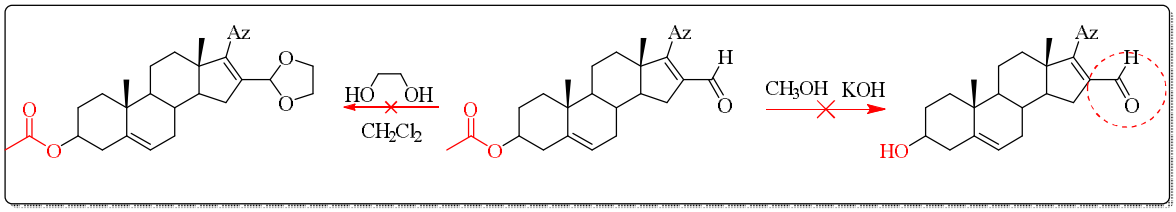


Figure 5.1 Hydrolysis reaction of aza-steroid

The C-17 aza-steroids **4a** and **4b** product obtained in the first part of this studies were then treated with $NaBH_4$ in methanol at room temperature in order to reduce the aldehyde to alcohol. Washing this product with sodium disulphide purified the product. Then the **5a, 5b** and **8** products were treated with *m*-CPBA to convert the double bond in the B ring into *cis*-diols.

Conversion of the double bond in the B ring of aldehyde containing azolyl steroids **4a, b** into *cis*- and *trans*-diols with *m*-CPBA or NBS was not achieved. All the spectral data obtained in this synthesis clearly shown that the expected product were not formed.

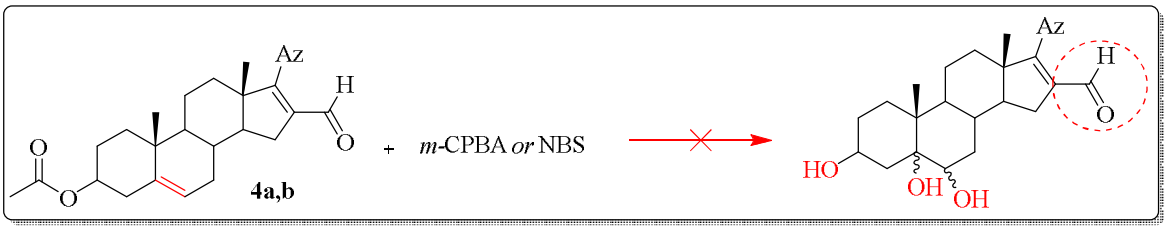


Figure 5.2 Failed reaction of *m*-CPBA and NBS

All the product synthesised in this study were characterised with the Infrared, ^{1}H-NMR and ^{13}C-NMR spectroscopic methods, melting point of all the products were measured unless those that decomposed before reaching their expected melting point.

In conclusion, all the compounds synthesised here, were believe to have possessed potential biological activity, since similar compounds in the literature were reported to be biologically active compounds.

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APPENDIX

Spectral Data

IR, ^{1}H-NMR and ^{13}C-NMR spectrum of 3β-acetoxyandrost-5-en-17-one **(2)**

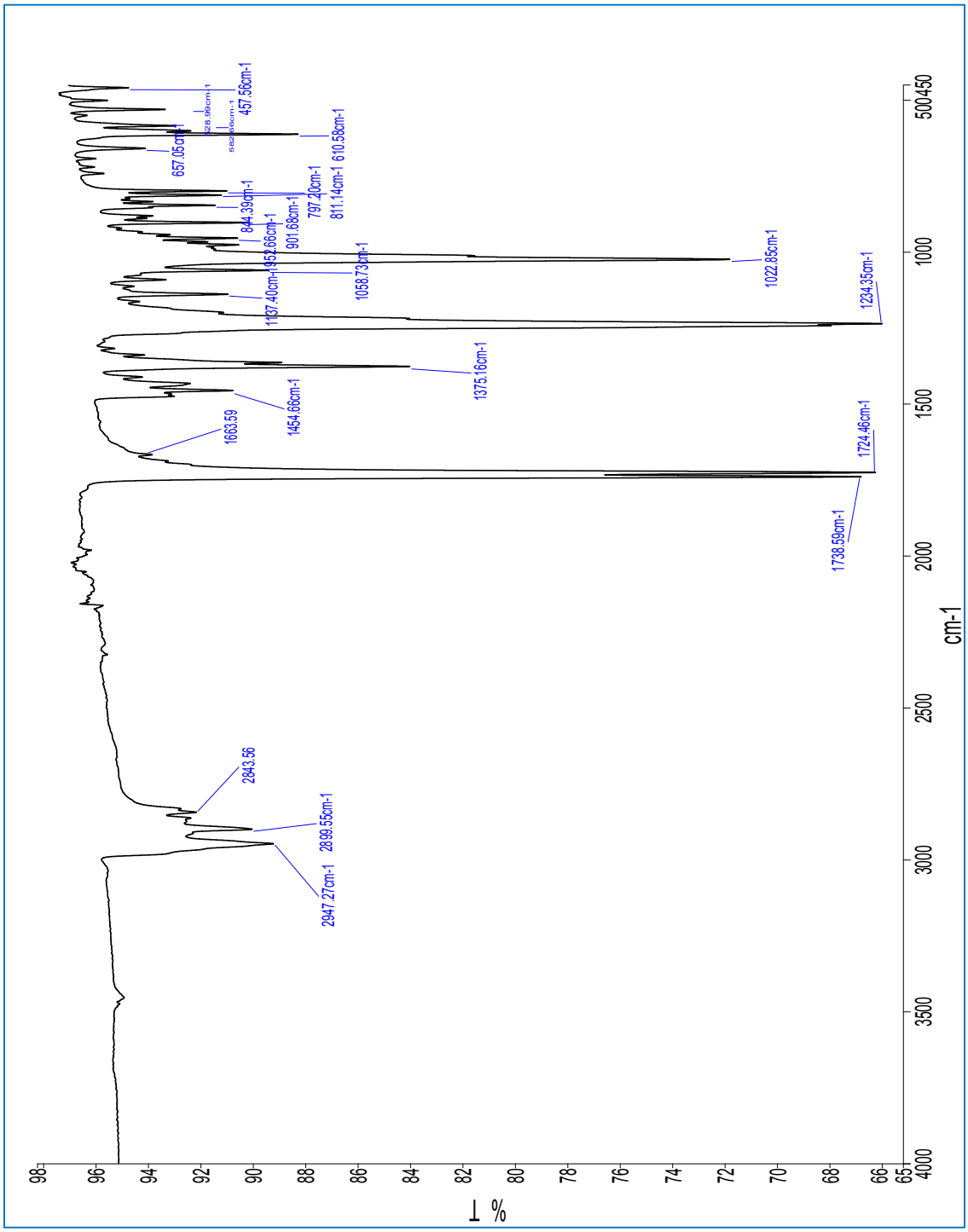

Figure A. 1 IR spectrum of 3β-acetoxyandrost-5-en-17-one **(2)**

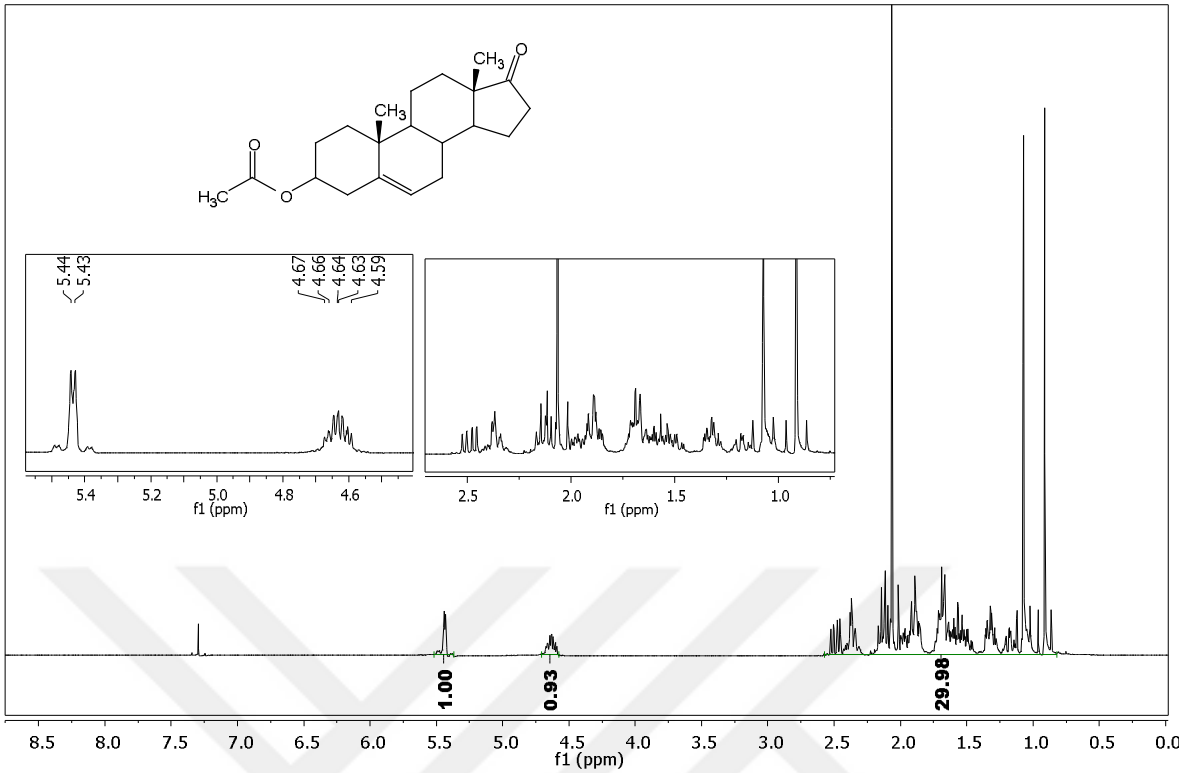

Figure A.2 [1]H-NMR spectrum of 3β-acetoxyandrost-5-en-17-one **(2)**

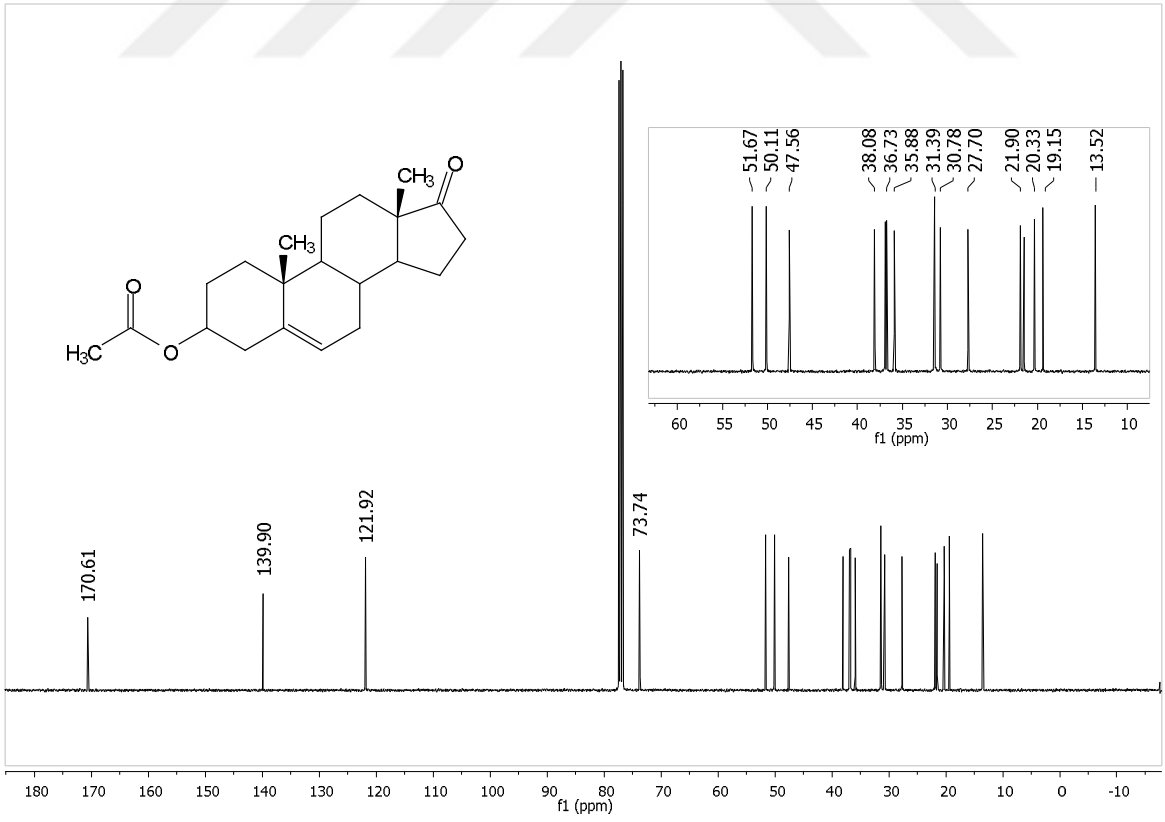

Figure A. 3 [13]C-NMR spectrum of 3β-acetoxyandrost-5-en-17-one **(2)**

IR, ^{1}H-NMR and ^{13}C-NMR spectrum of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene **(3)**

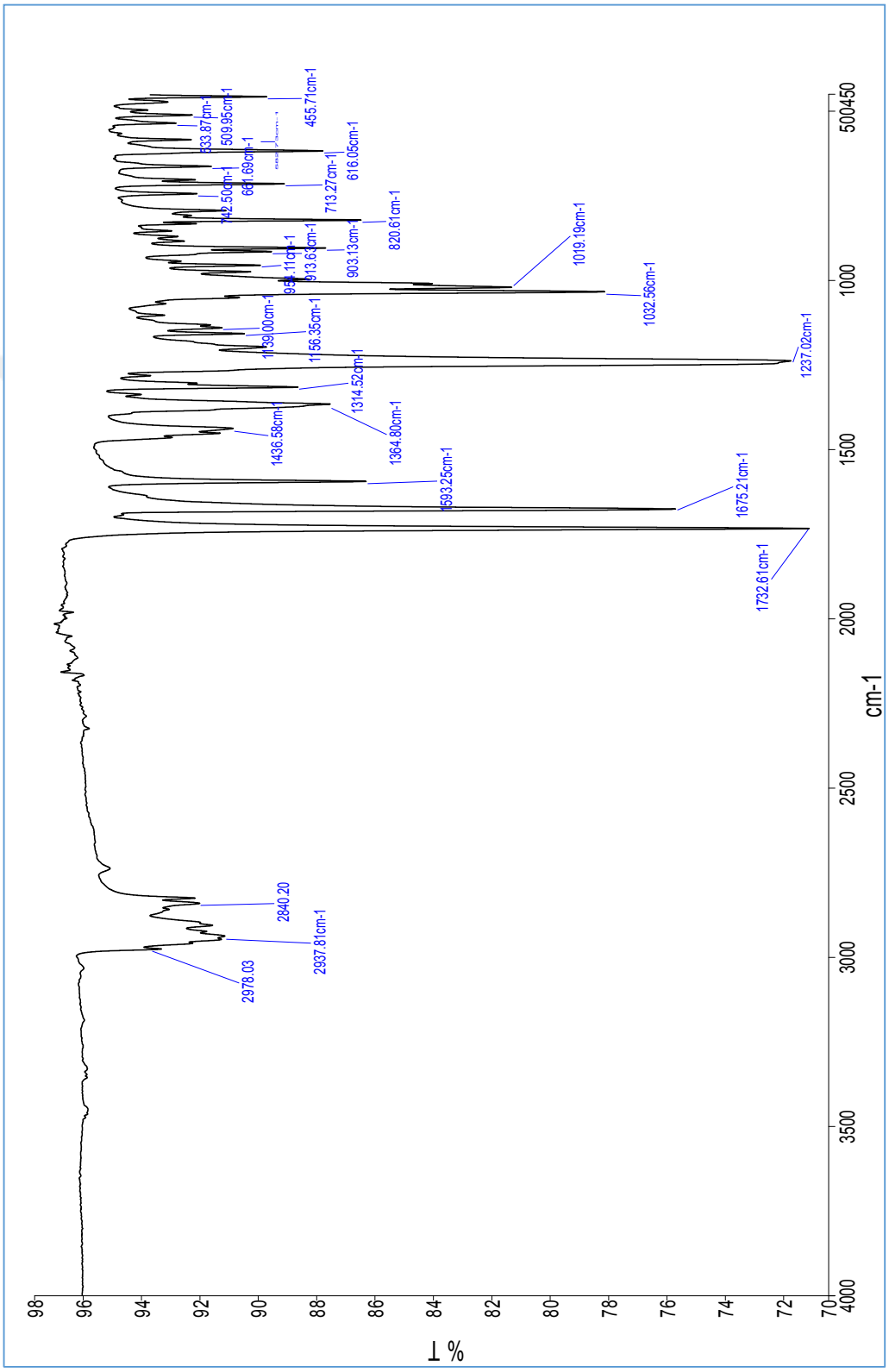

Figure A. 4 IR spectrum of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene **(3)**

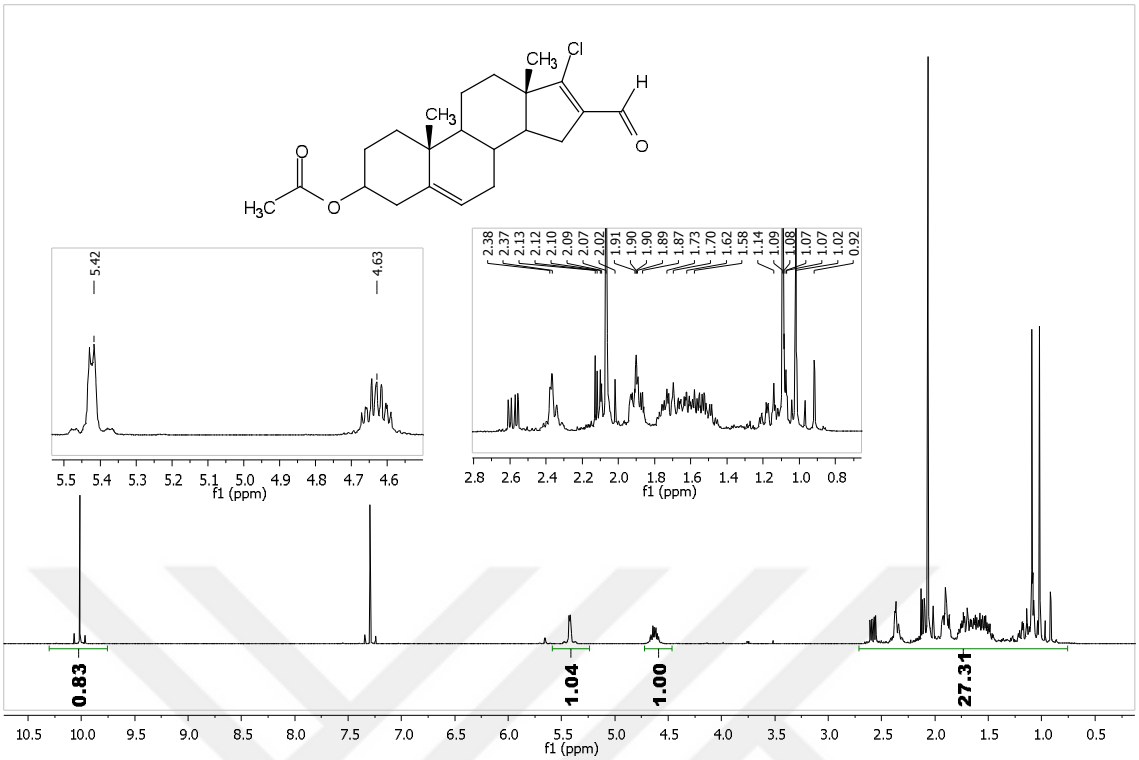

Figure A. 5 [1]H-NMR spectrum of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene **(3)**

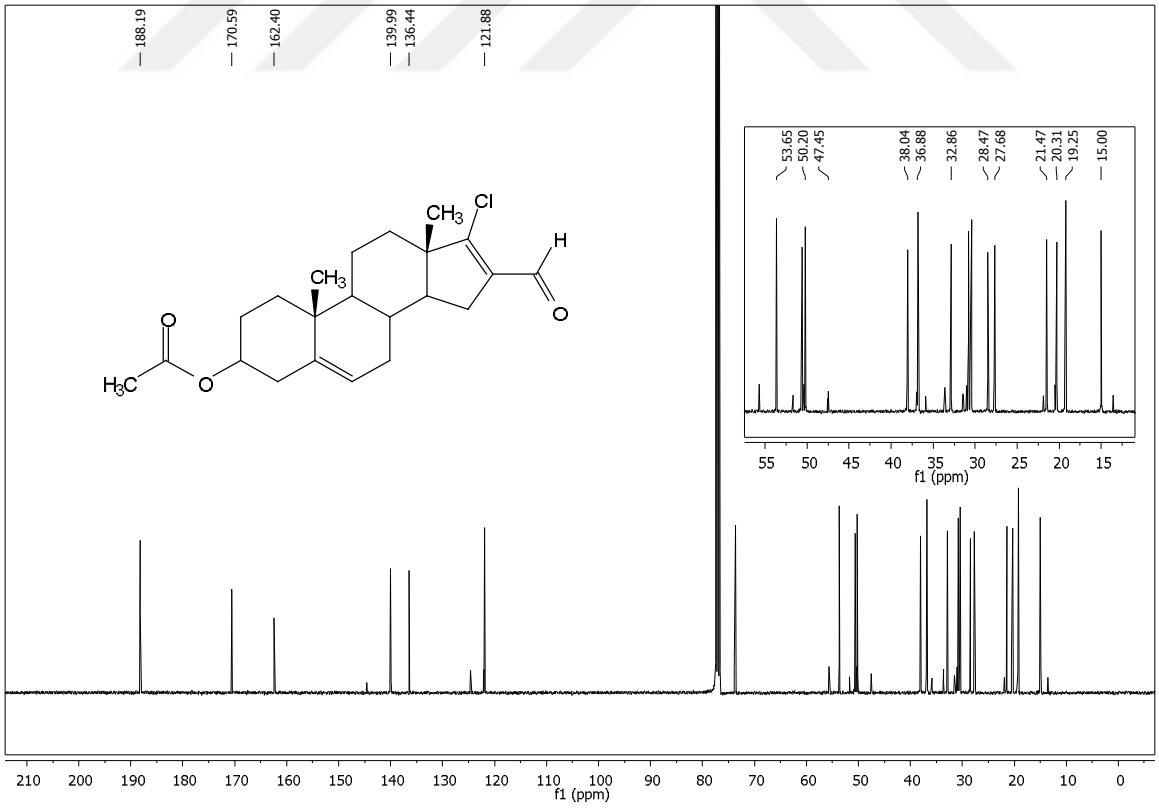

Figure A.6 [13]C-NMR spectrum of 3β-Acetoxy-17-chloro-16-formylandrosta-5,16-diene (**3)**

IR, ^{1}H-NMR and ^{13}C-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formylandrosta-5,16-diene **(4a)**

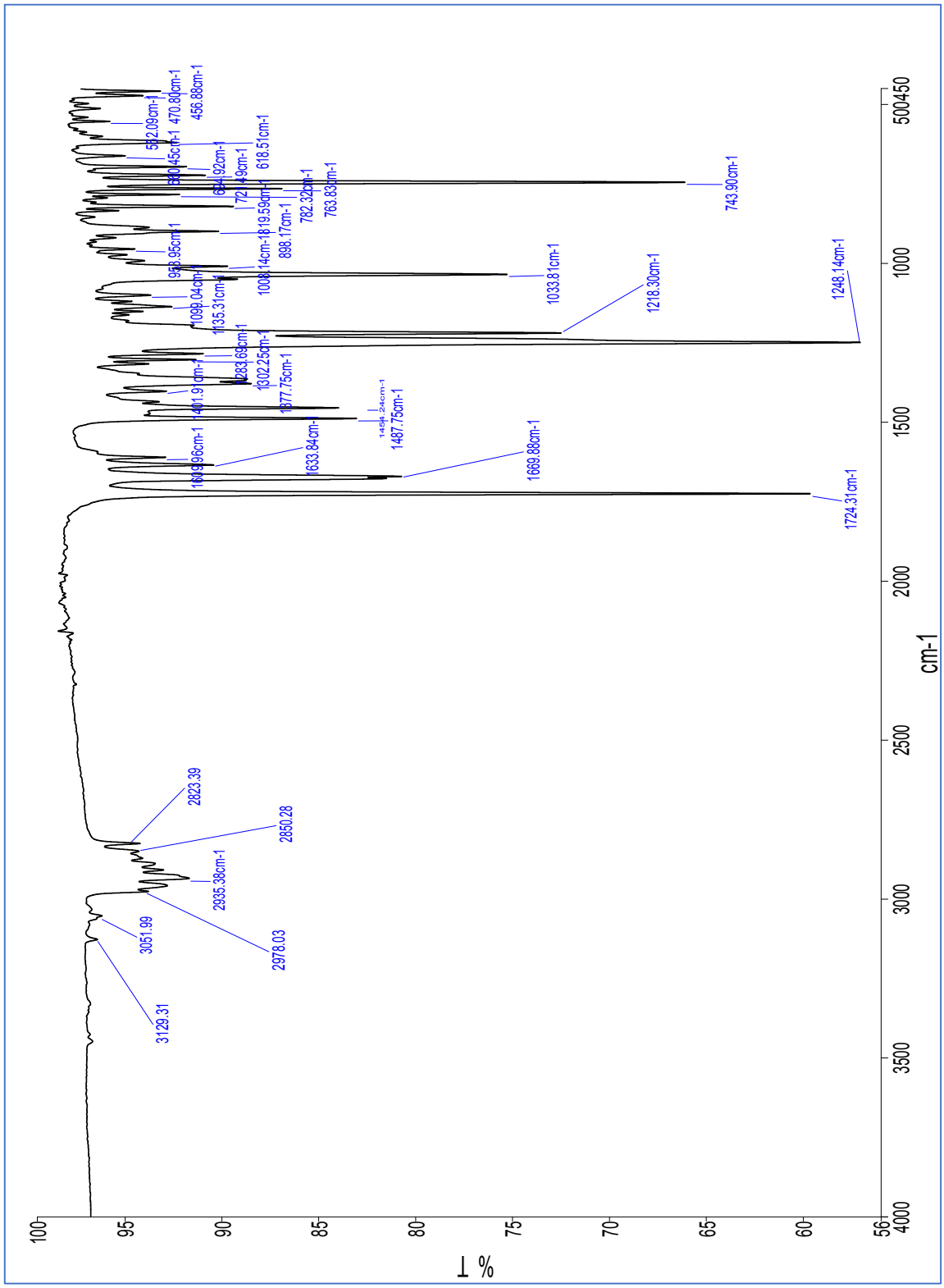

Figure A.7 IR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formylandrosta-5,16-diene **(4a)**

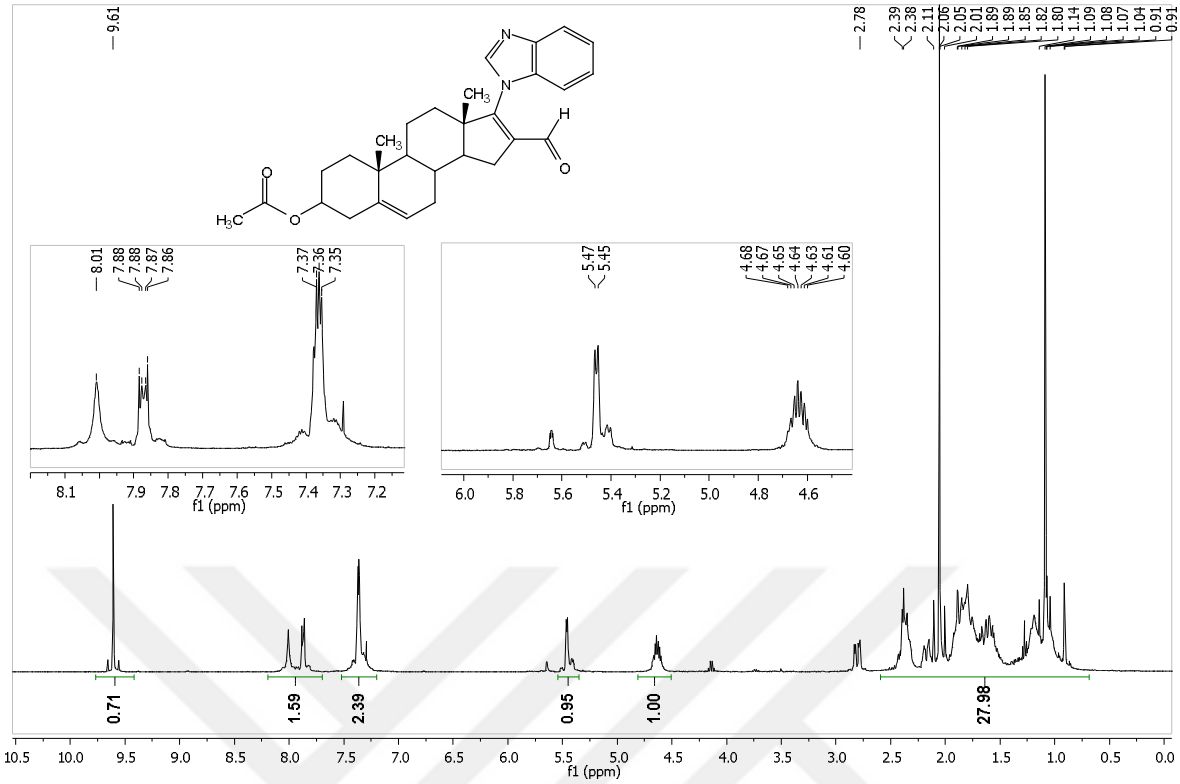

Figure A. 8 ^{1}H-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formylandrosta-5,16-diene **(4a)**

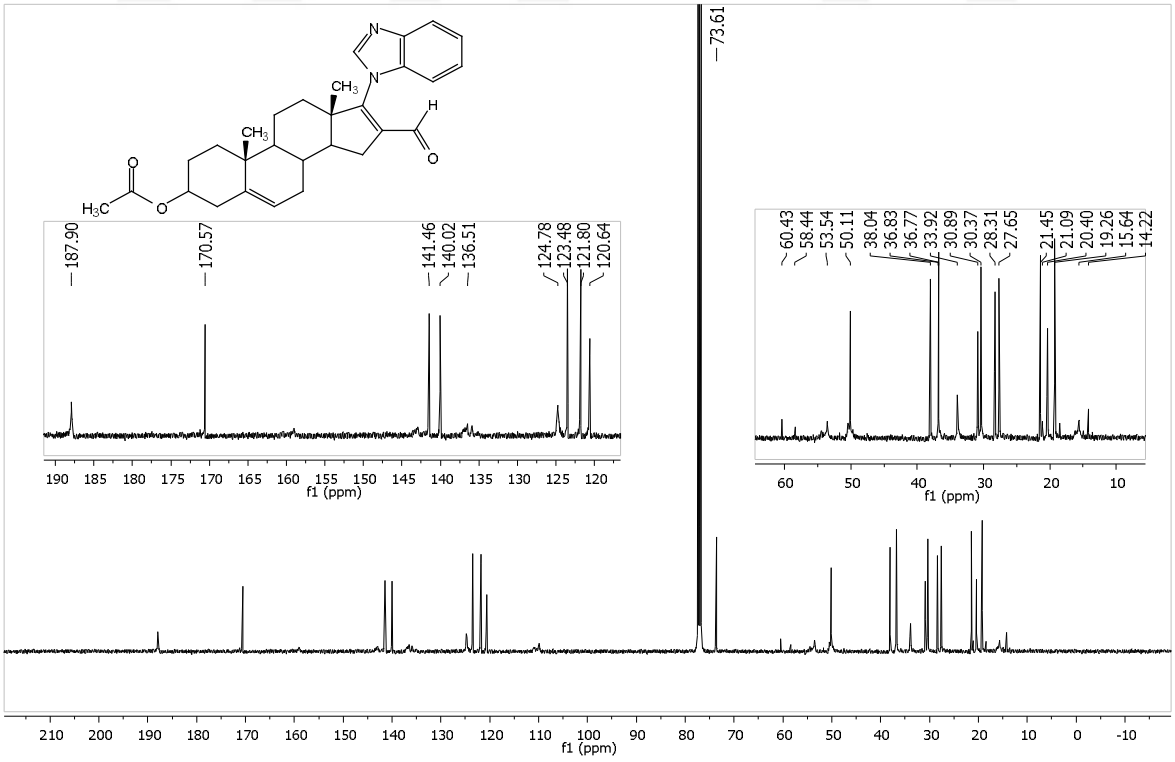

Figure A. 9 ^{13}C-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-formylandrosta-5,16-diene **(4a)**

IR, ^{1}H-NMR and ^{13}C-NMR spectrums of 3β-Acetoxy-17-(1H-imidazol-1-yl)-16-formylandrosta-5,16-diene **(4b)**

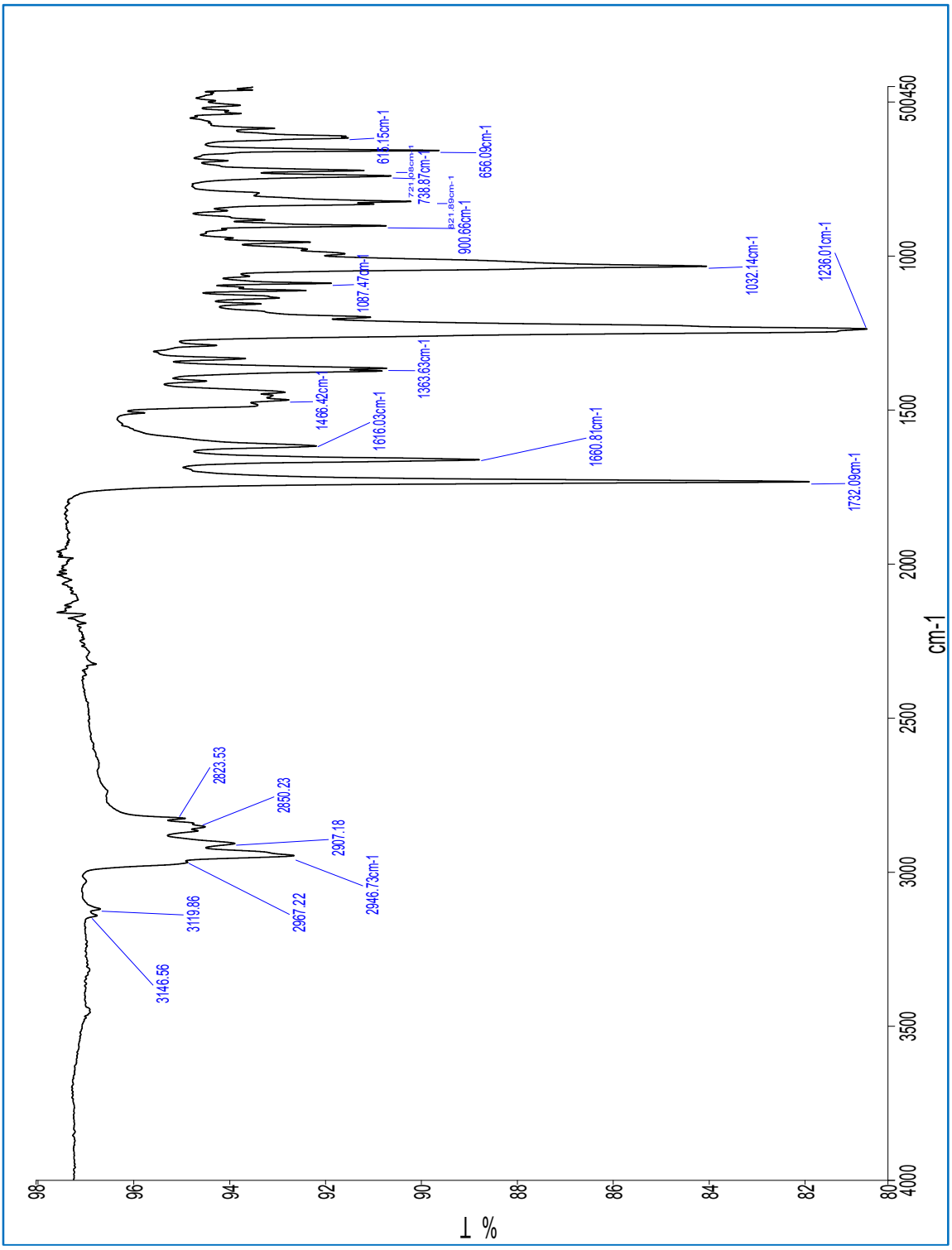

Figure A. 10 IR spectrums of 3β-Acetoxy-17-(1H-imidazol-1-yl)-16-formylandrosta-5,16-diene **(4b)**

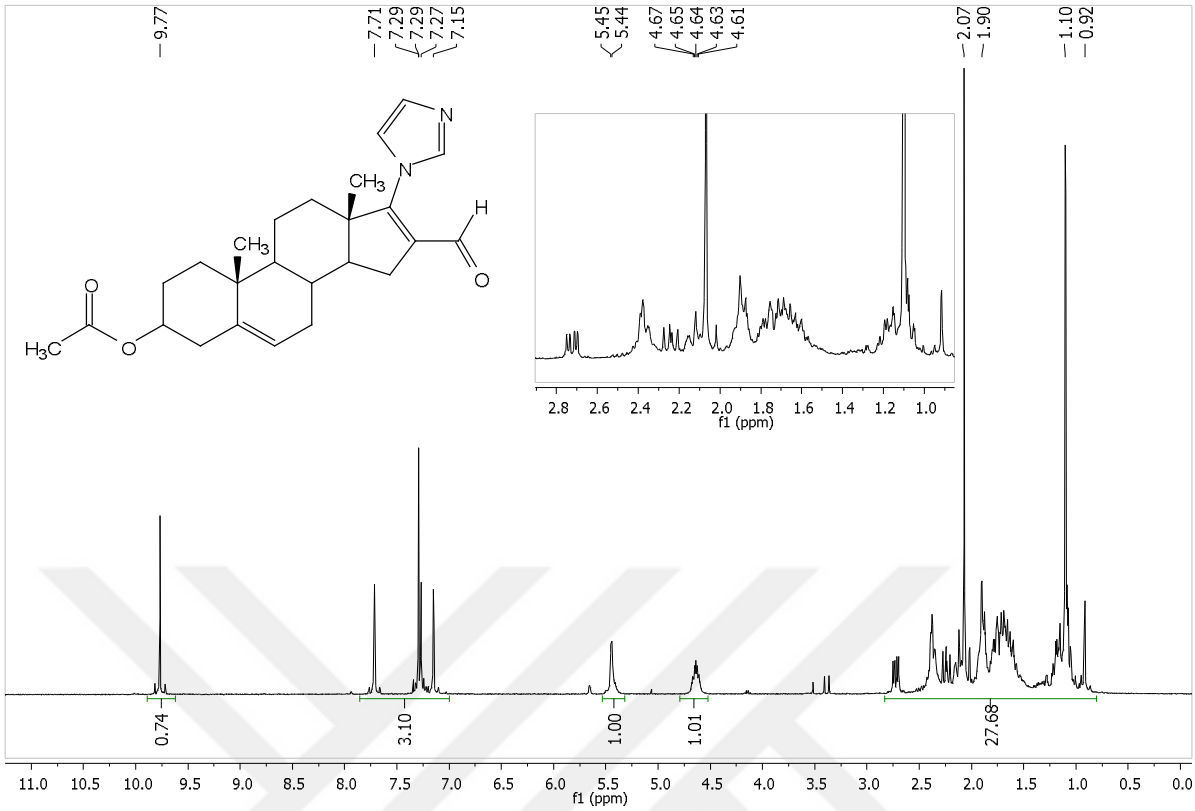

Figure A. 11 ^{1}H-NMR spectrums of 3β-Acetoxy-17-(1H-imidazol-1-yl)-16-formylandrosta-5,16-diene **(4b)**

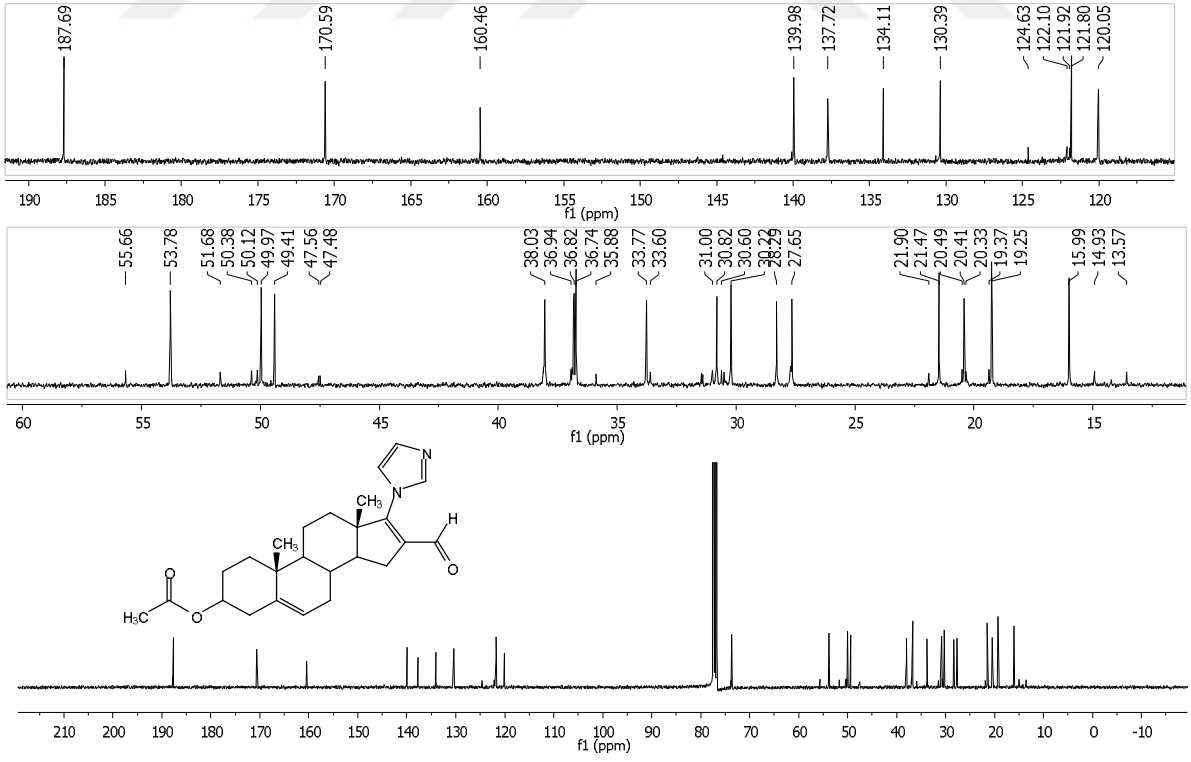

Figure A. 12 ^{13}C-NMR spectrums of 3β-Acetoxy-17-(1H-imidazol-1-yl)-16-formylandrosta-5,16-diene **(4b)**

IR, ^{1}NMR, and ^{13}CNMR spectrum of 16-(1*H*-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate (**8)**

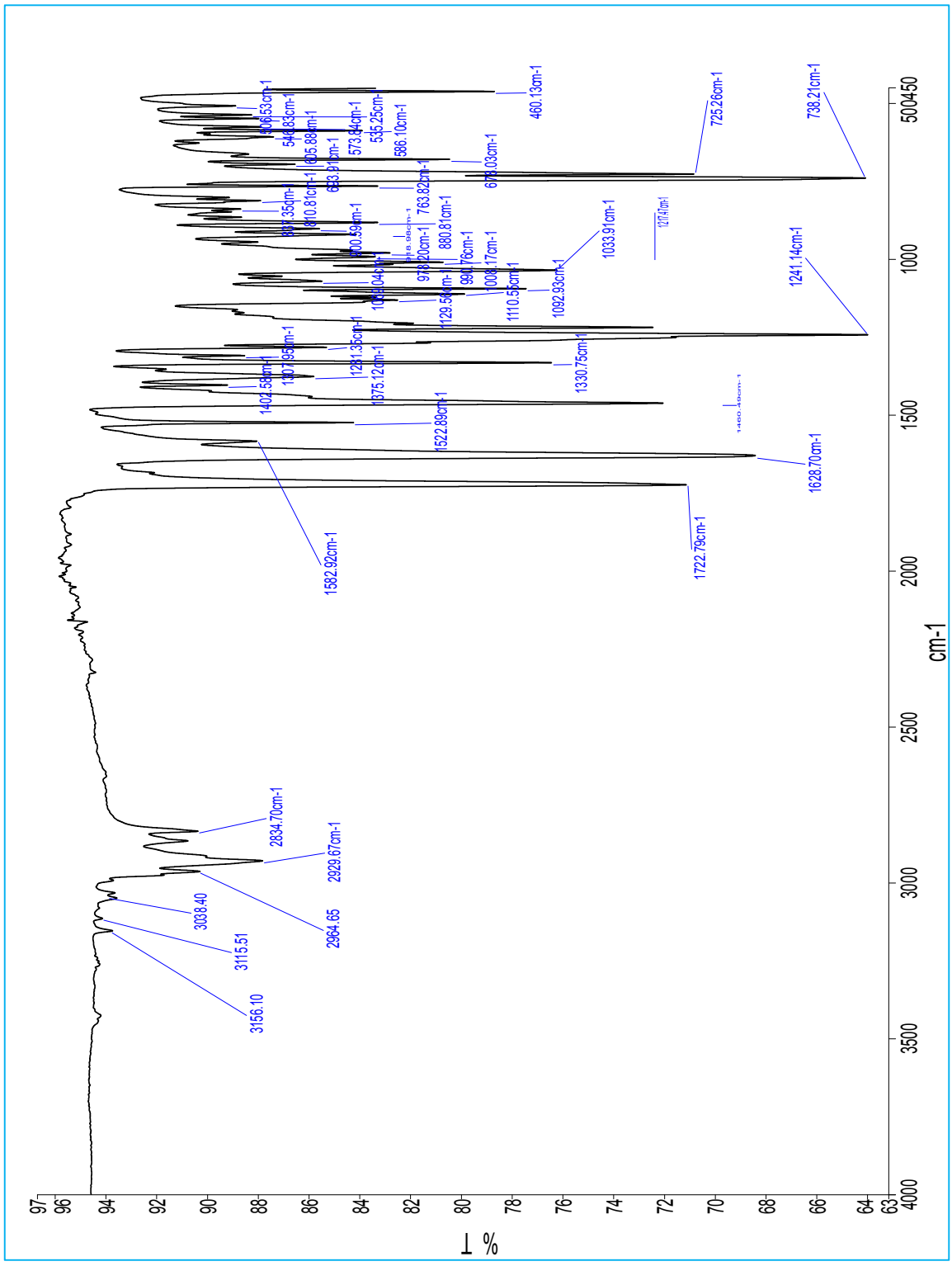

Figure A. 13 IR of spectrum of 16-(1H-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate **(8)**

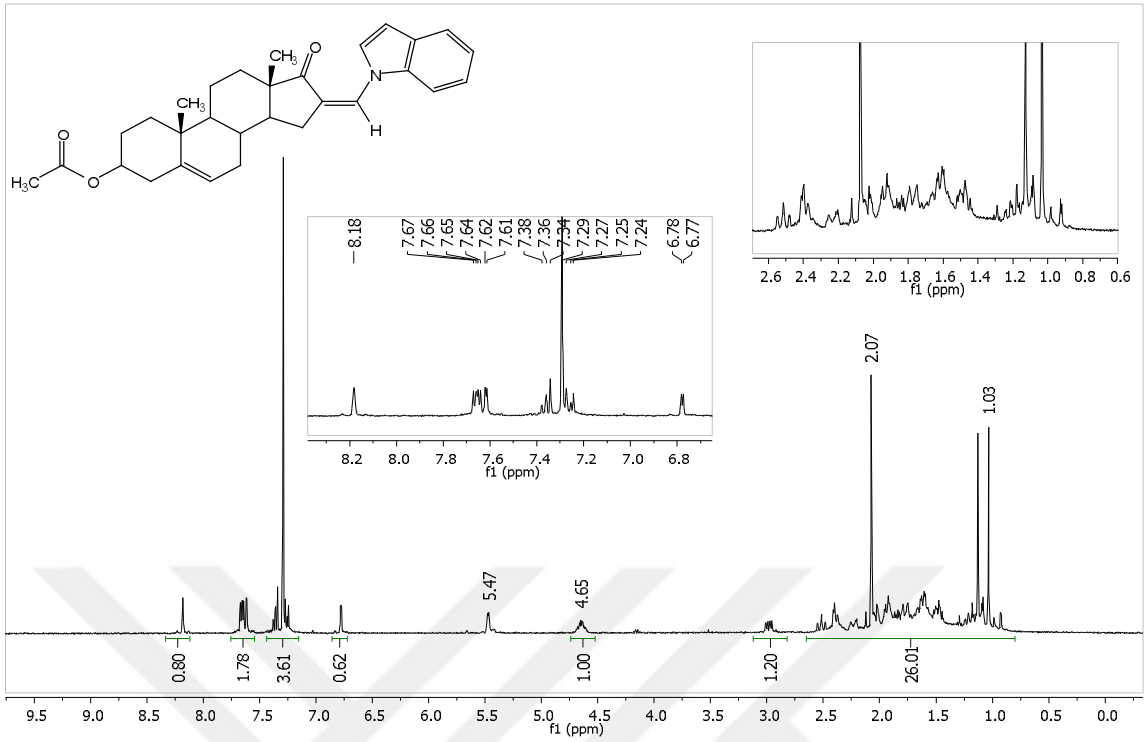

Figure A. 14 [1]H-NMR of spectrum of 16-(1H-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate **(8)**

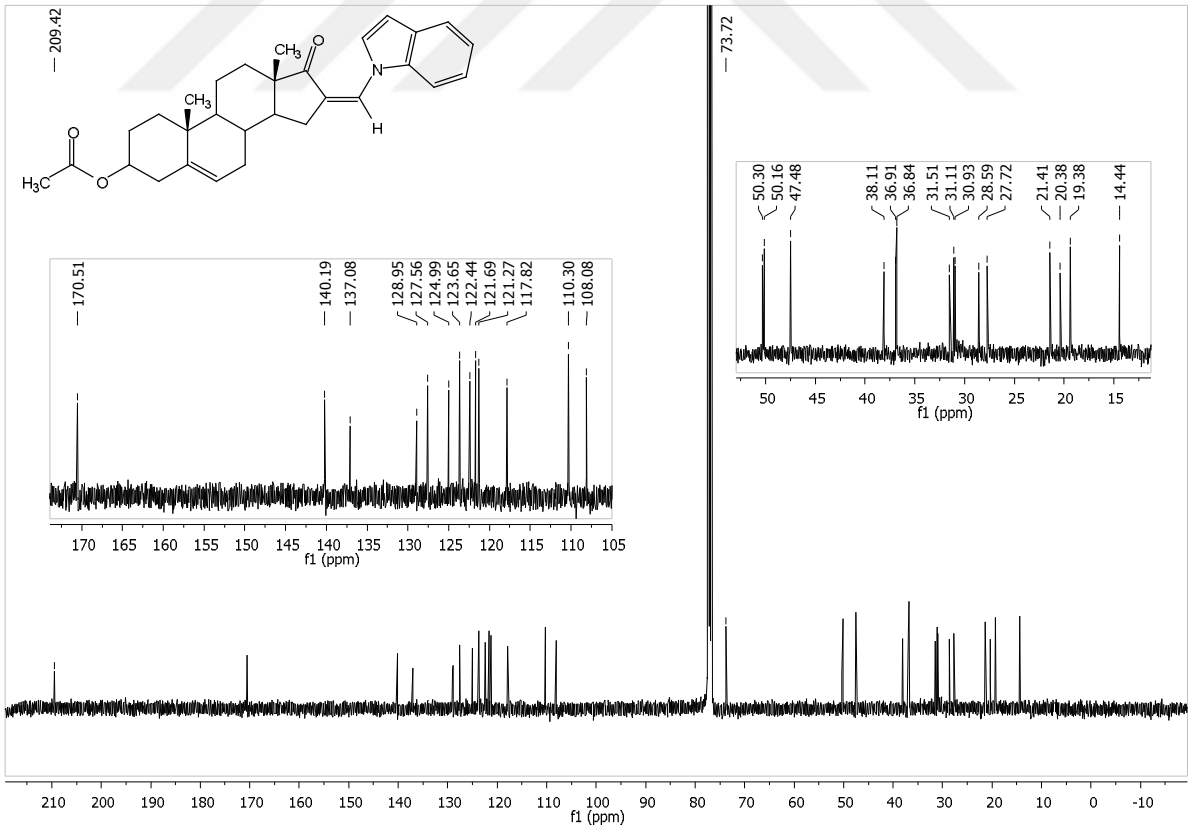

Figure A. 15 [13]C-NMR of spectrum of 16-(1H-indol-1-yl) methylen-17-oxoandrost-5-en-3β-ylacetate **(8)**

IR ^{1}H-NMR ^{13}C-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16-(hydroxymethyl) androsta-5,16-diene **(5a)**

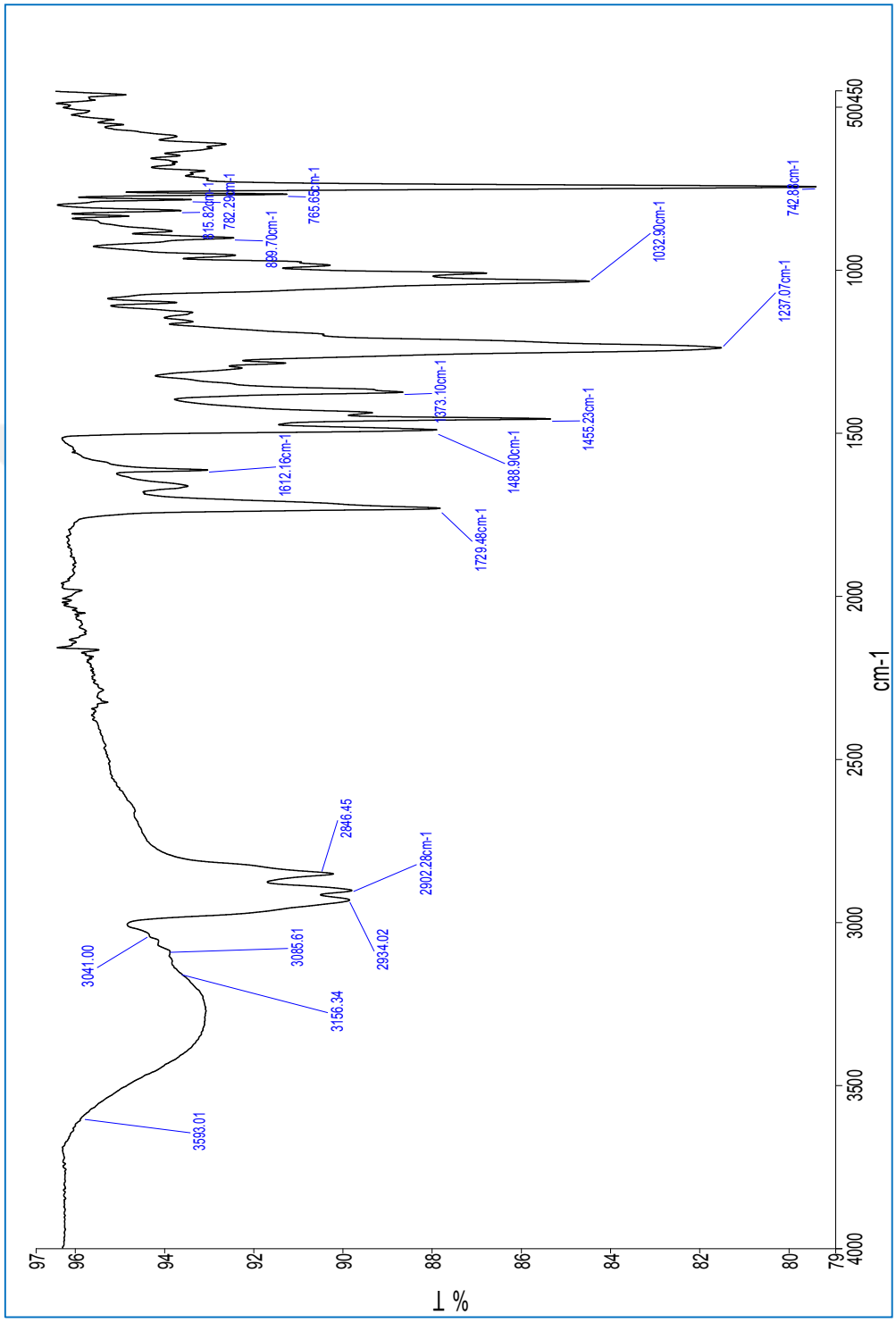

Figure A. 16 IR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16- (hydroxymethyl) androsta-5,16-diene **(5a)**

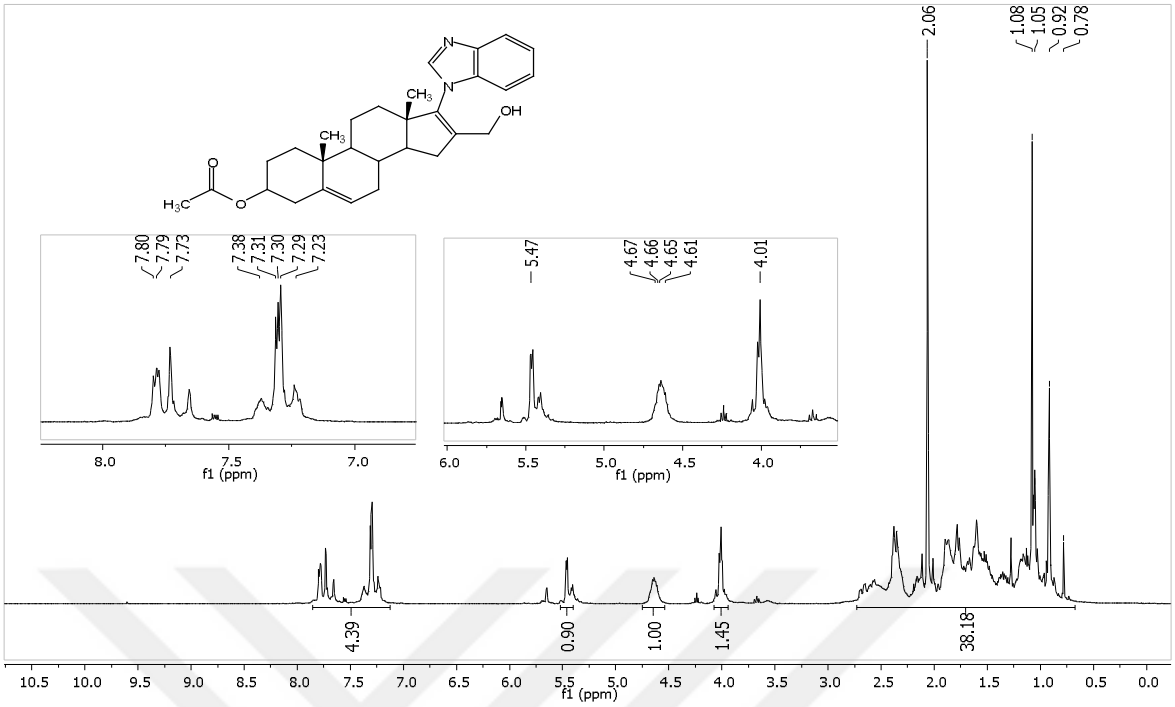

Figure A. 17 ^{1}H-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16- (hydroxymethyl) androsta-5,16-diene **(5a)**

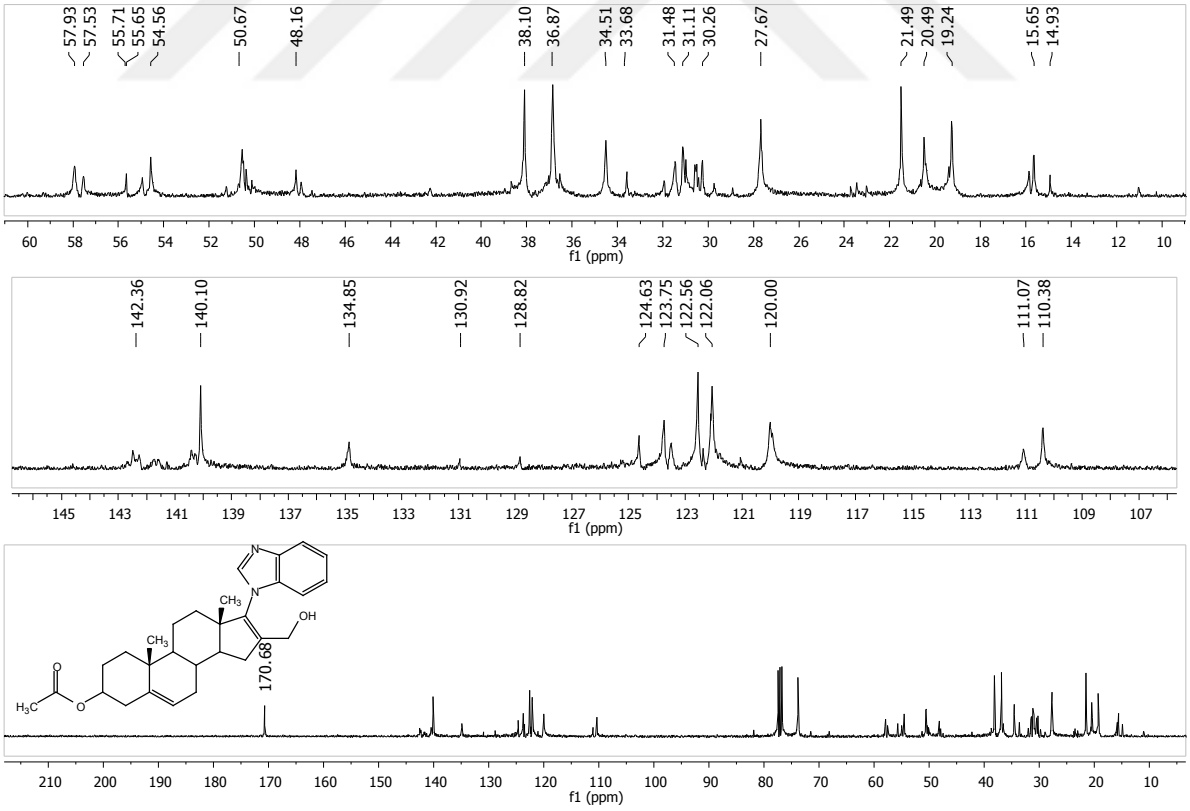

Figure A. 18 ^{13}C-NMR spectrum of 3β-Acetoxy-17-(1H-benzimidazol-1-yl)-16- (hydroxymethyl) androsta-5,16-diene **(5a)**

IR, ^{1}H-NMR and ^{13}C-NMR spectrum of 3β-hydroxyl-17-(1H-benzimidazol-1-yl) -5,6-dihydroxy -16-(hydroxymethyl)-16-ene **(6)**

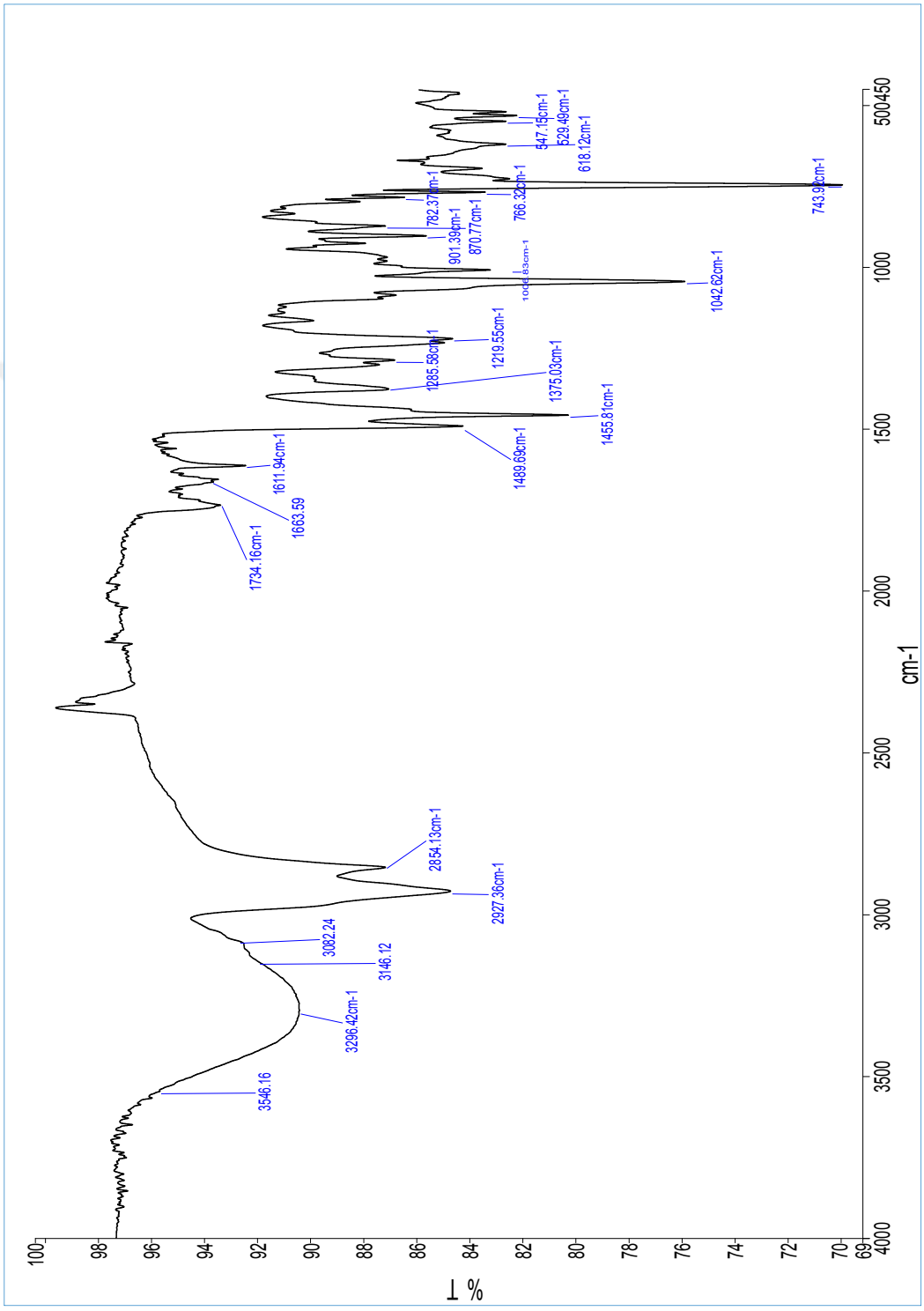

Figure A. 19 IR spectrum of 3β-hydroxyl-17-(1H-benzimidazol-1-yl) -5,6-dihydroxy -16-(hydroxymethyl)-16-ene **(6)**

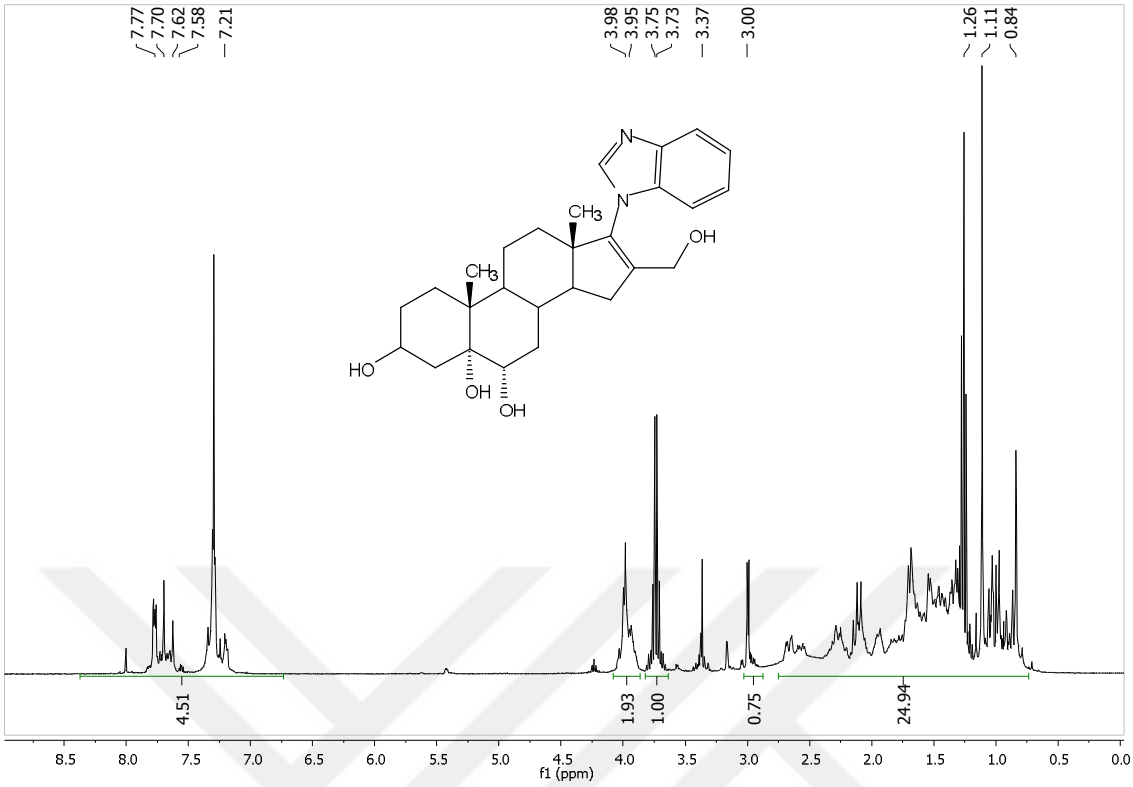

Figure A. 20 [1]H-NMRspectrum of 3β-hydroxyl-17-(1H-benzimidazol-1-yl) -5,6-dihydroxy -16-(hydroxymethyl)-16-ene **(6)**

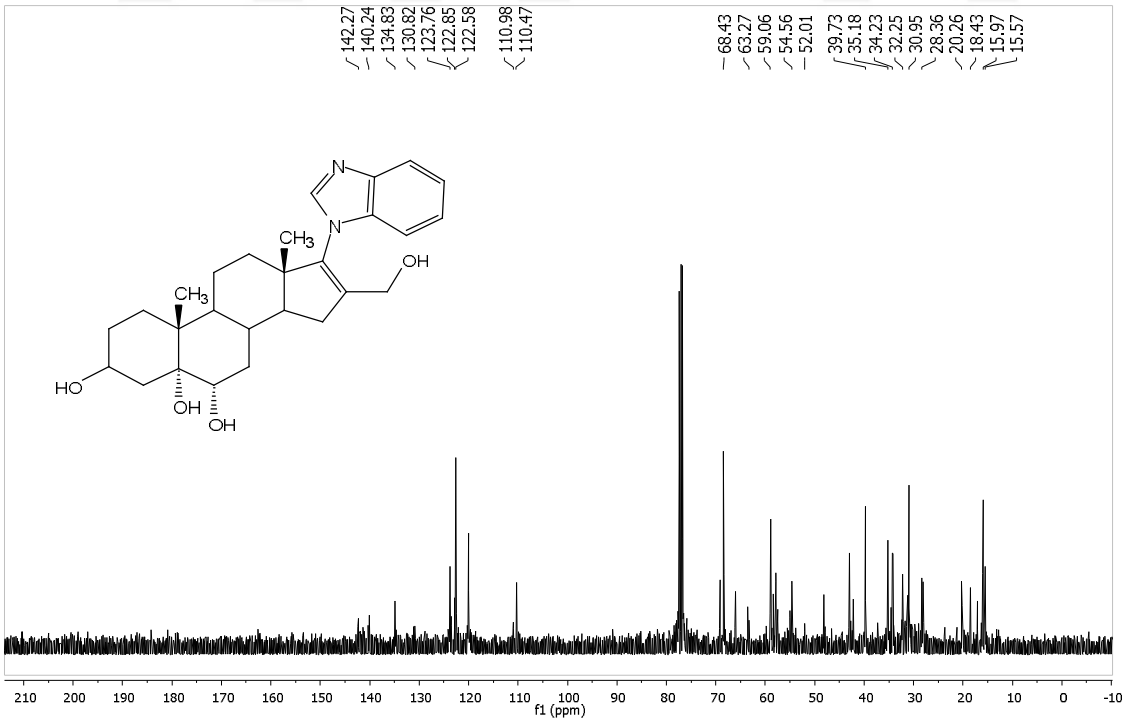

Figure A.21. 1H-NMRspectrum of 3β-hydroxyl-17-(1H-benzimidazol-1-yl) -5,6-dihydroxy -16-(hydroxymethyl)-16-ene **(6)**

IR ^{1}H-NMR ^{13}C-NMR spectrum of (16*E*) -(2-thiophen)-3β,5α,6β-trihydroxyandrostanone (**11a)**

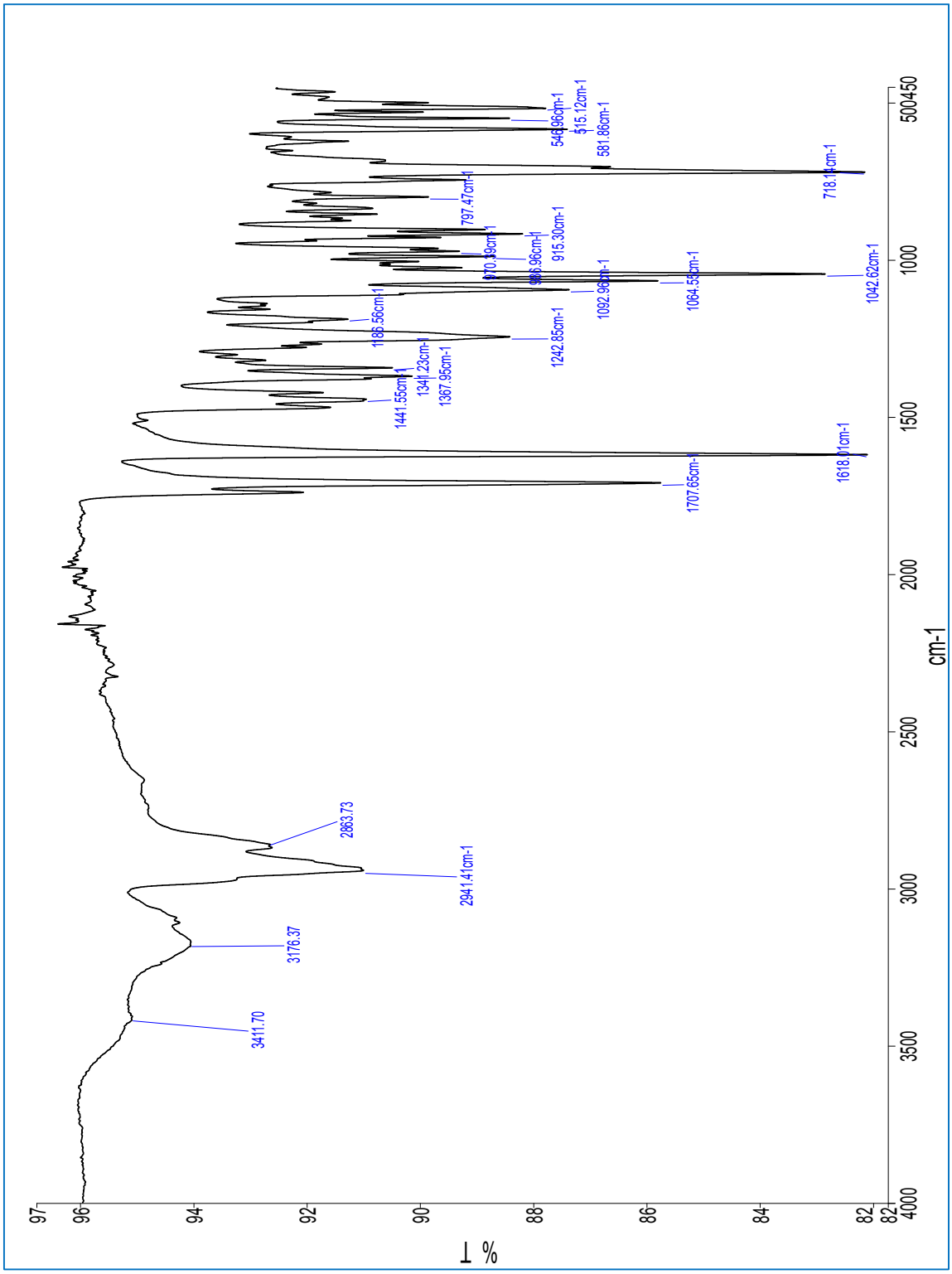

Figure A.22 IR spectrum of (16E) -(2-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11a)**

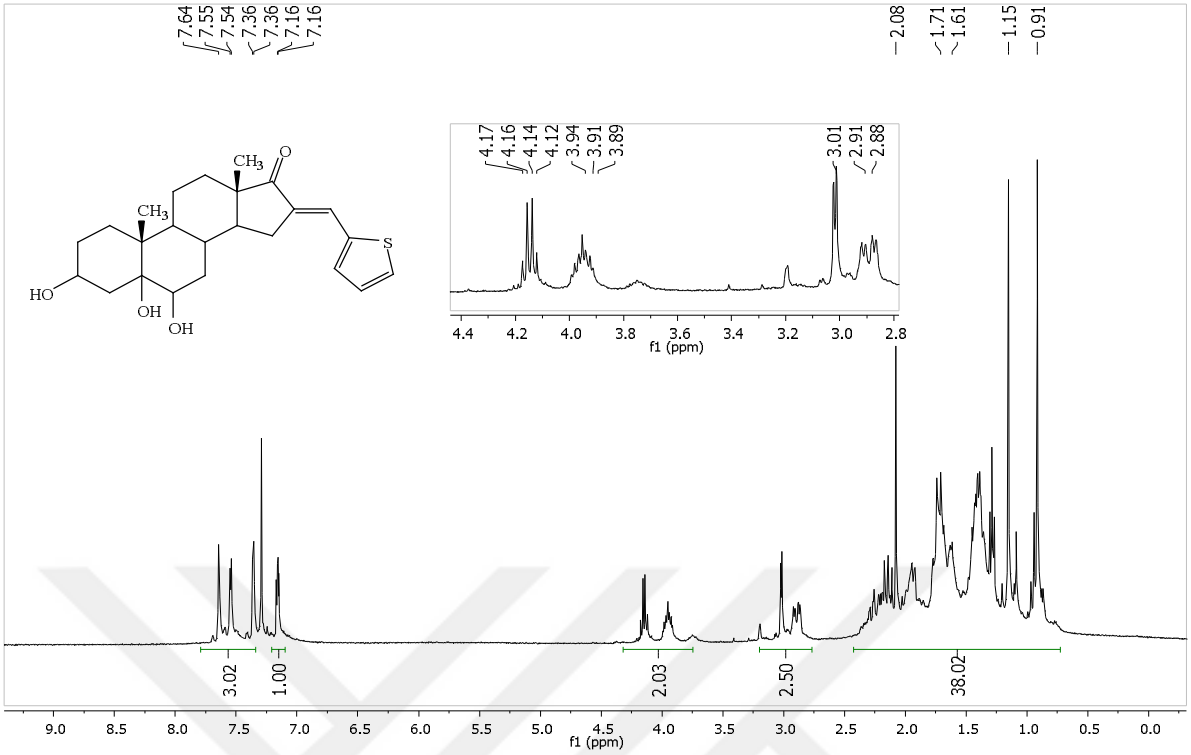

Figure A.23 ^{1}H-NMR spectrum of (16E) -(2-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11a)**

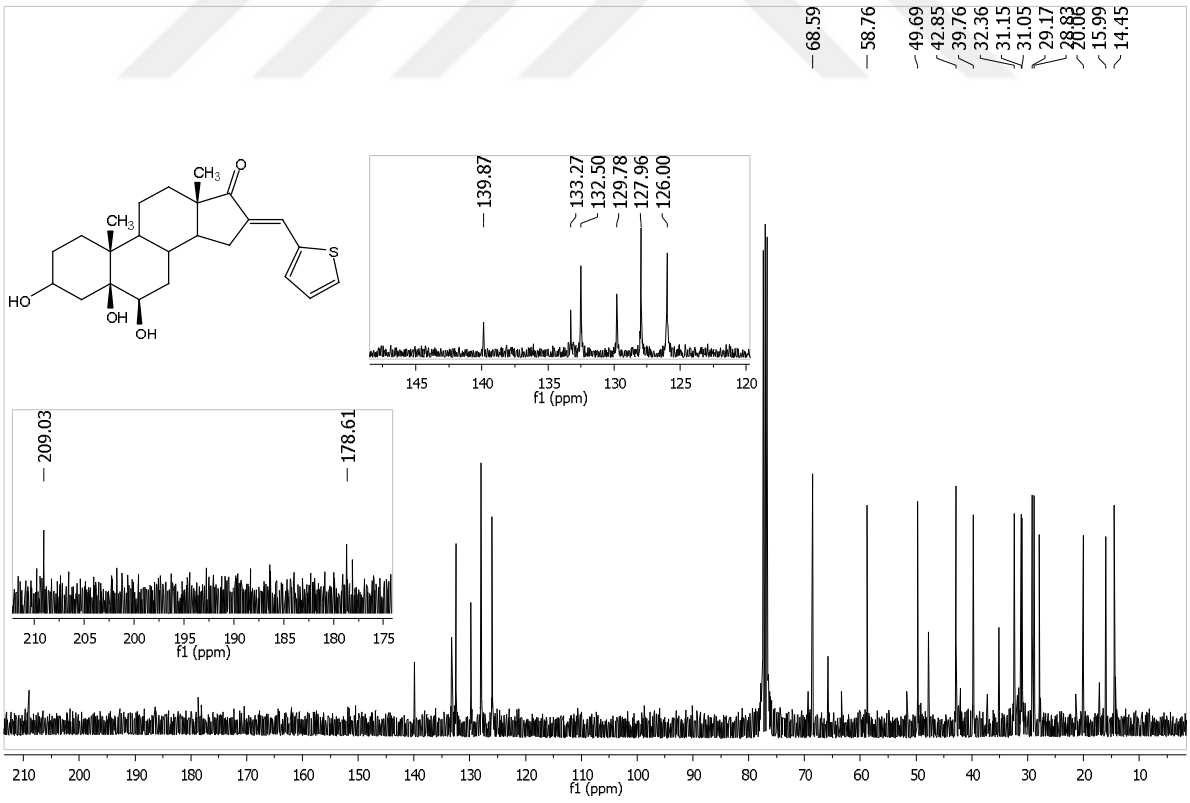

Figure A. 24 ^{13}C-NMR spectrum of (16E)-(2-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11a)**

IR ^{1}H-NMR ^{13}C-NMR spectrum of (16*E*) -(3-thiophen)-3β,5α,6β-trihydroxyandrostanone (**11b)**

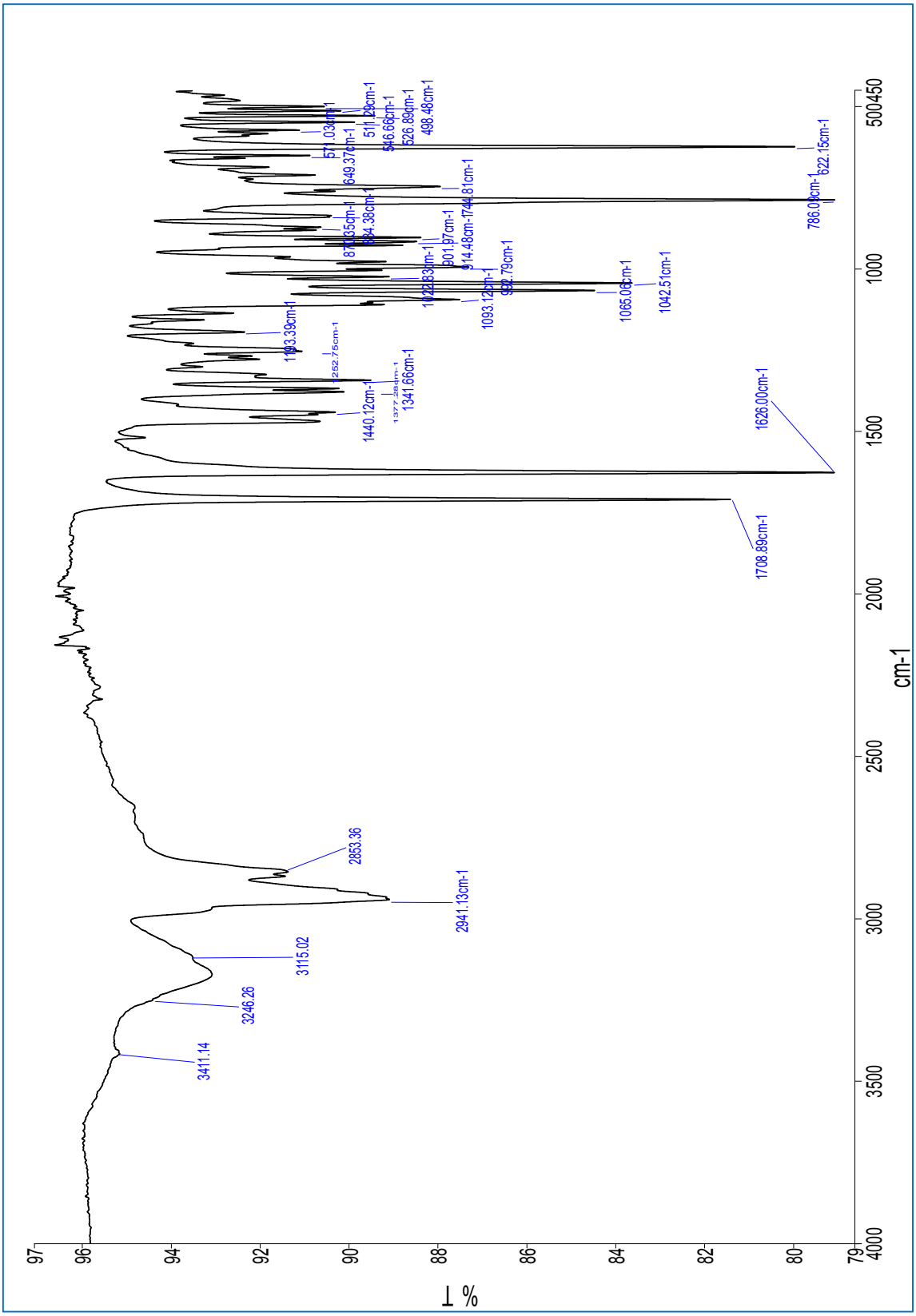

Figure A.25 IR spectrum of (16E) -(3-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11b)**

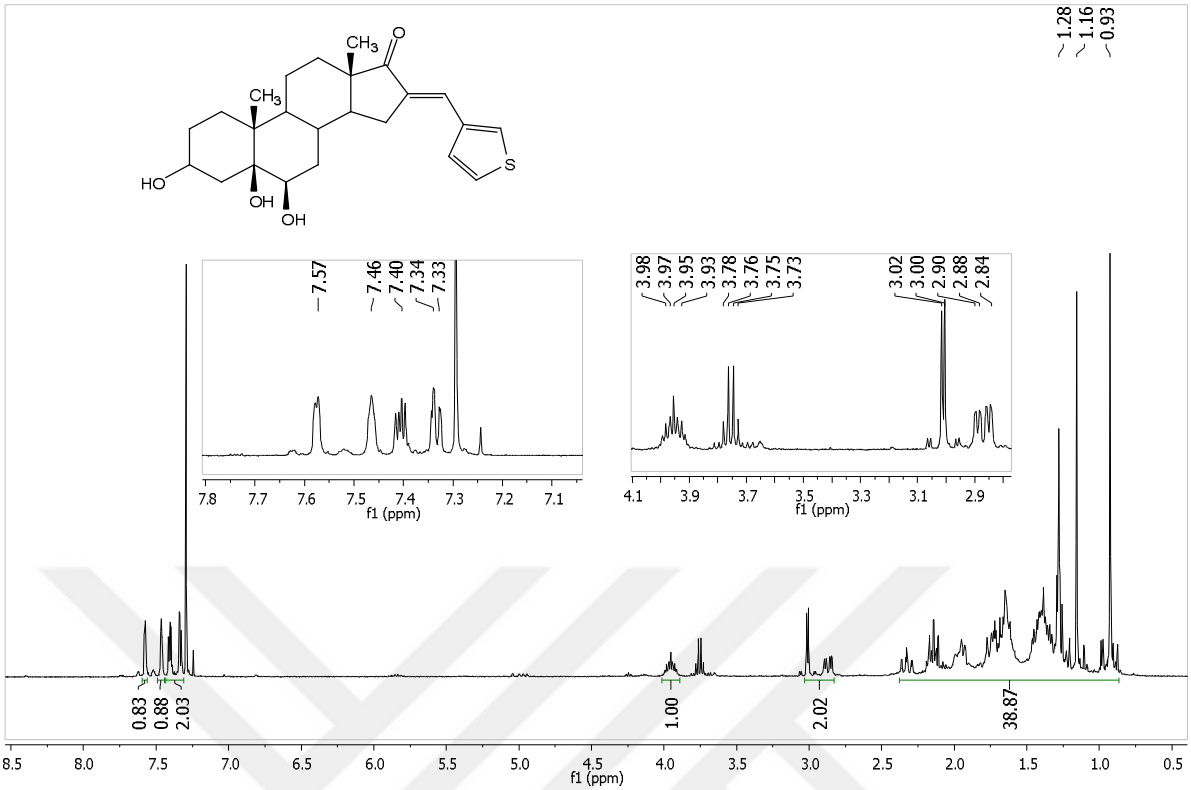

Figure A. 26 ^{1}H-NMR spectrum of (16E) -(3-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11b)**

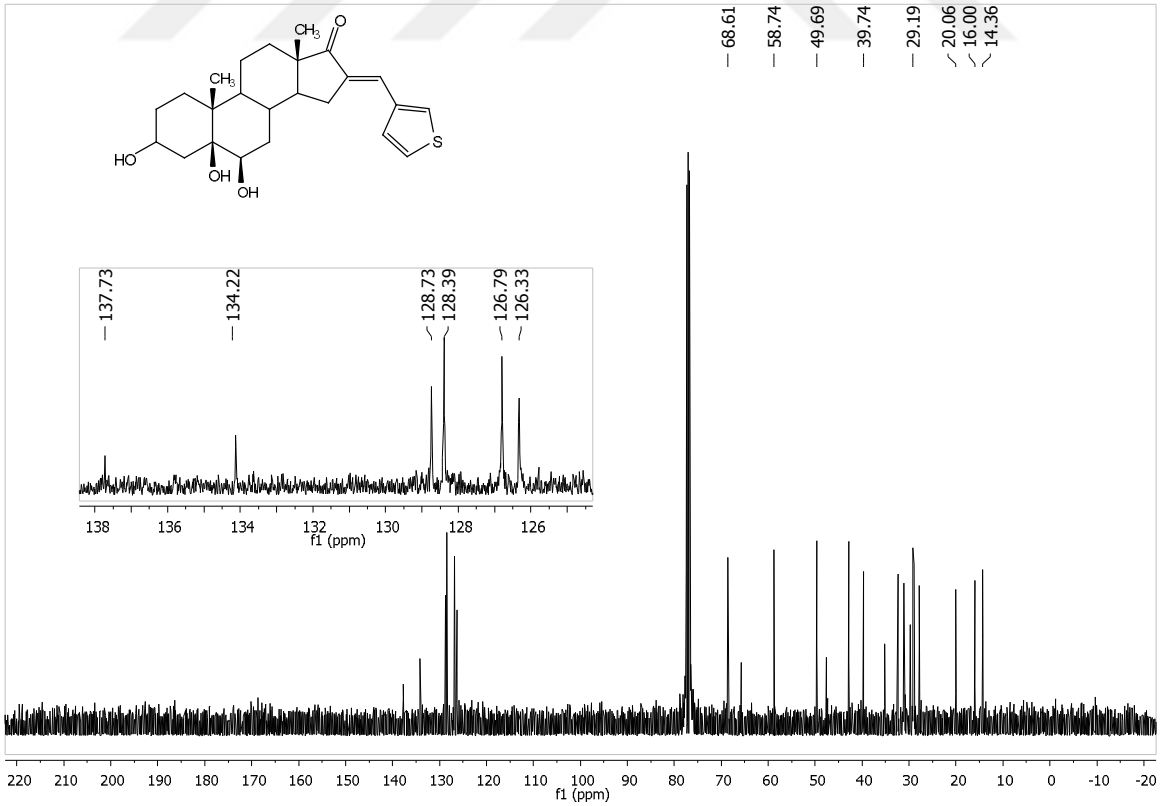

Figure A. 27 ^{13}C-NMR spectrum of (16E) -(3-thiophen)-3β,5α,6β-trihydroxyandrostanone **(11b)**

IR ^{1}H-NMR ^{13}C-NMR spectrum of 3β, 5α,6β-triacetoxyandrost-5-en-17-one **(13)**

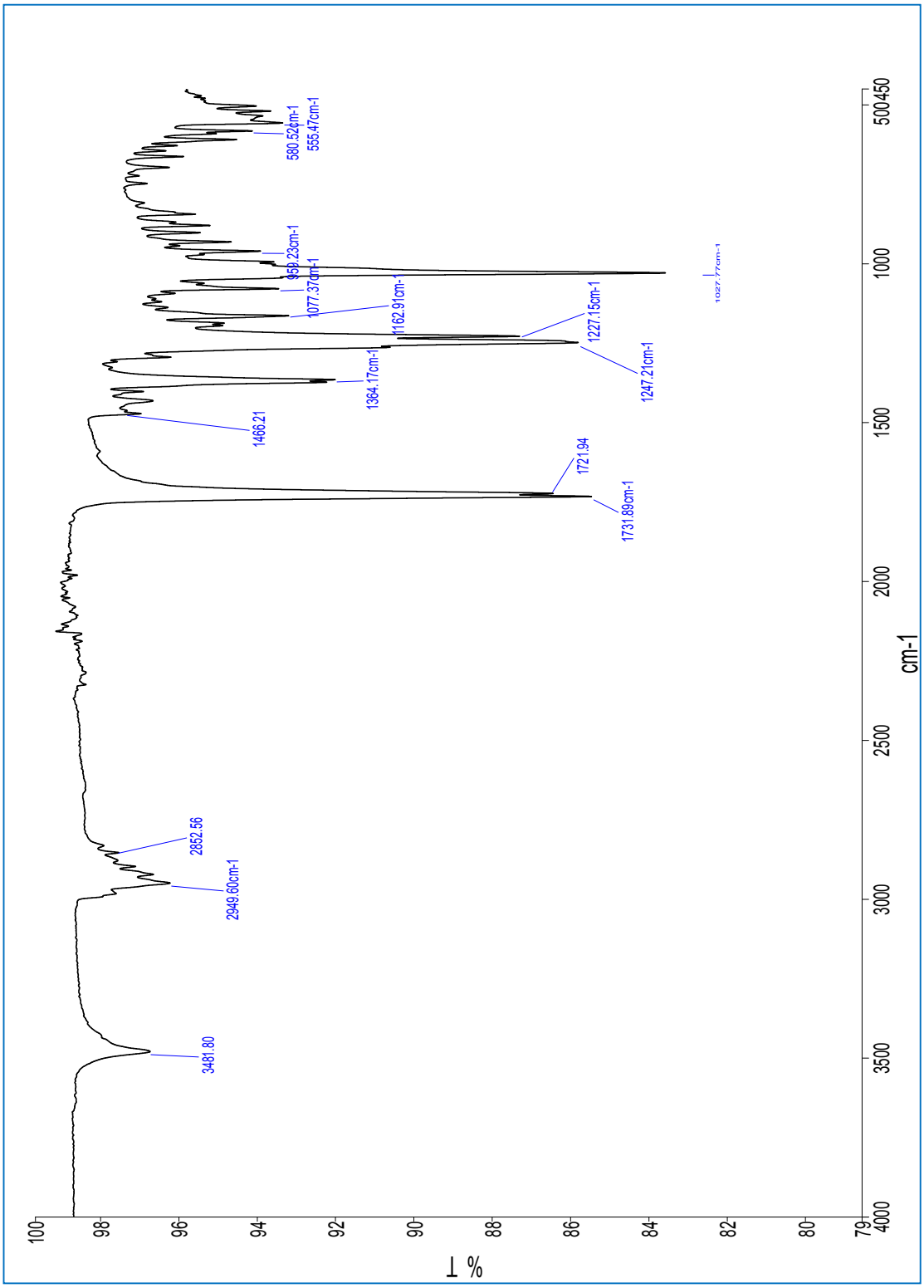

Figure A. 28 IR spectrum of 3β, 5α,6β-triacetoxyandrost-5-en-17-one **(13)**

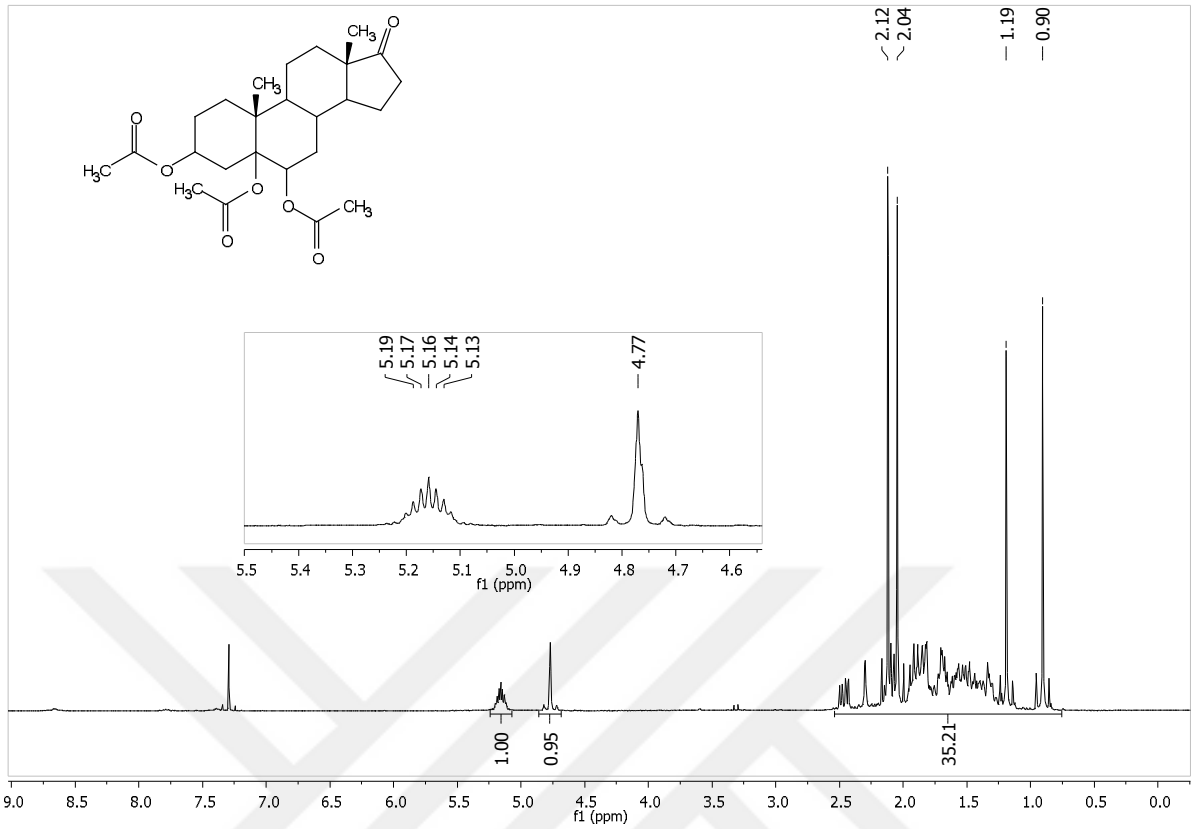

Figure A. 29 ^{1}H-NMR spectrum of 3β, 5α,6β-triacetoxyandrost-5-en-17-one **(13)**

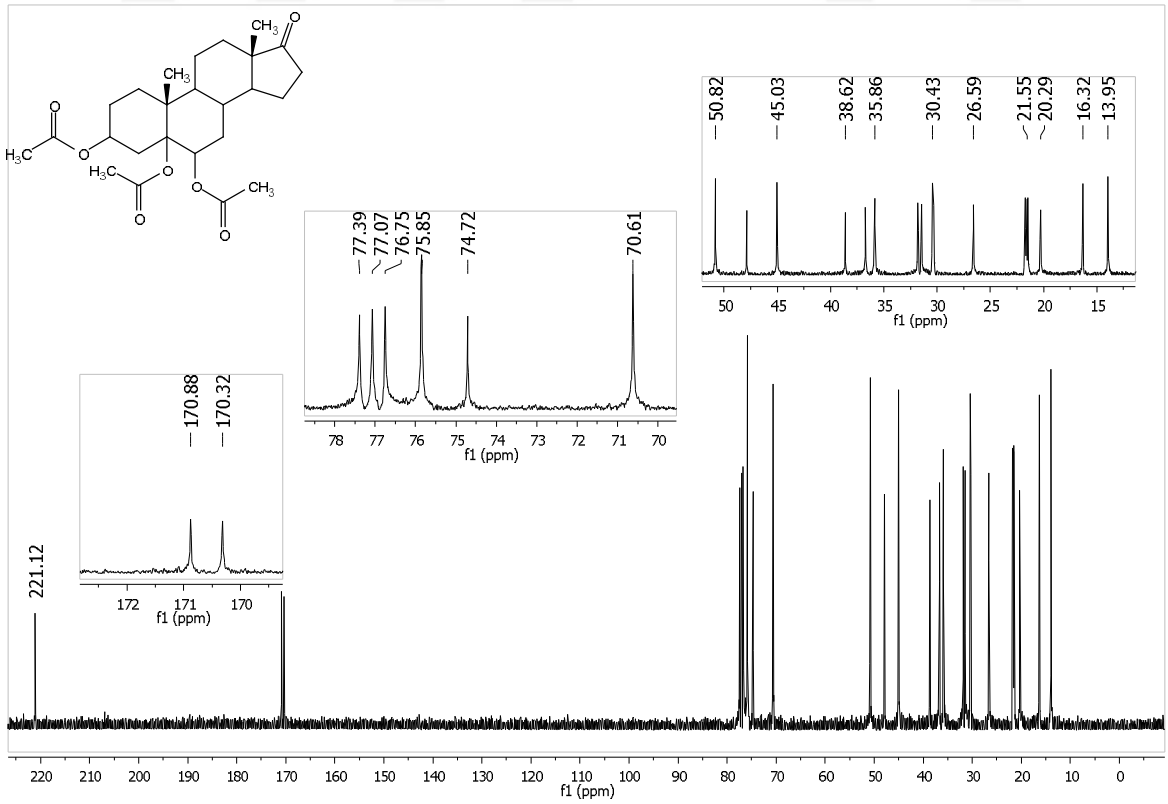

Figure A. 30 ^{13}C-NMR spectrum of 3β, 5α,6β-triacetoxyandrost-5-en-17-one **(13)**

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