Design, Synthesis and Evaluation of Novel Non-steroidal Molecules as Potential Anti-Prostate Cancer Agents

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Submitted By

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Statement by the candidate

This is to submit that this written submission in my project report entitled "Design, Synthesis and Evaluation of Novel Non-steroidal Molecules as Potential Anti-Prostate Cancer Agents" represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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This is certified that the work described in this project report entitled "Design, Synthesis and Evaluation of Novel Non-steroidal Molecules as Potential Anti-Prostate Cancer Agents" has been carried out by Paranjeet Kaur at the School of Pharmaceutical Sciences, Lovely Professional University, Punjab.

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Paranjeet Kaur

List of Abbreviations

S.No.	Abbreviations	Meaning
1	AR	Androgen Receptor
2	ARA	AR coregulators
3	AREs	Androgen responsive elements)
4	AF1/2	Activation function ½
5	BRCA1/2	Breast cancer gene ½
6	CRT	Conformal radiation therapy
7	CYP	Cytochrome
8	CNS	Central nervous system
9	DBD	DNA-binding domain
10	DHT	Dihydrotestosterone
11	FDA	Food and Drug Approval
12	HSPs	Heat shock proteins
13	LBD	Ligand binding domain
14	IMRT	Intensity modulated radiation therapy
15	NTD	N-terminal domain
16	PC	Prostate Cancer
17	PCR	Polymerase chain reaction
18	STDs	Sexual transmitted diseases
19	T	Testosterone
20	TURP	Transurethral resection of the prostate
21	HDAC	Histone deacetylase

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ABSTRACT

Prostate cancer is a carcinoma of prostate gland and is most commonly diagnosed cancer in men. It tends to develop in men over the age of fifty. Worldwide, it is second major concern for cancer related deaths in men. Although prostate cancer incidence rates are lower in Asian countries but in India it has increased recently. Progression of prostate cancer mainly related to androgens and androgen receptor (AR). Currently treatment include: steroidal and non-steroidal drugs but due to limitation of steroidal antiandrogen, focus shifted towards non-steroidal antiandrogen and efforts are devoted to develop the pure anti-androgens that would work effectively in both wild type and mutant type AR.

In-silico molecular docking analysis is proven a valuable tool in drug discovery and development and we explored it to identify non-steroidal potential anti-androgen that modulate AR. Based on the structural features of *R*-Bicalutamide, new pharmacophore of choice is designed with conformationally restricted structure to *R*-Bicalutamide.

The novel designed ligands possess two electron deficient rings linked with heterocyclic linker. The designed ligands were studied through molecular docking at Autodock Vina interface. Some of them showed comparative good binding affinity as to *R*-Bicalutamide which may serve as a potential androgen receptor modulator and could emerge as successful agent in management of androgen dependent prostate cancer. Also the potency is being affected by the geometry of these novel compounds where *cis* isomers have shown better binding affinity than the *trans* isomers. Some of the derivatives were synthesized by taking into consideration of their binding affinity as well as the feasibility of synthesis.

Chapter 1: INTRODUCTION

1.1. **Prostate gland**: It is male reproductive part and it is an exocrine gland. It is located in front of rectum and below the urinary bladder. It consists of 3 lobes; anterior, posterior, lateral. The size of prostate varies with the age. In younger men, it is about the size of walnut, but it can be much larger in older men.

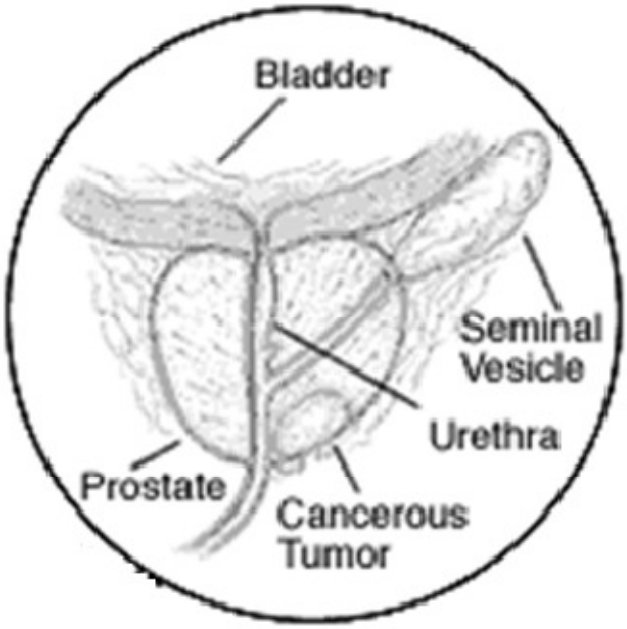


Figure 1. Prostate gland[1]

The function of prostate is to make prostatic fluid that helps in growing sperm cells in semen, making semen more liquid. Seminal vesicles are also located behind the prostate and it also provides fluid for semen's nourishment. The urethra passes urine and semen out of the body through the center of the prostate.

Prostatic fluid is slightly alkaline that usually contain 30% volume of semen and spermatozoa which is expelled in prostatic fluid for better motility, longer survival and better protection of genetic material. It also contains some smooth muscles that helps in releasing semen during ejaculation and is transported into male urethra through ejaculatory ducts which is located in prostate gland.

The Prostate begins to grow before birth. It develops rapidly during puberty, filled by androgens in the body. The male hormone, testosterone is made in the testicles. Testosterone gets converted

into dihydrotestosterone (DHT) by enzyme 5-alpha reductase. DHT is the main signal for the prostate to grow.

Prostate cancer is carcinoma of prostate gland and the most commonly diagnosed cancer in men. There are various types of cells in prostate. But mainly the prostate cancers develop from gland cells. Gland cells forms the prostate fluid which is added to the semen. Cancer in gland cells is called adenocarcinoma.

There are other types of cancer in the prostate gland, which are sarcomas, small cell carcinomas and transitional cell carcinomas. But they are so rare. Prostate cancer tends to develop in men over the age of 50.

Secretion: Proteolytic enzymes, prostatic acid phosphotase, prostate specific antigen (PSA).

Regulation: To function properly prostate need male hormones (testosterone). This is produced mainly by testicles. Some male hormones are produced by adrenal gland. However dihydrotestosterone is the main hormone that regulates the prostate.

1.2 Symptoms of Prostate Cancer:

1. Decreased urinary stream
2. Urinary frequency
3. Hematuria
4. Bone pain
5. Numbness or weakness
6. Badder/bowel incontinence

1.3 Risk Factors for Prostate Cancer:

1. Age – Rare before 40; 65% over the age of 65
2. Race - More common in African-American men
3. Family History - 1st degree relatives, father, brother
4. Nationality - North America and North West Europe, Africa
5. Genetics – BRCA1 and BRCA2 mutation increase risk
6. Obesity, Diet, Exercise, prostatitis, sexually transmitted diseases (STDs)

1.4 EPIDEMIOLOGY OF PROSTATE CANCER:

Prostate cancer is distributed worldwide. More than 6,70,000 men are diagnosed with prostate cancer every year. In 2013 in United States: 233,000 newer cases of cancers were found and 29,480 deaths were reported. Although PC incidence rates are lower in Asian countries but in India it has increased recently.[3]

Table 1: Prostate cancer cases and death by sex, US ,2014[2]

Estimated new cases			**Estimated new cases**		
Both sexes	male	Female	Both sexes	Male	Female
233,000	233,000	-	29,480	29,480	-

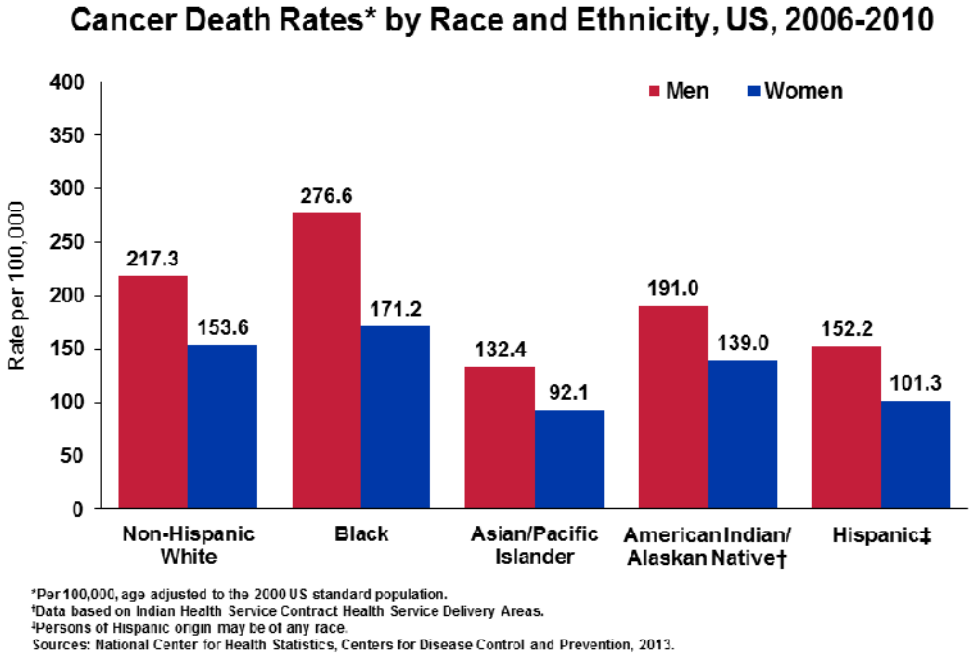


Figure 2. Prostate Cancer Incidence and Death Rates by Site, Race, and Ethnicity, US,2006-2010[2]

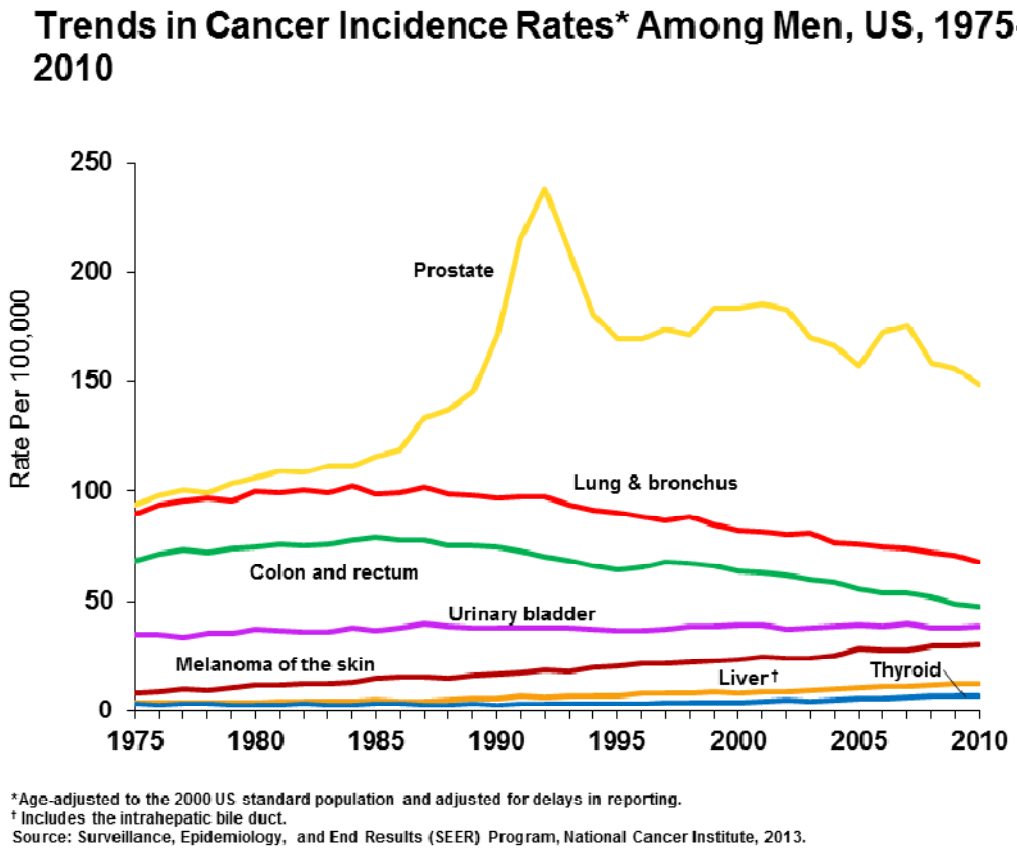


Figure 3. Comparative analysis of various types of cancer[2]

Table 2. Table showing relative proportion (&) of prostate cancer incidence, rank among top ten leading sites of all cancers, respective crude rate (CR) and age adjusted rate (AAR) per 100,000 population for different population based cancer registries of India.

s.no.	City	Relative proportion (%)	Rank	Respective crude rate (CR)	Age adjusted rate	Duration
1.	Bangalore	6.7	3rd	5.3	8.9	2008-2009
2.	Bhopal	5.2	5th	3.8	6.6	2009-2010
3.	Chennai	5.9	4th	6.3	7.0	2009
4.	Delhi	6.8	2nd	5.2	10.7	2008-2009
5.	Mumbai	6.8	3rd	4.8	7.8	2009-2010

6.	Kamrup	4.6	6th	-	11.1	2009-2011
7.	Ahmedabad rural	2.9	7th	-	2.6	2009-2010
8.	Ahmedabad urban	3.5	7th	-	5.4	2009-2010
9.	Kolkata	7.5	2nd	7.6	6.9	2008-2009
10.	Kollam	4.8	5th	6.2	5.7	2009-2010
11.	Nagpur	3.2	9th	-	3.4	2008-2009
12.	Pune	8.6	2nd	4.5	7.2	2009-2010
13.	Thiruvanantha purm	6.4	2nd	9.1	8.5	2008-2011
14.	Wardha	2.9	9th	-	2.0	2010-2011

1.5 DETECTION OR IDENTIFICATION OF PROSTATE CANCER:

Prostate specific antigen (PSA) : prostate make the protein called PSA , which is measured by blood test. If PSA level is high ,then cancer is more likely to grow. But large sized prostate cause high level of PSA.

Prostate ultra sound (transrectal ultrasound) : In this type of detection. an ultrasound probe is placed into a rectum in close contact to prostate. Biopsy is often used with ultrasound for cancer detection.

Prostate biopsy: A needle is inserted into prostate to make tissue out to check for prostate cancer. This is usually done through rectum.

Digital rectal examination (DRE): Lubricated, gloved finger is inserted into rectum and prostate is felt. If prostate is large or lumped then cancer can be easily detected.

1.6 TREATMENT OF PROSTATE CANCER:

When prostate cancer metastasizes beyond the prostate gland, it starts affecting other regions of the body and reaches to the stage where it can no longer be cured. However, treatment is usually

given to control the cancer for several years, to overcome its symptoms and to improve the quality of life. These treatments include:

1.6.1 **Surgery**: The nerves that control erections (which run along either side of the prostate) usually removed during the surgery which results in causing sexual impotency in man. However, to remove the prostate gland isn't suitable for men with advanced prostate cancer, but occasionally a transurethral resection of the prostate (TURP) can help to relieve problems with passing urine.

1.6.2 **Radiation therapy**: Radiation therapy has become so advanced that it aims the radiations so very precisely then in the past. Conformal radiation therapy (CRT), intensity modulated radiation therapy (IMRT), and proton beam radiation are currently used techniques. These technologies are so specific that only prostate gland is targeted rest other neighboring tissues are avoided.

1.6.3 **Hormonal therapy**: various hormone therapies have been emerged in recent years. For example; abiraterone (Zytiga) and enzalutamide (Xtandi). Another new drug being studied, known as *orteronel*, this acts similarly as abiraterone. This drug may act on CYP17 more precisely. There are some available drugs in the market that blocks the transformation of testosterone into dihydrotestosterone (DHT) for example: 5-alpha reductase inhibitors, such as finasteride (Proscar) and dutasteride (Avodart) . These drugs minimize the prostate size in men with benign prostatic hyperplasia.

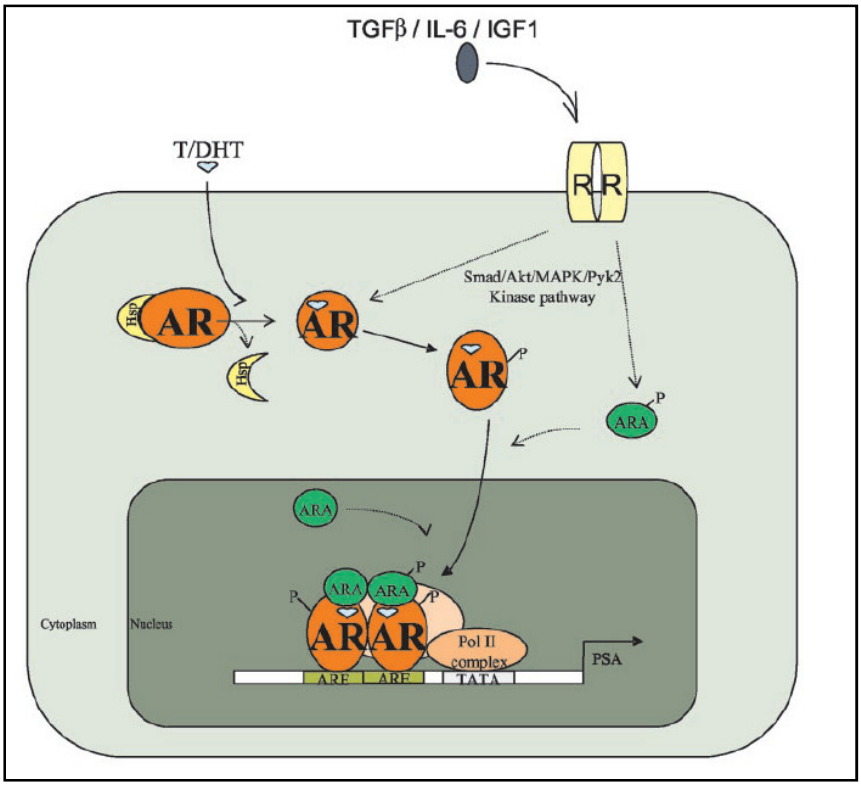
1.6.4 **Chemotherapy**: It is the most preffered treatment for controlling the cancer growth not only at early stage but also at advanced stage. Recent studies have shown that many drugs affect in such a way that improves the quality of patient's life. such as docetaxel (Taxotere) and cabazitaxel (Jevtana) have shown their results in increasing longevity of men's life. Other new drugs and combinations of drugs are continuing being studied.

1.6.5 **Immunotherapy: Vaccines:** In this type of therapy vaccines are generally used to boost the body's immune response to prostate cancer cells are. These vaccines help in treating and preventing prostate cancer. Major advantage of these types of therapies is that they seem to have very limited side effects. An example of this type of vaccine is sipuleucel-T (Provenge). Other drugs such as ipilimumab (Yervoy) which acts on white blood cells that helps to control the immune system of the body and to treat men with advanced prostate cancer.

1.7 ANDROGEN RECEPTOR AS A MOLECULAR TARGET:

1.7.1 **Androgen Receptor (AR):** Prostate cancer mainly progresses in response to androgens and androgen receptors (AR). The androgen receptor is steroidal member of nuclear receptor superfamily,[4] which comprises of around 100 members and continues to grow. Binding of these androgens on androgen receptor causes conformational changes of the receptor which affect receptor-protein interaction and receptor-DNA interaction. Androgen receptor works as agonists for hypogonadism while as antagonists for prostate cancer therapy. The various known AR ligands are classified as steroidal or nonsteroidal on the basis of their structure or as agonist or antagonist based on their ability to activate or deactivate transcription of AR target genes. However these steroidal AR ligands have limited use in prostate cancer therapy due to their low oral bioavailability, poor pharmacokinetic properties, and side effects. However, these newer discoveries in the class of nonsteroidal AR ligands provide the new insights to achieve the specificity and selectivity in tissue targeting as estrogen receptor ligands.Main function of AR is DNA-binding transcription factor that regulates gene expression. These regulated genes play significant role in development and maintenance of male sexual phenotype. The development of male sexual characterstics occurs upon testosterone interactions with androgen receptor in some cells and testosterone conversion to dihydrotestosterone in other cells. Dihydrotestosterone has more potency then testosterone. Testosterone responsible for primary male characterstics by acting in “wollfian duct” whereas dihydrotestosterone is responsible for secondary male characteristics by acting in urogenital sinus, urogenital tubercle and hair follicles.

Mechanism of action[5]: These AR regulates the gene transcription. Androgen when binds to AR, it results in changing conformations in receptors, that causes heat shock protein (HSP) to dissociate and then it transports from cytosol into nucleous and results in causing AR dimer. AR dimer then bind to specific sequence of DNA known as androgen response element (ARE). It then interact with other proteins in nucleous results in up or down regulations of specific gene transcription. Activation of transcription (up regulation) causes increase in synthesis of mRNA, which in turn translated by ribosomes to produce specific protein: protein specific antigen (PSA). Insulin like growth factor 1 is one of the known target gene for AR activation.



T (Testosterone) , DHT (Dihydrotestosterone), AR (Androgen receptor) , ARA (AR coregulators), AREs (Androgen responsive elements)

Figure 4. Mechanism of AR action[5]

1.7.2 STRUCTURE AND FUNCTION OF ANDROGEN RECEPTOR:

Androgen receptor mainly comprises of four functional domain : N-terminal domain (NTD), DNA-binding domain (DBD) and ligand binding domain (LBD) and C-terminal domain,[6] out of these four, three are major functional domain : LBD, DBD, NTD.

N-terminal domain contains N-terminal activation function-1 that play primary role in target gene transcription. AF-1 lies between residues 101 and 370, that function in causing full length transcriptional activity and is not conserved in sequence as other steroid receptors. Its dimerization surface involves 1-36 residues (FxxLF motif where F= phenyl alanine, L = leucine, X= any amino acid residues) and 360-494 both of which intramolecularly interact with ligand binding domain.

Ligand binding domain contains activation function-2 that induces its activity in the presence of bound agonist. It contains 5 exons. There is small flexible hinge region that combines LBD with DBD.

C-terminal functions in a ligand dependent manner and is more conserved in sequence.

Human AR LBD has same sequence with human progesterone receptor, glucocorticoid receptor and mineralocorticoid receptor (around 50% similarity). In the conservative mutations human AR LBD shares 88% identical sequence to progesterone receptor. Whereas LBD of steroid receptors have comparatively low sequence identity, but they all have similar 3-dimentional structures with conserved structural features, including "charge clamp" a helical features. These conformation similarities provide the structural basis for cross reactivity with synthetic steroids.

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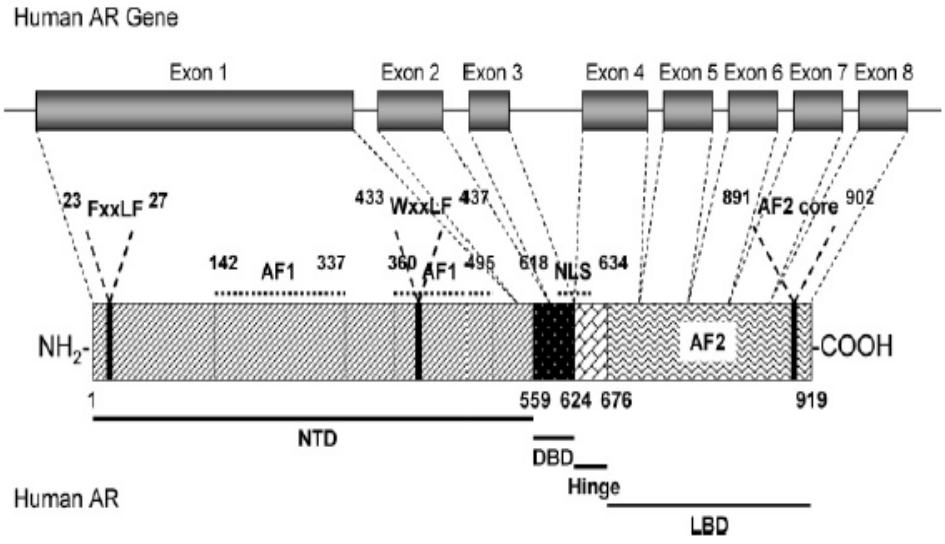


Figure 5. Structural organization of the AR gene and protein[8]

1.7.3 Physiology and clinical application of androgens:

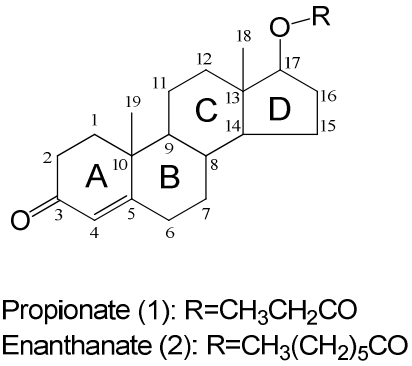
Physiologically, functional AR is responsible for development and maintenance of male sexual phenotype as well as male pubertal changes. AR is mainly expressed in androgen target tissues, such as the prostate, skeletal muscle, liver, and central nervous system (CNS), with the highest expression level observed in the prostate, adrenal gland, and epididymis [12] In adult males, androgen functions in maintaining libido, spermatogenesis, muscle mass and strength, bone

mineral density, and erythropoisis.[13,14] Its activity in reproductive tissues, including prostate, seminal vesicle, testis, and accessory structures, is called androgenic effects, whereas nitrogen retaining effects of androgen in muscle and bone is called anabolic effects. The mutation in AR gene causes: androgen insensitivity syndrome and prostate cancer. Androgen receptor also convert some AR antagonists into agonists

Chapter 2: REVIEW OF LITERATURE

2.1 STEROIDAL ANDROGENS:

2.1.1 TESTOSTERONE ESTERS: Esterification of male hormone, testosterone esters (Figure 6) results in controlling prostate cancer growth and is usually through parentral route for its prolonged activity.



Propionate (1): R=CH_3CH_2CO
Enanthanate (2): R=$CH_3(CH_2)_5CO$

Figure 6. Testosterone esters.

2.1.2 **17α-Alkylated Androgens:** The 17-position substitution of these male hormones like testosterone and DHT was alkylated to block the metabolism of the 17α-hydroxyl group, which results in improving the oral bioavailability of these compounds, such as 17α methyltestosterone (Figure 7). 17α-Methyltestosterone has similar affinity as testosterone.[16]

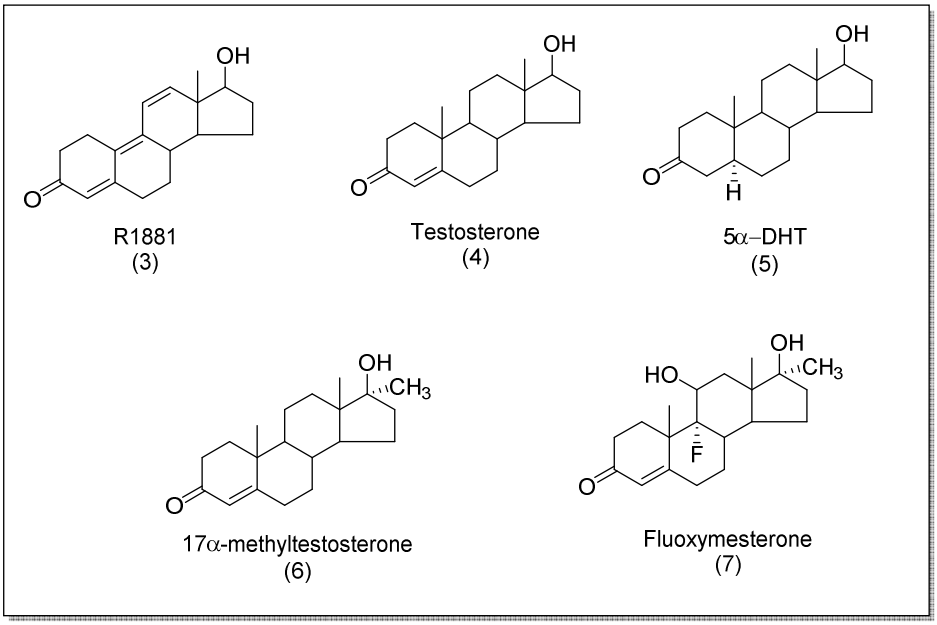


Figure 7. Steroidal AR agonists

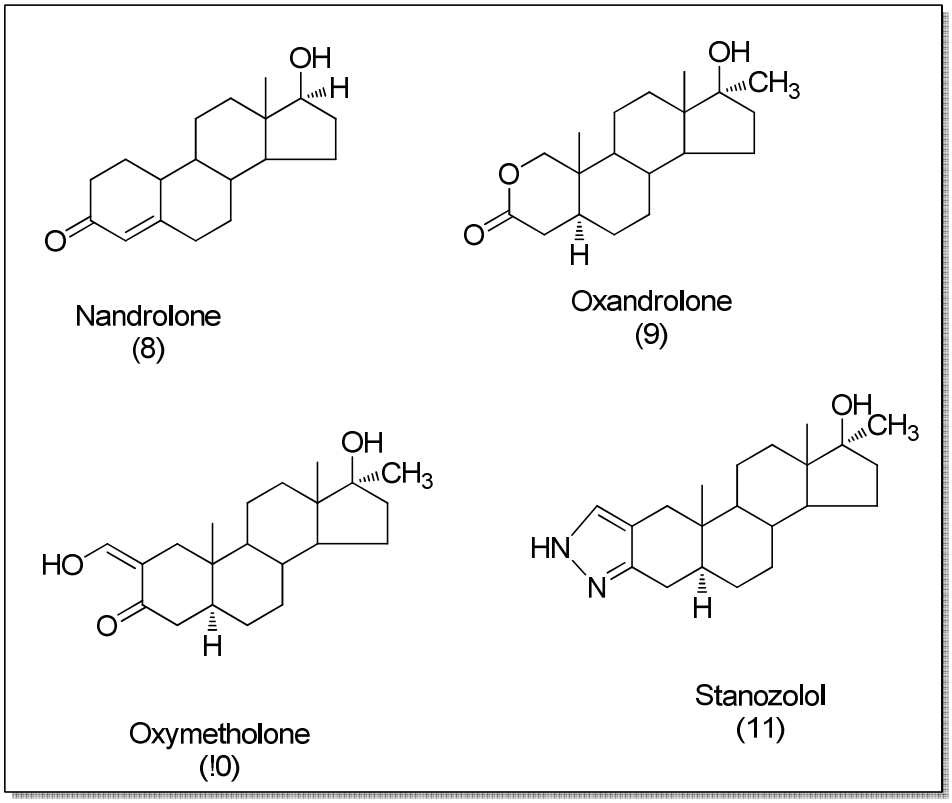


Figure 8. Anabolic steroids

2.1.3 **Anabolic steroids**: These are the synthetic androgens. These were generated as an effort to overcome unsuitable side effects associated with testosterone and its esters. These agents posses the stronger anabolic activity than androgenic activity. Commercially available anabolic steroids in the market are: oxandrolone, oxymetholone, stanozolol, and nandrolone (Figure 8).[8] But only limitation with these compounds is incomplete separation of androgenic and anabolic activity.

2.1.4 **Structure – activity relationship:** structural modification of these steroidal analogs led to the discovery of newer steroidal compounds that seems to be favorable for separation of anabolic and androgenic activity. Alkylation of 17α-OH group , results in improving oral bioavailabilty and prolong elimination half life of these ligands. But major side effects associated with 17α –alkylated androgens is hepatotoxicity. Still complete separation of androgen and anabolic activity has not been achieved with synthetic steroids. Other limitation associated with steroidal androgens is its structural modification which tend to cross react with other steroidal receptor.

2.2 **STEROIDAL ANTIANDROGEN:**

Antiandrogens are the ompounds which antagonize the action of testosterone or DHT by competitively inhibiting their binding to AR. These compounds have greater potential to treat prostate cancer. These ligands include: cyproterone acetate, oxendolone and spironolactone (figure 9).

Cyproterone is steroidal antiandrogen that compitively antagonize the action of testosterone or DHT on AR of prostate cancer cells as well as hypothalamus and pituitary. Thus in hypothalamic and pituitary pathway it blocks its negative feedback mechanism and results in increasing (LH) serum level, finally increases the testosterone level. This leads to lower the ability of cyproterone to block the androgenic stimulation. To overcome this problem, esterification of cyproterone was done, which competes with DHT to bock androgenic stimulation by inhibiting gonadotrophin secretions: androgens, estrogens and LH levels. But latter it was found to have certain limitations i.e loss of libido, sexual potency and liver hyperplasia. Other disadvantages of using these steroidal antagonists are: poor oral bioavailability, potential hepatotoxicity, lack of tissue selectivity, cross reaction with other steroid receptors, structural modification of the steroidal ligands is limited. Such serious

side effects gave an incentive to the pharmaceutical companies to look for alternative non steroidal 'pure' antiandrogens that would not show such severe side effects.

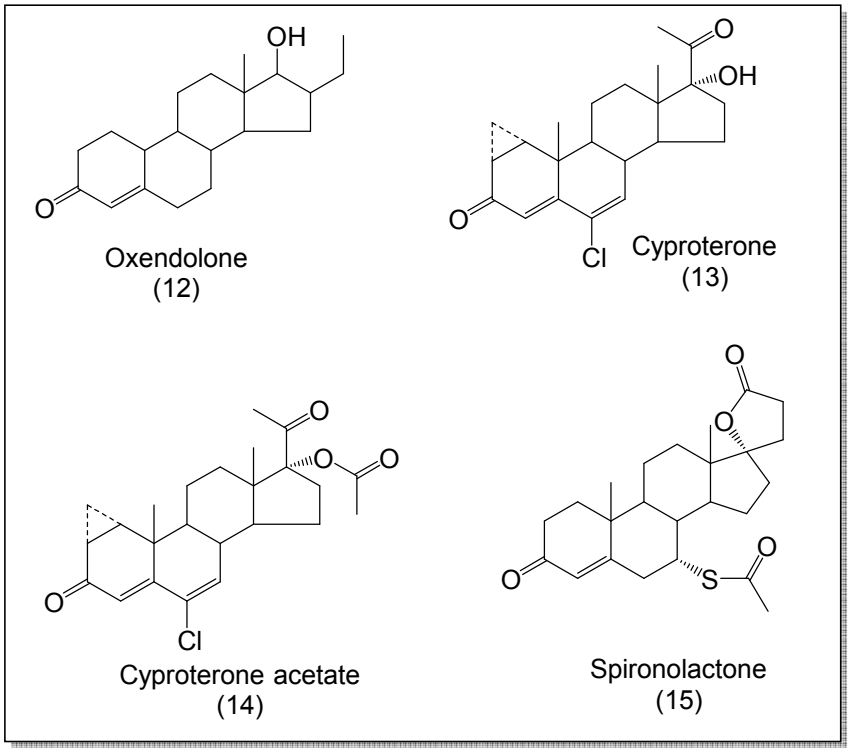


Figure 9.Steroidal antiandrogens

2.3 NONSTEROIDAL ANDROGEN RECEPTOR LIGANDS:

The non-steroidal ligands have high AR specificity, improve oral bioavailability, and allow more flexible structural modifications as per requirements.[17]

The first developed class of non-steroidal antiandrogen is known as toluidides. It includes: *R*-bicalcutamide, flutamide, hydroxyflutamide, nilutamide, 3-trifluoromethyl-4-nitroaniline. (Figure 10).[51] Flutamide was the first non-steroidal anti-androgen. Later bicalutamide and nilutamide were generated. The advantages of these compounds that they did not affect libido or potency like the other steroidal compounds.

Flutamide: (arylpropionamide analog) flutamide is generally given orally. It undergo extensive first pass metabolism . upon hydrolysis gives its active form 2-hydroxy flutamide , which is even more potent than flutamide.[18] Its elimination half life is 8 hour.[19] As flutamide

has lower binding affinity to AR as compared to hydroxyflutamide, so it is generally given at higher doses to achieve complete AR blockage.

Nilutamide: It is nitro aromatic hydantoin analog of flutamide. It is eliminated by reduction of aromatic nitro group.[20] It has comparatively longer half life i.e two days. It is associated with hepatotoxicity during nitro reduction from nitro anion free radical.[21,22]

Bicalutamide: It is an aryl propionamide analog and it is known as first choice antiandrogen for prostate cancer treatment. It is less hepatotoxic in comparison to other drugs.[23] It also has longer half life i.e 6 days.[24,25] It allows once a day administration at lower dose (50 mg/day). It has chiral carbon in structure so given in its racemic form. Its androgenic activity resides almost in its *R*-enantiomer.

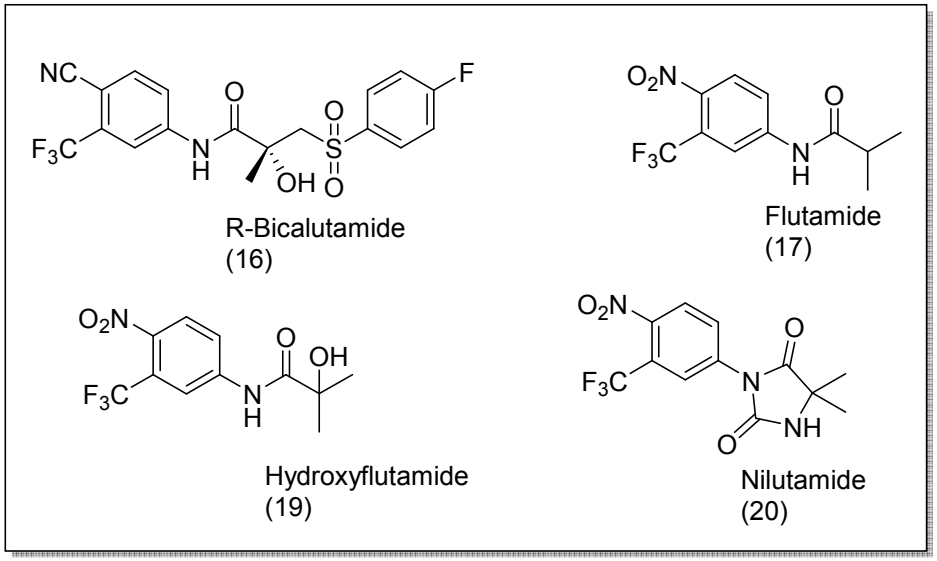


Figure 10. Non-steroidal antiandrogens as toluidides derivatives

Antiandrogens play significantly role in the treatment of prostate cancer in its early stage. During continued antiandrogen therapy or androgen abscission therapy, disease start progressing and reaches to a state where androgen independent prostate cancer develops or androgen begins to support the tumour growth known as anti-androgen withdrawal syndrome.[26] For example, hydroxyflutamide and bicalutamide appears to be agonist in particular mutants such as T877A and W741C[27,28] respectively. Therefore, researchers devoted their efforts towards the development of new generation of pure antiandrogens" that could work in both wildtype and mutant AR.

2.4 ADVANCEMENTS IN NON-STEROIDAL ANTIANDROGENS:

2.4.1 Hydantoin derivatives:

Hydantoin analogues were designed through structural modification of commercially available non-steroidal antagonist, nilutamide, which led to the generation of newer classes of non-steroidal antiandrogens such as Hydantoin (bicyclic-1*H*-isoindole-1,3- (2*H*)-dione) analogues (Figure 11).[51,29-32] in this category BMS-1[32] and BMS- 15121[31] are the lead compounds that bind to the wild-type AR with high affinity. On other hand increased size of the ring system 2.2.2 (BMS- 337143 vs BMS-434681)[29-32] provides more steric interactions and bind to mutant type AR with strong affinity. Beside this, the combined structural features of bicalutamide and BMS-434681 led to the generation of BMS-5 (azahydantoin). The modified [2.2.1]-bicyclic hydantoins (**26-29**) were designed and based on the biological activity as well as through molecular modeling several of these compounds founds better than to bicalutamide (Table 3).[33]

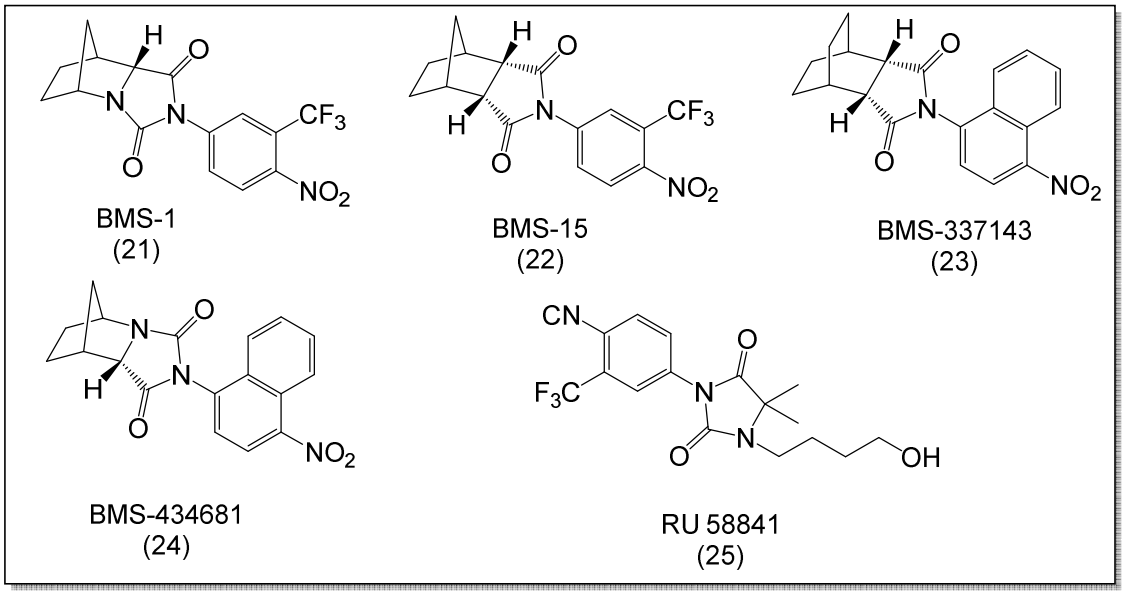
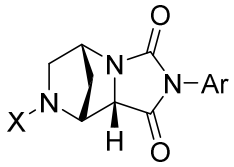


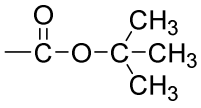
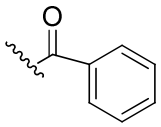
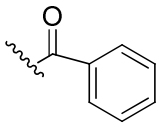
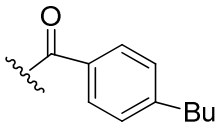
Figure 11. Non-steroidal antiandrogens as hydantoin analogs

Based on these studies, a series of other modified hydantoins were developed on the basis of compound BMS-5. The activity of these newer derivatives was evaluated on the ability of these compounds to bind to (Ki) and functionally antagonize (IC_{50}) the WT AR found in the MDA-453 cell line as well as antagonize the MT AR (T877A) found in the LNCap cell line as shown in table below. Thus the series of modified hydntoin compounds showed very

potent binding affinity towards AR in addition to an antagonist profile in vitro against the WT AR, in comparison to bicalutamide and flutamide, a clinically used antiandrogens.[34]

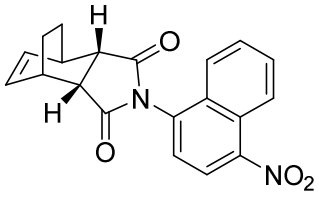
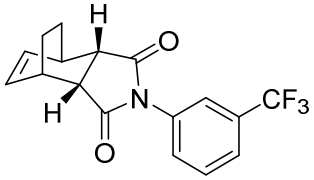
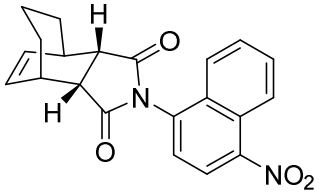
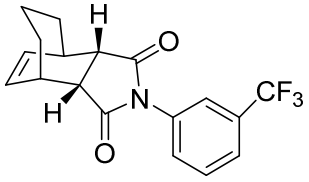
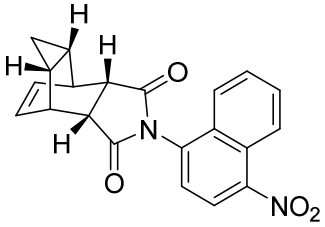
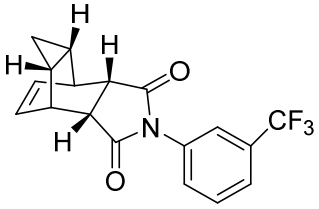
Table 3: Bicyclic hydantoin derivatives



Compound	X	Ar	Ki[a] (µM)	MDA-453 IC_{50}[b] (µM)	LNCaP IC_{50}[b] (µM)
26		4-nitro-naphthyl	0.237	0.252	2.39
27		4-cyano-3 trifluoromethylphenyl	0.124	0.300	2.60
28		4-nitronaphthyl	0.012	>5.00	2.54
29		4-cyano-3-trifluoromethylphenyl	0.693	17.9	33.7

Docking of these compounds into the AR model revealed that these compounds were found better and were successful in forming critical hydrogen bond with N705 residue, relative to that of bicalutamide. Due to the presence of 4-notronephthyl in bicyclo [2.2.2] octane and bicyclo [3.2.2] nonane **30, 32** and **34** tend to make these compound as agonist whereas on introduction of 3-trifluoromethyl aniline, antagonist activity is observed for compounds **31, 33** and **35** with little change in binding and functional activity to T877A AR and MT AR. Similarly on introduction of 4-nitronaphthyl group, as ring size is increased , the binding affinity is also increased. Whereas when 3-trifluoromethyl aniline is used, loss of binding and functional activity is observed. Thus, SAR suggested that upon modification of active portion such as aniline led to the discovery of newer promising and challenging molecules. [35]

Table 4: advanced Bicyclic hydantoin derivatives

Compound	Structure	MDA-453 Ki, nM[a]	MDA-453 IC_{50} nM[b]	LNCaP Ki, nM[a]	LNCaP IC_{50} nM[b]	PC_a 2b IC_{50}, nM[a]
30		19	224	0.5	Ag[d]	Ag[d]
31		100	196	19	66	0.4
32		9	49	9	Ag[d]	Ag[d]
33		2810	658	20	23	179
34		3	786	NT[e]	Ag[d]	Ag[d]
35		3200	1700	9	30	148

2.4.2 **Phthalimide derivatives**:

Hashimoto et al developed the various phthalimide derivatives through lead compound *N*-[(3,5-dimethyl-4-isoxazolyl)methyl] phthalimide (DIMP). The structural similarities between DIMP and thalidomide (a hypnotic/sedative drug) were taken consideration to treat prostate cancer due to its effect on cytokine production. Phthalimide ligands are shown in figure 12.[51, 36,37]

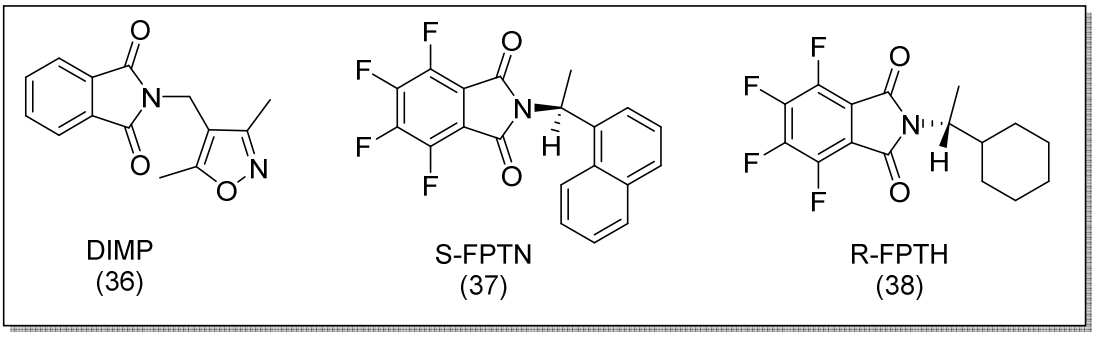


Figure 12. Non-steroidal antiandrogens as phthalimide derivatives.

2.4.3 **Dodecaborane derivatives**:

In the year 2004 another new class of non-steroidal antagonist was generated which upon inserting novel hydrophobic core (carborane)[38] containing hydrophilic functional group between two polar groups, a carbonyl group and hydroxyl group at hydrophobic ends (Figure 13). These carboranes interacts with the hydrophobic surface of LBD of nuclear receptor sue to their spherical hydrophobic surface. Hydrophobic interaction along the spherical carborane cage produces stronger interaction to Era then that of *17b*-estradiol for example: 1-hydroxymethyl-12-(4-hydroxyphenyl)-1,12-dicarba-closo-dodecaborane (BE120).[39,40] thus, the usage of this carborane cage has made it possible to develop wide variety of nuclear receptor ligands. Thus, the compounds **39** and **40** showed potent antiandrogenic activity as compared to hydroxyflutamide which was seen by growth promotion/inhibition assay using androgen-dependent SC-3 cells.[41]

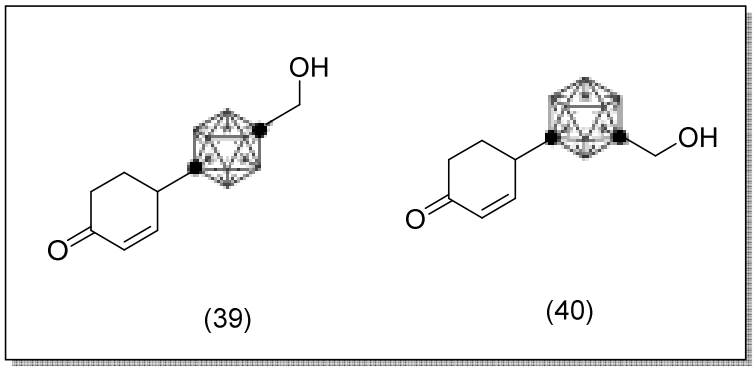


Figure 13. Dodecarborane derivatives

2.4.4 **Selective androgen receptor modulators (SARMs):**

Newer class as selective androgen receptor modulator emerged as one of the successful class that contains molecules which are highly specific and selective in their action. One of the major advantages of this class of drug is that, they are orally active and target AR in different tissues as well. Commercially available small molecules today in market shows undesirable side effects caused due to nonselective inhibition of AR action. In order to minimize these side effects, newer class of tissue selective androgen receptor has been emerged as an approach for treatment of prostate cancer. These ligands are proposed to work as antagonist in the prostate while agonist in other target tissues or having no effect in other tissues.[42,43] The progress has been made in identifying novel non-steroidal antiandrogens. Structurally modified bicalutamide led to the first generation of selective androgen receptor modulators (Figure 14).[51]In this class Lead compounds are S1 and S2 that shows their role in tissue selectivity and bind AR with high.[44,45] these compound shows partial agonist activity in prostate but fully agonist in levator ani muscle. Further the suppressive effects of this class of SARMs as studied, suggested that such compounds might be useful for male contraception.[46] Various important classes fall in the category of SARMs that have shown tissue selectivity. One of them is hydantoin derivatives containing lead compound BMS 564929 binds AR with high affinity and high specificity.[47] later developed a series of tetrahydroquinolin (THQ) derivatives which shows little tissue selectivity and work as a strong agonist in the prostate and levator ani muscle. [48]

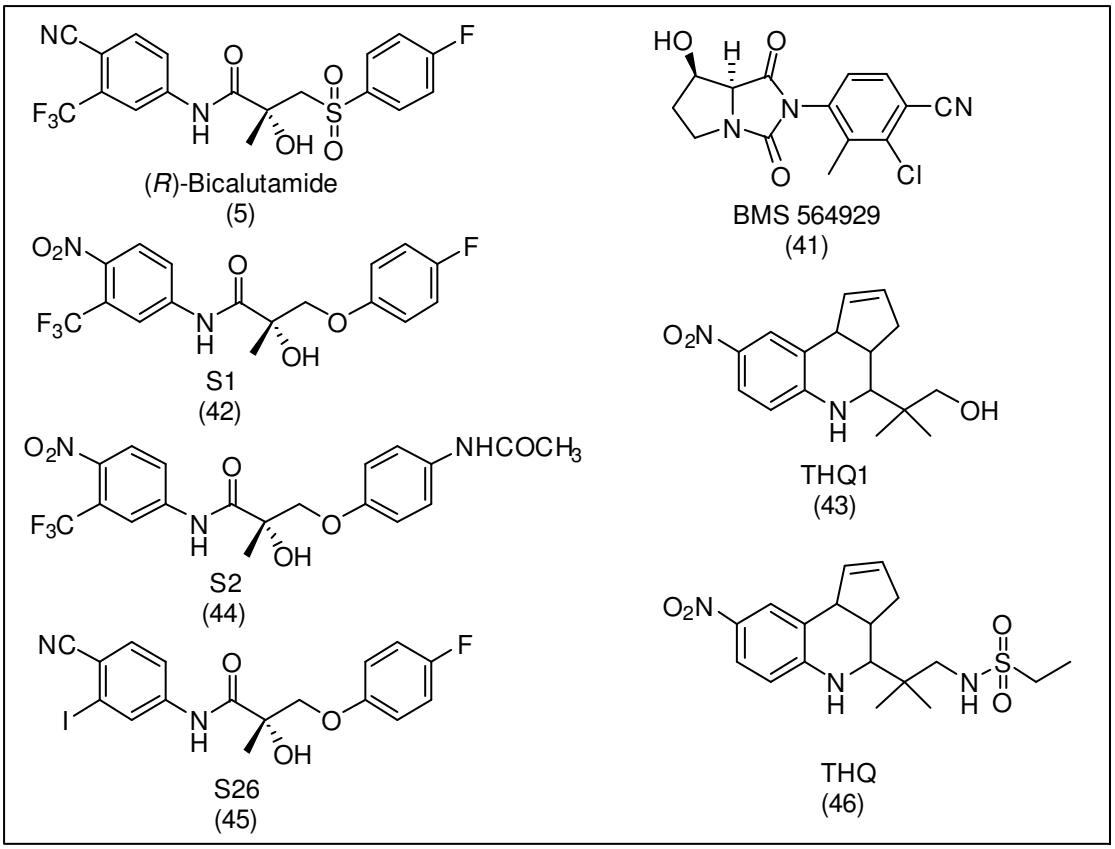


Figure 14. Some selective androgen receptor modulators (SARMs)

2.4.5 Isoxazolone derivatives:

Compounds with two aromatic groups connected with two to four carbon unit mimics as a spacer are candidate ligands for AR. Based on this knowledge T. Ishioka et al. designed a generic structure **47** as a possible androgen antagonist skeleton (Figure 15). Data supporting the idea that a structure related to **47** can be a ligand for AR were obtained with the help of computer assisted molecular design. Among the candidate compounds presented by the computer, there was (Z)-4-(4-N,N-diethylaminophenylmethylene)-3-methyl-5(4H)-isoxazolone (**48**) which was found to be moderate potent antagonist by biological assays. This was selected for the further modification.

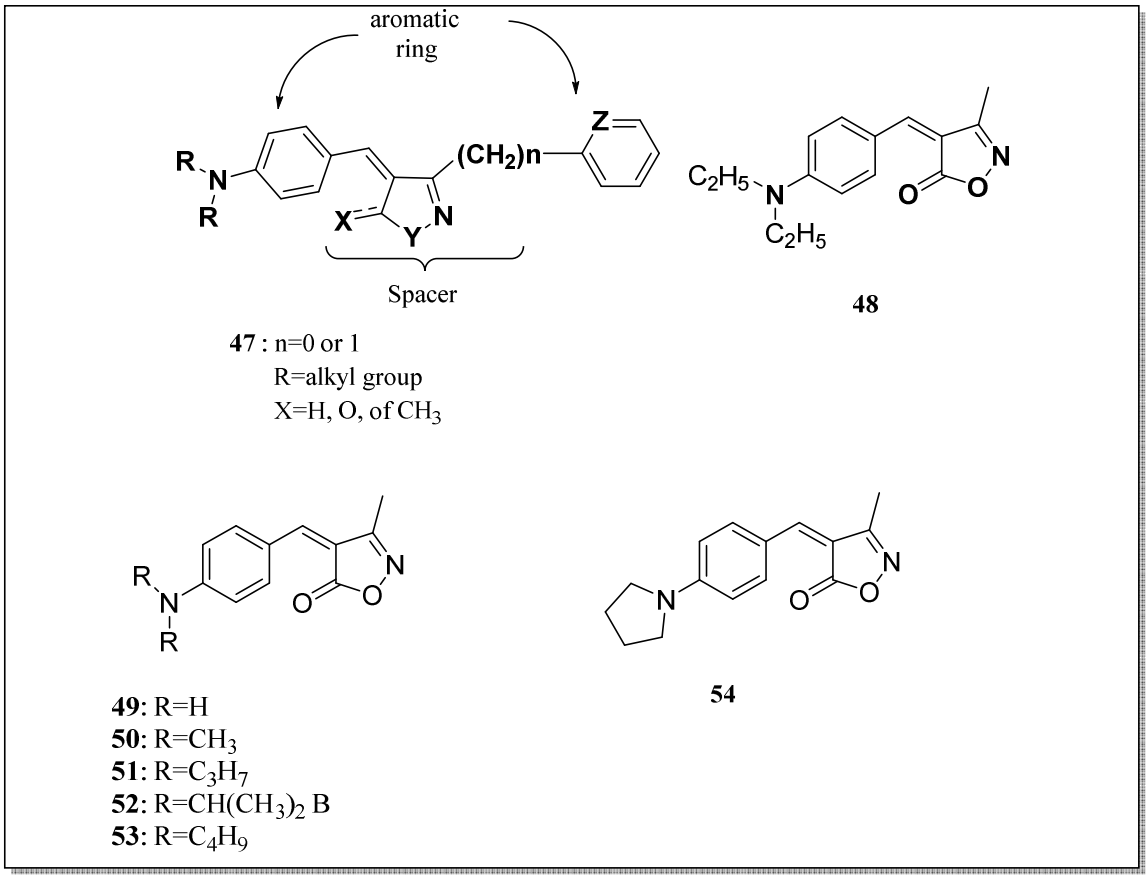


Figure 15: Molecular design of generic structure of isoxazolone

Androgenic and anti-androgenic activities of the compounds of this class were assessed by receptor-binding assay and growth promotion/inhibition assay using androgen-dependent SC-3 (Shionogi carcinoma) cells. It was observed that the non-alkylated derivative and hydroxyl analogue **49,50** were inactive, whereas two alkyl groups on amino group (**51**–**53**) resulted in active analogues. Cyclic analogue at amino resulted in an increase of the activity (**54**). (Z)-4-(4-pyrrolidinophenylmethylene)-3-phenyl-5(4H)-isoxazolones (**55-61**)

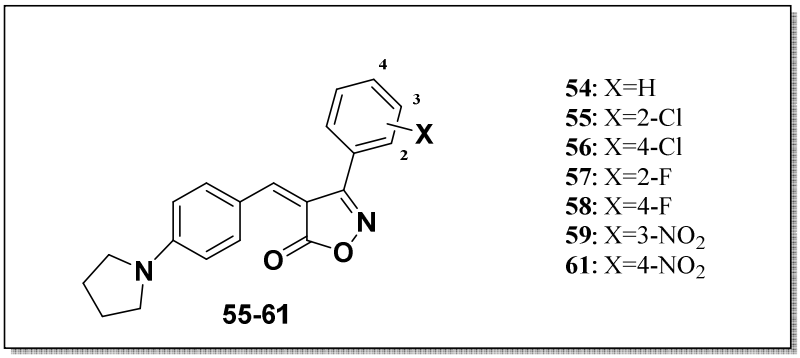


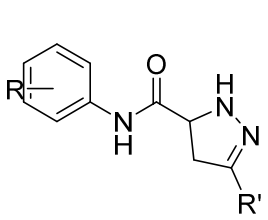
Figure 16 : (Z)-4-(4-pyrrolidinophenylmethylene)-3-phenyl-5(4H)-isoxazolones and related compounds

The effect of subtituent at 3-phenyl group of (Z)-4-(4-pyrrolidinophenylmethylene)-3-phenyl-5(4H)-isoxazolones was investigated. Introduction of a halogen atom (fluorine or chlorine) resulted in increased activity (**55-61**). The introduction of chlorine at the para-position of **57** increased the activity almost twice than that of ortho-substitution (**56**). But this order was found to be reversed in case of fluorine derivatives (figure 16).[49]

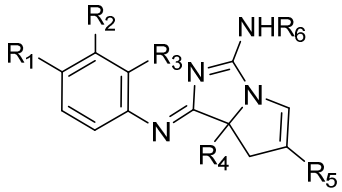
2.4.6 Imidapyrazole:

later the novel series of pyrazolines came into the history as selective androgen receptor modulators upon modification of lead compounds S1 and BMS 564929.[50] Due to the metabolic instability of amide bond in pyrazoline structure **(62)** led to the replacement with an amidate linkage and series of amidate compounds were formed. In structure **62**, amide bond was replaced by cyano amidate moiety. When test was carried on the rats , the commercially available drug, Bicalutamide tend to reduce prostate weight and levator ani muscle weight by 60% at 30mg/kg dose whereas Compound **63** when given at same dose level tend to reduce prostate weight by 26% at same dose level in the presence of endogenous testosterone. Thus the data concluded that compound **63** is a high;y selective in their action with agonist activity on rat muscle and mixed agonist and antagonist activity on the rat prostate.[51]

Table 5: Imidapyrazole derivatives



62



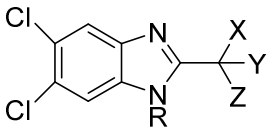
63

compound	R1	R2	R3	R4	R5	R6	Levater ani. Stimulation (%)	Prostate stimulation (%)	Prostate inhibition (%)
63(R)	CN	CF_3	H	Me	CF_3	H	91	36	30
63(S)	CN	CF_3	H	Me	CF_3	H	72	29	<10

2.4.7 **Dichloro-benzimidazole derivatives**:

It has been explored that by replacing proplionanilide moiety in bicalutamide and flutamide with benzimidazole found to be effective in acting against prostate. During screening of benzimidazole derivatives, trifluoromethyl group shows greater antagonist activity in prostate. Thus the following compounds in table below showed significant efficacy in the prostate in comparing well to bicalutamide in mature rats (Table 6).[52]

Table 6: 5,6-Dichloro-benzimidazole derivatives



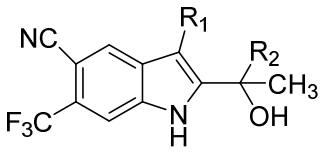
Compounds	R	X	Y	Z	P.W inh %[a]
64	H	H	OH	CF_3	96
65	H	H	H	CF_3	90
66	H	OH	OH	CF_3	94

substitution by small alkyl chains are tolerated, *N*-Methylation knocked out activity completely. On replacing one methyl group with a trifluoromethyl group in a compound (**64-**

66) gave good efficacy in comparison to clinically used drugs. Substitution with a heteroaromatic ring diminished activity. Insertion of an oxygen or sulfur into the alkyl group showed more activity in comparision. The hydroxyl analog was found to very potent antagonist.

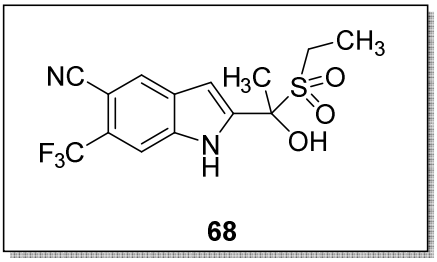
2.4.8 **Indole derivatives:** J. C. Lanter et al. established the indole carbinol moiety as a potent androgen receptor ligand by bioisosteric replacement of the anilide portion of bicalutamide. It has been identified that bioisosteric replacement of the amide moiety by indole carbinol in non-steroidal anilide antiandrogens. Through systematic modification of this scaffold, series of novel 2-(1H-indol-2-yl)-propan-2-ols have been developed and among these series, compound (**67**) shown in table below exhibited higher potency in vivo when dosed orally in an immature rats as shown in table 7 below.[53]

Table 7: Indole derivatives



67

Compound	R_1	R_2	ID_{50} (mpd)	% pros Wt. Redn[a]
67 (+)	H	CF_3	0.13	80
67 (-)	H	CF_3	0.43	76
Bicalutamide	-	-	0.29	75



68

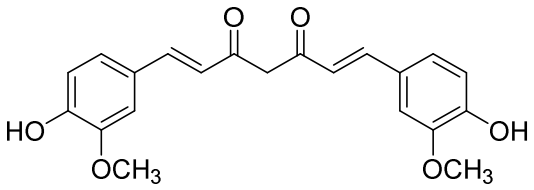
Figure 17. β-alkylthio indolyl carbinol as antiadrogen

Further modification of β-alkylthio indolyl carbinols i.e removal of the methylthio and hydroxy moieties resulted in reduction in activity, whereas substitution of alkyl group to indole nitrogen resulted in decreased activity. Oxidation of the sulfur or formation of sulfoxide reduced the activity while further oxidation to the sulfone (**68**) restored it with potent activity (Figure 17).[54]

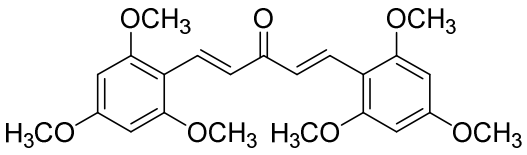
2.4.9 **Curcumin** :

Curcumin (**69**) is known to possesses an antimicrobial and anticancer activity and it is well explored by researcher to develop novel curcumin analogues. Around 24 compounds were synthesized, out of these, compound (**70**) showed potent growth inhibitory activities on prostate cancer lines with IC_{50} values in sub-micromolar range, fifty times more potent than curcumin (Table 8).[55]

Table 8: Curcumin derivatives



Curcumin (**69**)



70

Compound	PC-3 (IC_{50} μM)	LNCap (IC_{50} μM)	MCF- 7 (IC_{50} μM)	MDA-MD-231 (IC_{50} μM)
Curcumin (**69**)	19.8±2.1	19.6±3.7	21.5±4.7	25.6±4.8
70	2.1±1.1	0.5±0.1	0.4±0.1	0.6±0.1

2.4.10 Thiazolone-based pyrazoline derivatives:

To examine the anticancer activity several novel thiazolone-based compounds containing 5-aryl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl framework were obtained. It was found that combination of thiazolone and pyrazoline scaffold has greater influence on cancer activity (Table 9) . The structure activity relationship revealed that compound **71** was reported as active against prostate cancer cell line (DU-145) .[56]

Table 9: Thiazolone-based compounds containing pyrazoline

Compound	Mean growth (%)	Range of growth (%)	Sensitive cell line	Growth % of sensitive cell line	Activity
71	42.59	57.04	DU-145 (prostate cancer)	57.04	Active

2.4.11 Thioxoimidazolidinones derivatives:

Thioxoimidazolidinone containing compounds are well explored as antiandrogens (Figure 18). Recently thioxoimidazolidinones were approved by FDA for the treatment of CRPC. The important functionality for the better potency was found trifluromethyl and nitrile group in RD162 (**72**) and MDV3100 (**73**).[57] Khatik et.al reported another variant of the same, selectively inhibiting the androgen receptor. On the basis of structure based approach, considering the structures of the clinically used non-steroidal anti-prostate cancer agents, a confirmationally restricted new molecule is designed having salient features as that of these commercially available non-steroidal anti-prostate cancer agents. Compound **74** demonstrated cytotoxicity better than doxorubicin and flutamide on PC-3 (IC_{50} = 092µM) and LNCaP (IC_{50} = 0.500 µM) cells respectively.[58]

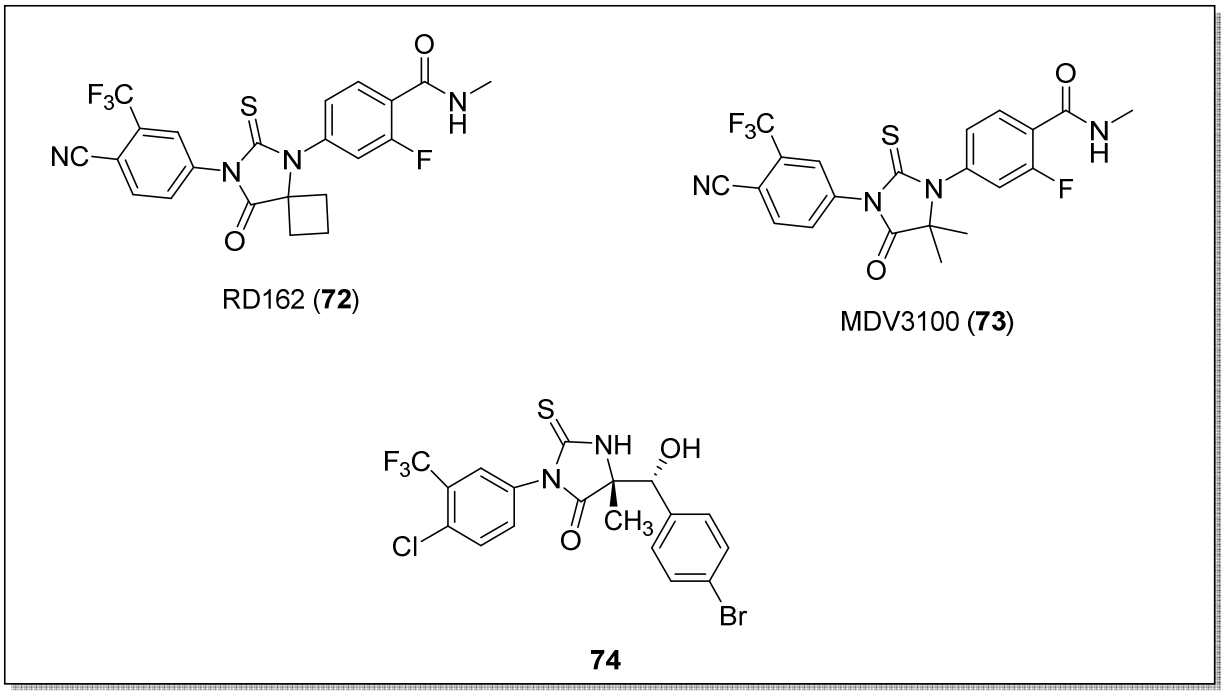
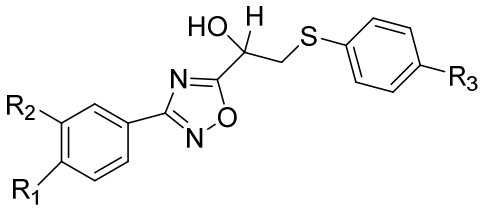


Figure 18. Thioxoimidazolidinones derivatives as antiandrogens

2.4.12 Oxadiazoles derivatives:

Recently it was reported that 1,2,4-oxadiazoles derivatives (**75-80**) containing Sulfide and sulfonyl group showed greater potency against prostate cancer cell lines; PC-3 cells and LNCaP cells as shown in table 10. These compounds were found to be potential anti-prostate cancer agent, exhibited excellent potency against the androgen independent and dependent prostate cancer cells.[59]

Table 10: 1,2,4 oxadiazole derivatives



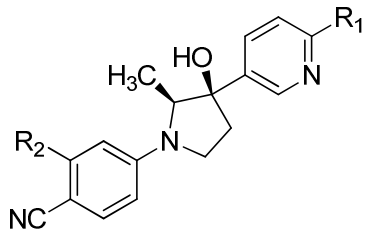
Compound	R1	R2	R3	IC_{50} on PC-3 cells (µM±SD)	IC_{50} on LNCaP cells (µM ±SD)
75	F	Cl	F	11.50±0.43	>100
76	F	Cl	Cl	10.00±0.13	26.40±0.40

77	NO_2	CF_3	H	3.50±0.12	9.67±0.50
78	NO_2	CF_3	F	5.50±0.59	8.15±0.84
79	NO_2	CF_3	Cl	3.00±0.23	>100
80	NO_2	CF_3	Br	51.00±0.40	40.00±0.57
Bicalutamide	-	-	-	77	1.71±0.12

2.4.13 Phenylpyrrolidines derivatives:

It was reported that novel 1-arylmethyl-4-phenylpyrrole and 1-arylmethyl-4-phenylpyrazole and 1-aryloxy-4-phenylpyrazole effective against PCs as orally available AR antagonists, including CRPC. Structure–activity relationship (SAR) studies for these compounds showed that the cyanophenyl group a and the arylmethyl/aryloxy moiety contributes to the strong AR antagonistic activity. The compounds (**81, 82**) shown below in table 11 exhibited greater potency against prostate cancer cells.[60]

Table 11: 3-aryl-3-hydroxy-1-phenylpyrrolidines

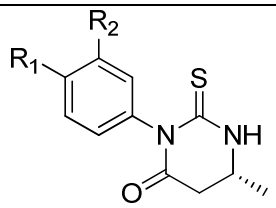


Compound	R_1	R_2	Binding IC_{50} (µM)		Receptor antagonist IC_{50} (µM)		LNCaP Inhibition	
			Wild	T877A	Wild	T877A	1µM	10µM
81	CONHMe	Cl	0.044	0.10	0.025	0.099	39	101
82	CONHMe	F	0.83	1.7	0.29	1.7	41	35

2.4.14 Thioxotetrahydropyrimidin-4(1H)-one derivatives:

Kumar et al recently disclosed the new class of antiandrogen thioxotetrahydropyrimidin-4(1H)-ones. On the basis of molecular modeling studies most potent derivatives of 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1H)-ones identified. Stereoselectively synthesized 3-aryl-6-methyl-2-thioxotetrahydropyrimidin-4(1H)-one derivatives were screened in vitro against prostate cancer cell lines, PC-3 and LNCaP and the most potent derivatives.[61]

Table 12: 3-aryl-3-hydroxy-1-2-thioxotetrahydropyrimidin-4(1H)-ones



Compound	R_1	R_2	PC-3 IC_{50} (µM)	LNCaP IC_{50} (µM)
83 (R)	CN	CF3	1.25	0.8
84(S)	CN	CF3	34	13
85(R)	CN	Cl	90	14
86 (S)	CN	Cl	100	39

CHAPTER 3: RATIONALE

Need to develop a pure Antiandrogen:

The existing drugs show side effects such as: Hepatotoxicity, Gynecomastia. They are also ineffective in case of resistance through mutation. Hydroxyflutamide act as agonist on T877A AR mutant: Hydroxyflutamide and cyperoterone acetate treatment may result in T877A point mutation. This mutation has also been discovered in patients with androgen withdrawal syndrome (AWS) being treated with these compounds. Bicalutamide act as agonist on W741L and W741C AR mutants, bicalutamide treatment resulted in 2 LBD mutations, W741L and W741C, causing bicalutamide to be ineffective.

Efforts must be put forward to the development of new generation of "pure antiandrogens" that would effectively work out in both wild-type and mutant AR resulted prostate cancer.

Upon the examination of various parameters and bonding interactions it was revealed that the Bicalutamide (**1**) acquires the conformation similar to structure **2** and the pharmacophore that has been designed to meet these requirements are **2** which establish the interactions, its impact and the conformation adopted.

Concept of conformationally restricted structure similar to Bicalutamide structure **1 (Figure 19)** was utilized to design the novel potential anti-prostate cancer agents.

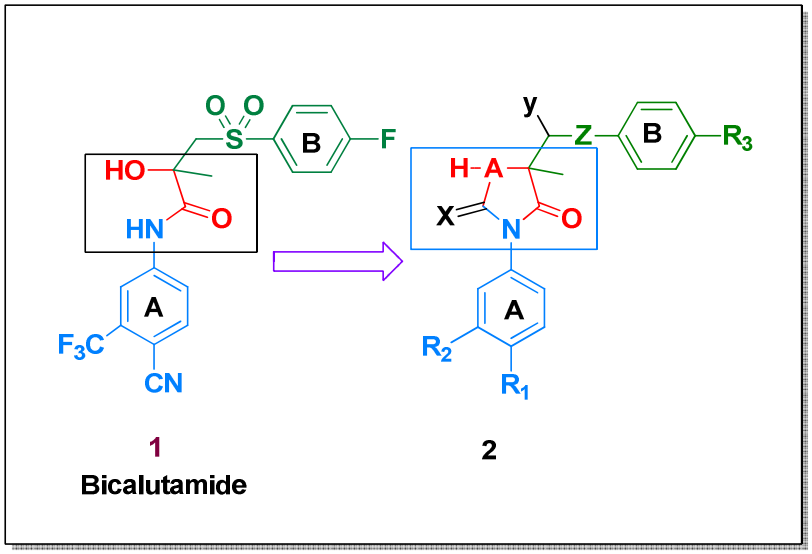


Figure 19. Design of novel series of compounds based on Bicalutamide.

These cyclic structures interlocked the amide nitrogen and the hydroxyl oxygen by carbonyl functionality, which led to the design of structure **2,** an oxadiazole/triazole derivative (**Figure 19**).

Literature study leads to the design of new pharmacophore for antiandrogen (figure 20). These two electron deficient rings can be connected with the linker which may a heterocycle. Two ring A & B may be attached with an electron withdrawing functional groups which render them electron deficient.

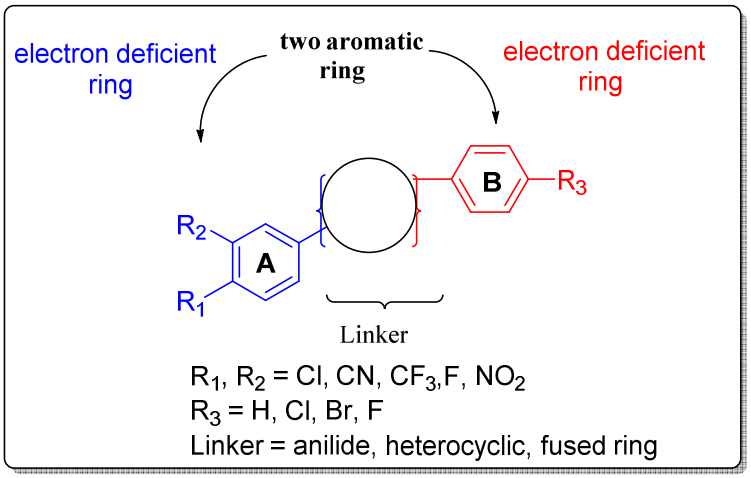


Figure 20. Design of novel pharmacophore for antiandrogens

CHAPTER 4: OBJECTIVES

This research project aims in identifying a potential anti-androgen that inhibits the androgen receptor (AR) and could emerge as a successful clinical candidate for the treatment of androgen dependent prostate cancer.

The specific objectives of the project are:

- Design of novel 1,2,4-oxadiazole/triazole derivatives.
- Study the binding interactions of the 1,2,4-oxadiazoles/triazole by molecular docking and identify the most potent compounds.
- Synthesis of most potent compound as identified through molecular docking.
- Characterization of synthesized compound through spectroscopic techniques.
- Submission of synthesized compounds for *In vitro* studies on human prostate cancer cell lines for determining cytotoxicity at laboratory of pharmacology and toxicology, NIPER, SAS Nagar (Punjab).

CHAPTER 5: WORK PLAN

To develop new anti-prostate cancer agent (antiandrogen), it is necessary to have the knowledge of the binding interactions of existing drugs with the wild type/ mutated AR. By taking into consideration of various aspects of these ligand-receptor interactions, new scaffolds will be designed to develop new AR antagonist as given below:

Ligand preparation: Database of various ligands will be prepared and geometry as well as energy will be minimized through ChemDraw program. All the optimized ligands will be saved in pdb format.

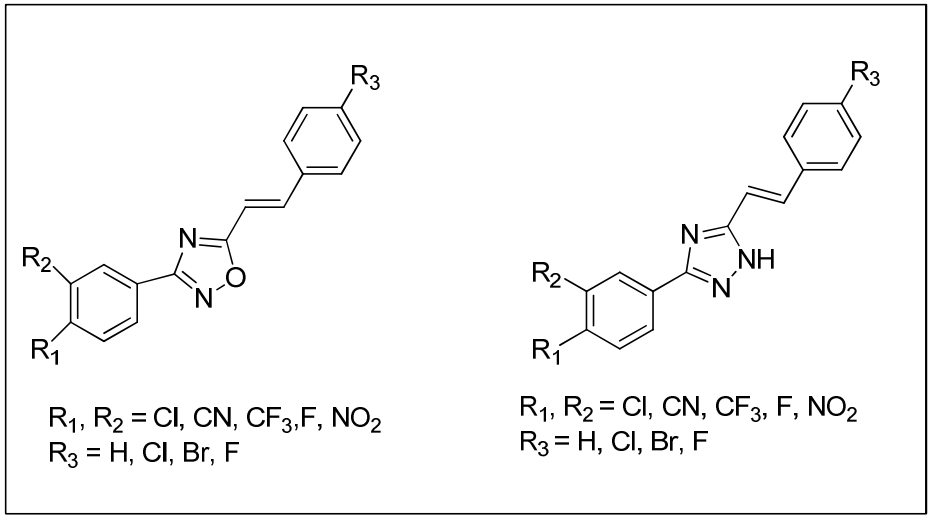


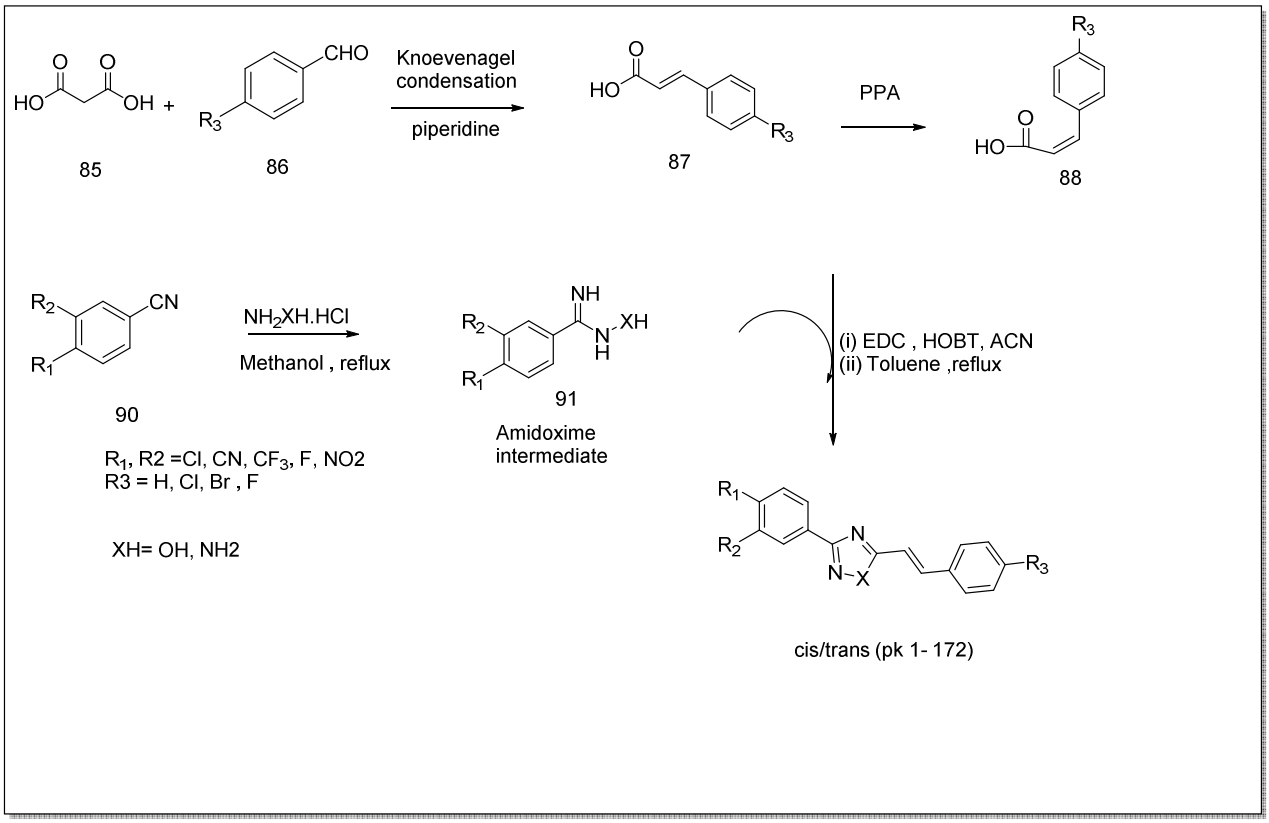
Figure 21. Proposed oxadiazole/triazole ligands

Protein preparation: Protein structures will be downloaded from protein data bank and prepared prior to docking in order to add hydrogen atoms, optimize hydrogen bonds, remove atomic clashes, and perform other operations by selecting the protein chain, heteroatoms, ligands and waters present in pdb file.

Docking study: Setup the docking parameters and start docking calculations by selecting protein and ligand from library and by analyzing the interactions between protein and ligand.

Analysis of docking result: Analysis of results will be carried out by comparison of docking results and ranking them with their docking score as calculated by binding affinity toward the androgen receptor (with RMSD 0-1).

Synthesis of synthetically feasible most potent compounds: Synthesis of most potent and synthetically feasible compound will be carried out as shown in the scheme depicted below:



Scheme 1. (*E/Z*)-3-(3,4-substituted phenyl)-5-(4-substituted styryl)-1,2,4-oxadiazole/triazole

Scheme 1 includes synthesis of proposed derivatives. Here Knoevenagel reaction employed for the synthesis of various *trans*-α,β-unsaturated aryl carboxylic acids by reacting aldehyde and malonic acid. Trans isomer will be converted by heating it in polyphosphonic acid (PPA) medium. Further substituted aryl amidoxime/hydrazide will be prepared by using condensation reaction of aryl nitrile with hydroxylamine or hydrazine hydrochloride. The synthesized amidoxime/hydrazide and unsaturated acid will be cyclized to afford the 1,2,4,-oxadiazole/triazole derivative as the desired compounds.

CHAPTER 6: RESULTS AND DISCUSSION

6.1 **Molecular modeling** : The X-ray crystal structure of *R*-Bicalutamide in WL AR LBD complex shows bent conformation for Bicalutamide, due to hydrogen bonding interactions. Therefore, it generated an idea to synthesize conformationally restricted model with similar structural properties to investigate the anti-prostate activity. With our current interests on heterocyclic scaffolds and anti-prostate cancer agents, we designed conformationally restricted oxadiazole (Series 1) and triazole (Series 2) derivatives with bioisosteric replacement with: Cl, CN, CF_3, F, NO_2, Br. The designed compounds were drawn in 3D structure by using ChemDraw and geometry was minimized by using molecular mechanics method. The all geometry minimized structure were then converted or transformed into readable .pdb format by using Autodock-vina software (ADT).

Molecular docking[62]: To determine most potentially active ligands towards AR modulator by using Autodock-vina. All designed molecules were prepared for docking purpose as discussed below:

6.1.1. Preparation of Protein (1z95)[63] : From the File menu, choose Read Molecule, highlight the PDB file for your protein, and click Open or, right click on Python Molecular Viewer (PMV) Molecules at the bottom of the window and choose the protein pdb file. Also fix any problems with the PDB files, such as missing bonds or atoms, and remove extraneous structures such as water molecules. Before beginning this section, inspect the PDB file to learn what such structures may be present. We want to keep only the protein and such cofactors as may be bound to it naturally. And then save it as pdbqt file (figure 22).

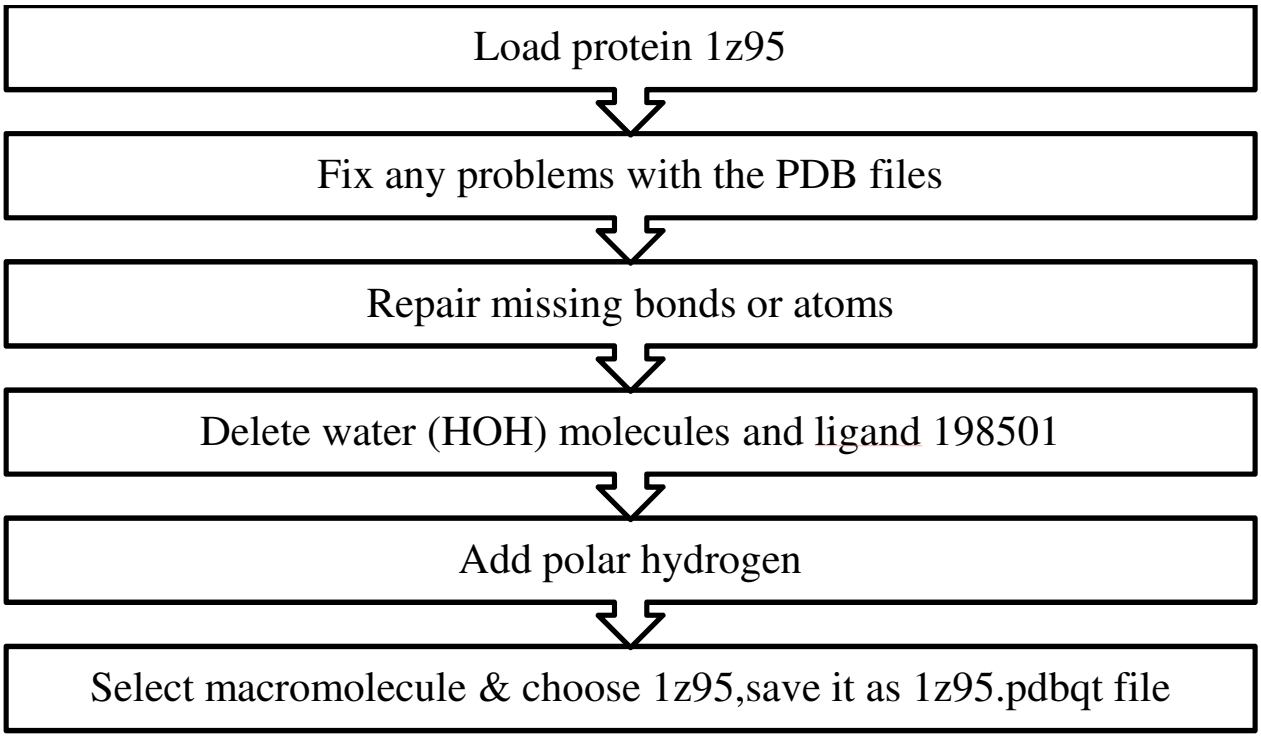


Figure 22: Schematic flowchart for protein preparation

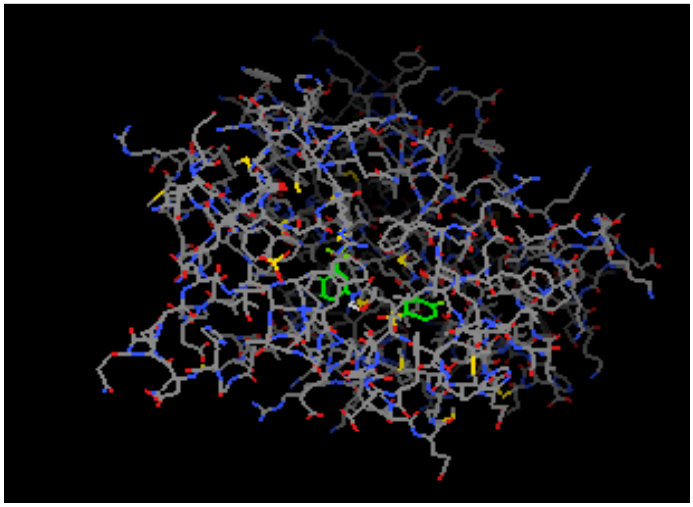


Figure 23. Visualization of protein

6.1.2 Preparation of Ligand : Go to ligand on menu bar, then click on input molecule and then open the ligand and select pdb files. Then choose the file containing the ligand, and click Open. Then message will be pop up on screen as:

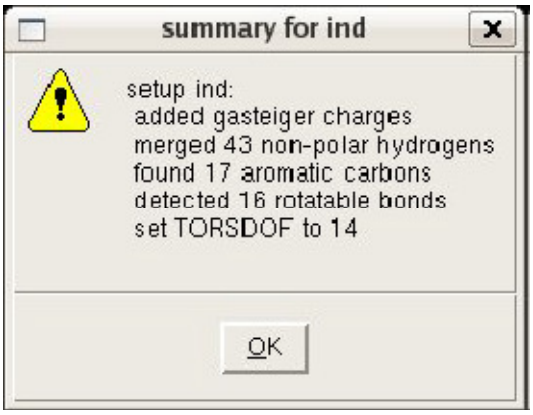


Figure 24. Pop up message while loading the protein

On the menu bar, select Ligand -> Torsion Tree -> Detect Root. A small dot will appear, marking the choice.

Next, select Ligand -> Torsion Tree -> Choose Torsions. The Torsion Count widget appears.

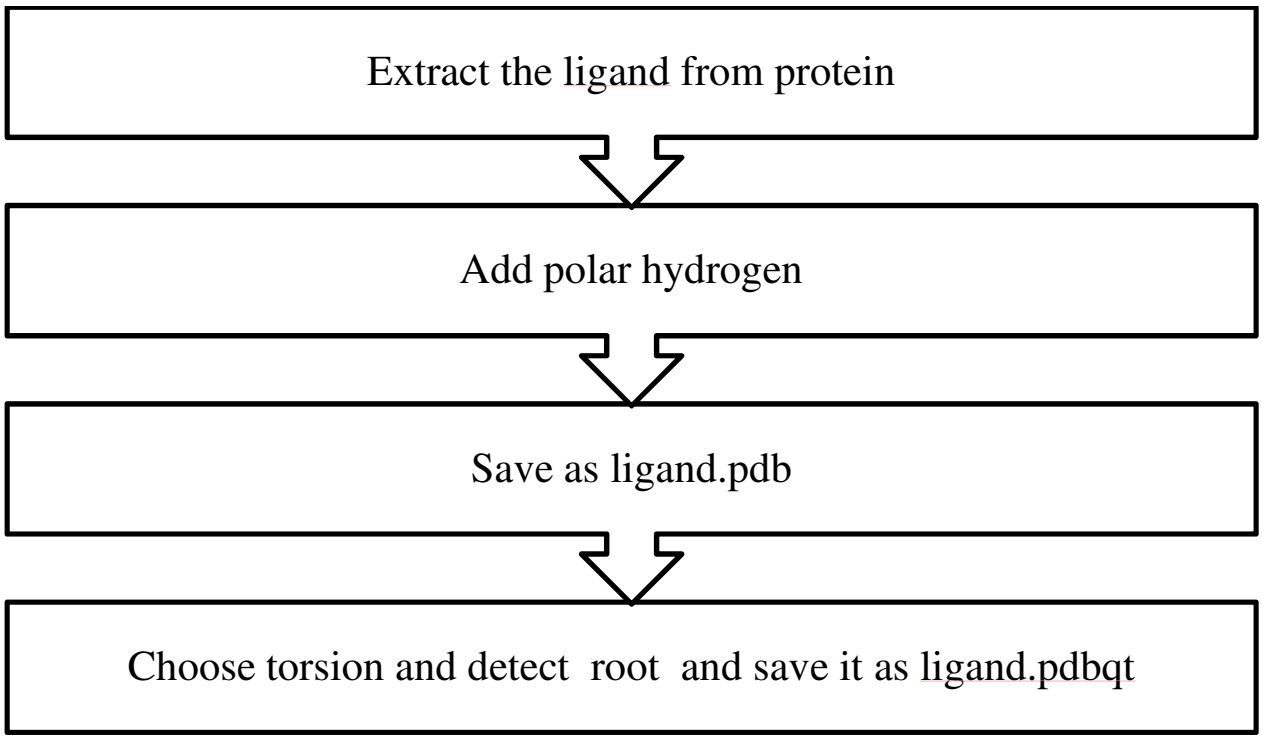


Figure 25: Schematic flowchart for ligand preperation

After manipulating torsions, press done. Display now looks like as shown below:

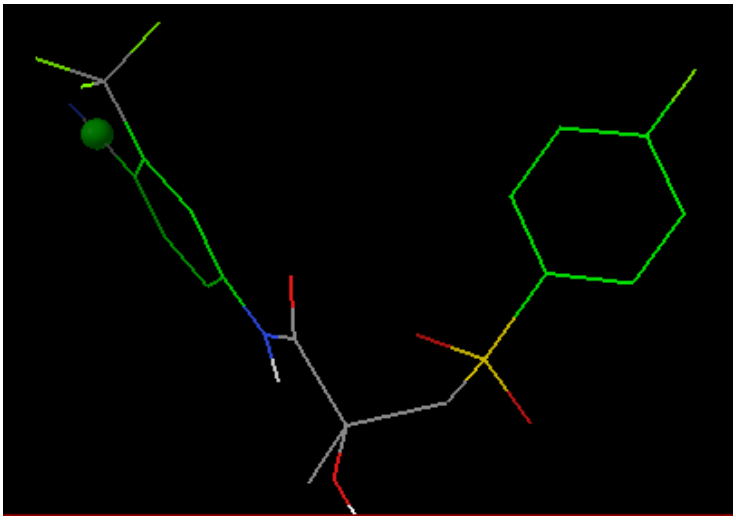


Figure 26. Detection of root on the ligand

6.1.3 **Docking and Validation of Protein:** Load ligand.pdbqt file and set it as map type by choosing ligand. After this centralize ligand by setting grid box and then save it by close saving current. Then save the protein as pdbqt file and then prepare configuration file and save it as conf.txt . then analyze the docking results in command prompt as shown below:

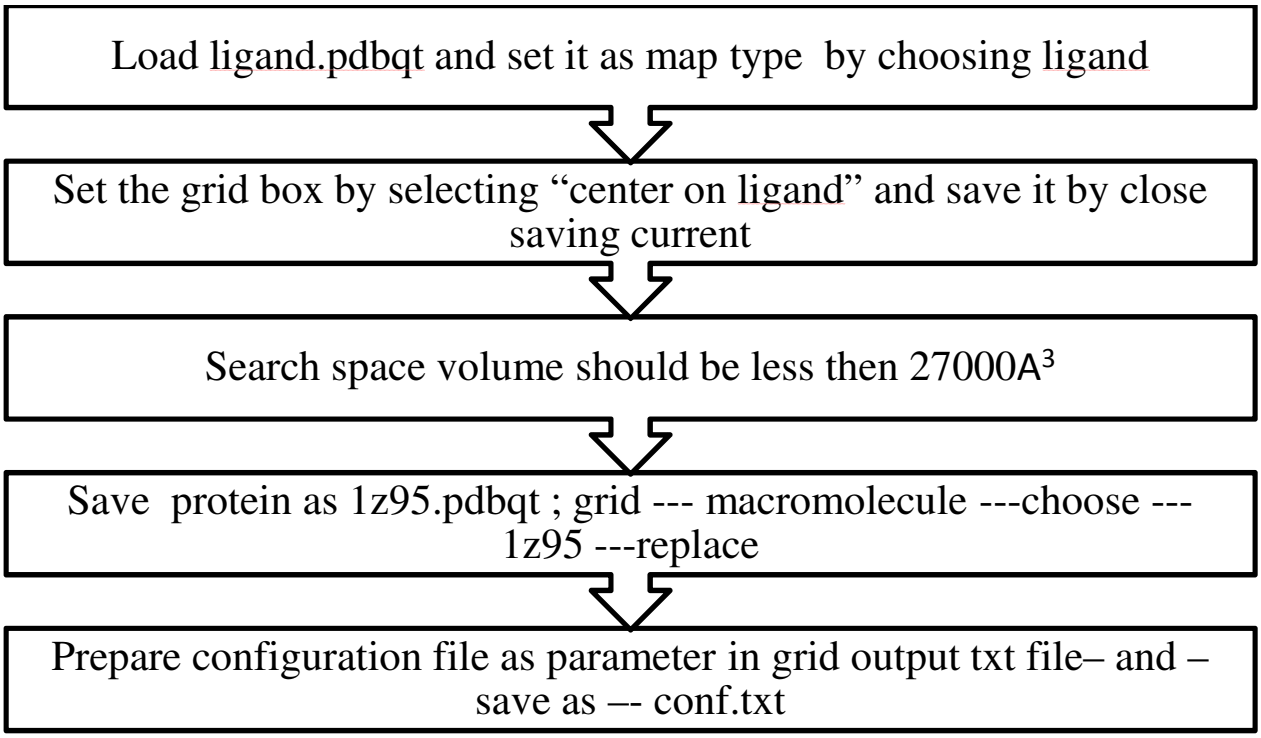


Figure 27: Schematic flow chart for preparation of grid for docking

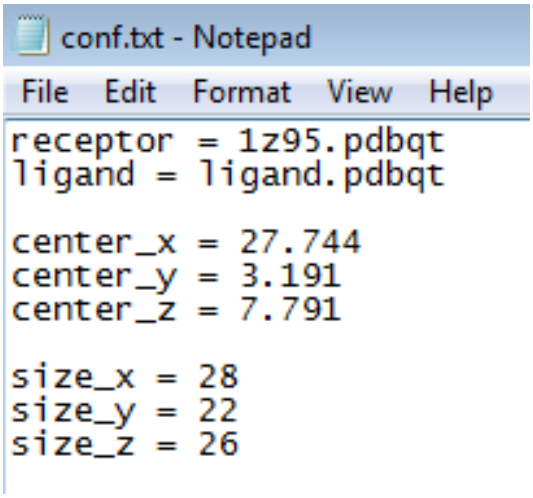


Figure 28. Preparation of configuration file for docking

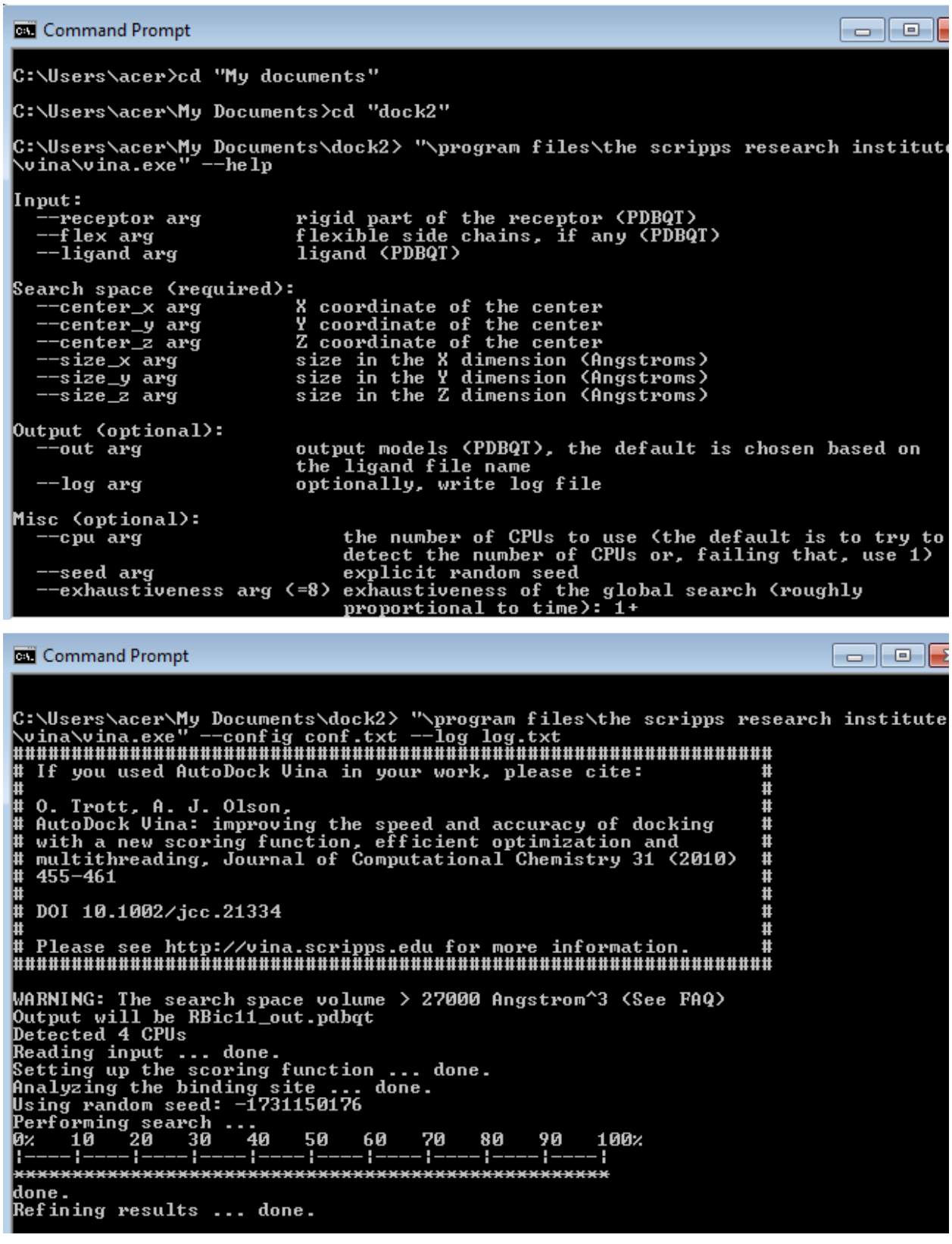


Figure 29. Docking via command prompt at vina interface

6.1.4 Overlay of docked ligand with crystallographic structure of *R*-bicalutamide

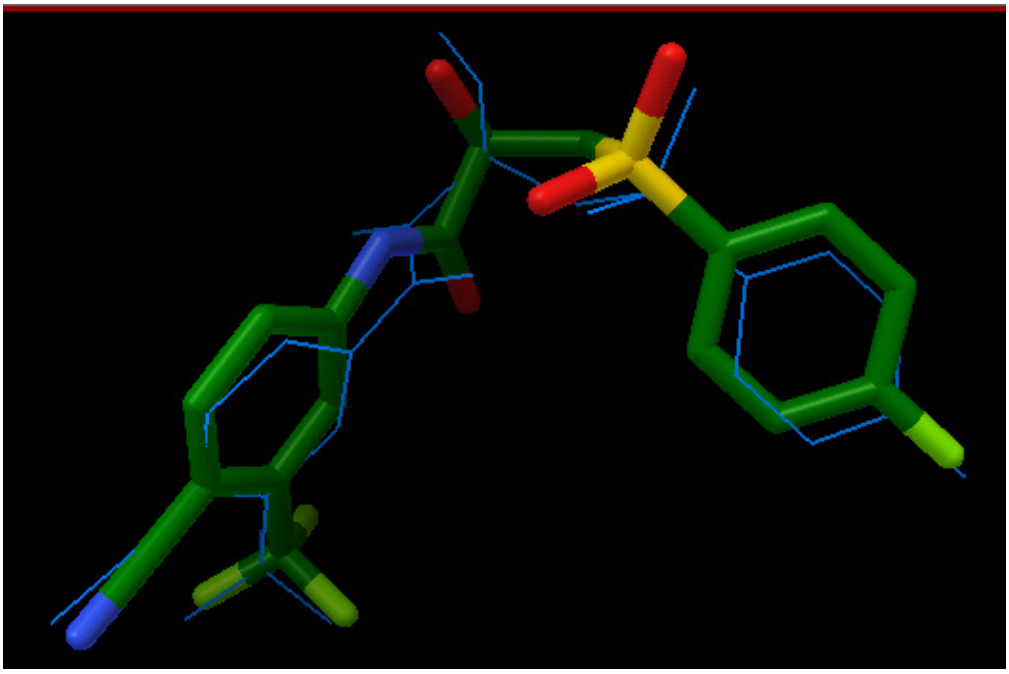


Figure 30. Ball & stick model: Crystal structure (Atom type colour)
Line model: Docked ligand (Blue colour)

6.1.5 Interactions of (*R*)-bicalutamide with the W741L mutant AR

A- ring

-CN forms H bonds with Q711, M745 & R752

-CF_3 situated in hydrophobic environment

B-ring

-The ligand adopts a greatly bent conformation

sterically disrupting the formation of AF2 region

Chiral hydroxy group

-OH forms H bonds with L704 & N705

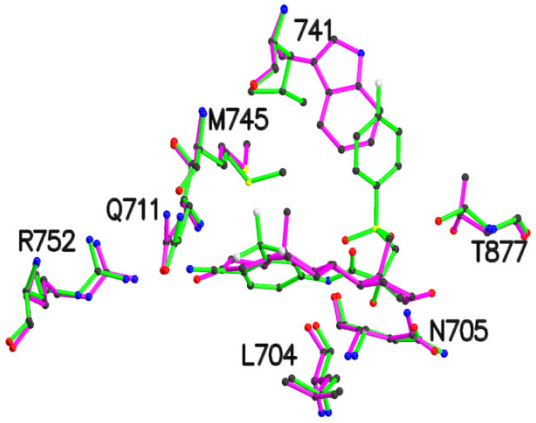
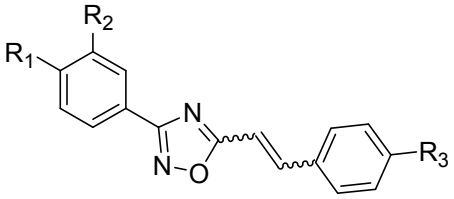


Figure 31. Interactions of (*R*)-bicalutamide with the W741L mutant AR

6.1.6 **Docking of various designed ligands :** 160 designed molecules from series '1' and '2' were docked and their results are given as such in table 13 and 14

Table 13 : Designed ligands of oxadiazole derivatives. *NA - not determined

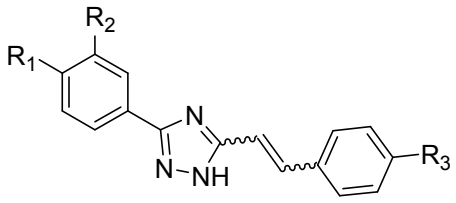


Series 1: Oxadiazole derivative								
S.No.	Code		R_1	R_2	X	R_3	Affinity score	
	Cis	*Trans*					*cis*	*Trans*
1	PK_1	PK_{81}	H	H	O	H	-9	-8
2	PK_2	PK_{82}	H	H	O	F	-9.3	-8.1
3	PK_3	PK_{83}	H	H	O	Cl	-9.4	-6.8
4	PK_4	PK_{84}	H	H	O	Br	NA*	NA*
5	PK_9	PK_{89}	F	Cl	O	H	-9.8	-8.5
6	PK_{10}	PK_{90}	F	Cl	O	F	-10.1	-7.9

7	PK_{11}	PK_{91}	F	Cl	O	Cl	-10.3	-6.9
8	PK_{12}	PK_{92}	F	Cl	O	Br	NA*	NA*
9	PK_{17}	PK_{101}	Cl	F	O	H	-9.6	-8
10	PK_{18}	PK_{102}	Cl	F	O	F	-10.1	-7.1
11	PK_{19}	PK_{103}	Cl	F	O	Cl	-10	-6.4
12	PK_{20}	PK_{104}	Cl	F	O	Br	NA*	NA*
13	PK_{25}	PK_{105}	Cl	CN	O	H	-10.5	-8.6
14	PK_{26}	PK_{106}	Cl	CN	O	F	-10.9	-7.8
15	PK_{27}	PK_{107}	Cl	CN	O	Cl	NA*	-7
16	PK_{28}	PK_{108}	Cl	CN	O	Br	NA*	NA*
17	PK_{33}	PK_{113}	Cl	CF_3	O	H	-10.4	NA*
18	PK_{34}	PK_{114}	Cl	CF_3	O	F	-11.1	-7.8
19	PK_{35}	PK_{115}	Cl	CF_3	O	Cl	-10.9	-7.3
20	PK_{36}	PK_{116}	Cl	CF_3	O	Br	NA*	-8.3
21	PK_{41}	PK_{121}	CN	CF_3	O	H	-10.6	-8.6
22	PK_{42}	PK_{122}	CN	CF_3	O	F	-11.6	-8.6
23	PK_{43}	PK_{123}	CN	CF_3	O	Cl	-11.5	-7.6
24	PK_{44}	PK_{124}	CN	CF_3	O	Br	NA*	NA*
25	PK_{49}	PK_{129}	NO_2	CF_3	O	H	-10.5	-8.2
26	PK_{50}	PK_{130}	NO_2	CF_3	O	F	-11.1	-7
27	PK_{51}	PK_{131}	NO_2	CF_3	O	Cl	-10.7	-6.1
28	PK_{52}	PK_{132}	NO_2	CF_3	O	Br	NA*	NA*
29	PK_{57}	PK_{137}	NO_2	F	O	H	-9.8	-7.6
30	PK_{58}	PK_{138}	NO_2	F	O	F	-10.3	-6.7
31	PK_{59}	PK_{139}	NO_2	F	O	Cl	-10.2	-5.3

32	PK_{60}	PK_{140}	NO_2	F	O	Br	NA*	NA*
33	PK_{65}	PK_{145}	CF_3	F	O	H	-10.2	NA*
34	PK_{66}	PK_{146}	CF_3	F	O	F	-10.4	-6.4
35	PK_{67}	PK_{147}	CF_3	F	O	Cl	-10.4	-5.9
36	PK_{68}	PK_{148}	CF_3	F	O	Br	NA*	NA*
37	PK_{73}	PK_{153}	CH_3O	CH_3O	O	H	-9.1	-7
38	PK_{74}	PK_{154}	CH_3O	CH_3O	O	F	-9.5	-6.7
39	PK_{75}	PK_{155}	CH_3O	CH_3O	O	Cl	-9.5	-6.2
40	PK_{76}	PK_{156}	CH_3O	CH_3O	O	Br	NA*	NA*
41			*R*-Bicalutamide				-10.9	

Table 14: Designed ligands of triazole derivatives. *NA - not determined



Series 2: Triazole derivatives								
S.No.	Code		R_1	R_2	X	R_3	Affinity score	
	Cis	*Trans*					*cis*	*Trans*
1	PK_5	PK_{85}	H	H	NH	H	-9	-7.9
2	PK_6	PK_{86}	H	H	NH	F	-9.3	-8.1
3	PK_7	PK_{87}	H	H	NH	Cl	-9.1	-6.8
4	PK_8	PK_{88}	H	H	NH	Br	NA*	NA*

5	PK_{13}	PK_{93}	F	Cl	NH	H	-9.8	-8.4
6	PK_{14}	PK_{94}	F	Cl	NH	F	-10.3	-7.8
7	PK_{15}	PK_{95}	F	Cl	NH	Cl	-10.3	-6.7
8	PK_{16}	PK_{96}	F	Cl	NH	Br	NA*	NA*
9	PK_{21}	PK_{97}	Cl	F	NH	H	-9.8	-6
10	PK_{22}	PK_{98}	Cl	F	NH	F	NA*	-7.8
11	PK_{23}	PK_{99}	Cl	F	NH	Cl	-10.2	-6.8
12	PK_{24}	PK_{100}	Cl	F	NH	Br	-10.1	NA*
13	PK_{29}	PK_{109}	Cl	CN	NH	H	-10.2	-8.5
14	PK_{30}	PK_{110}	Cl	CN	NH	F	-10.8	-7.5
15	PK_{31}	PK_{111}	Cl	CN	NH	Cl	-10.7	-6.5
16	PK_{32}	PK_{112}	Cl	CN	NH	Br	NA*	NA*
17	PK_{37}	PK_{117}	Cl	CF_3	NH	H	-10.8	-8.3
18	PK_{38}	PK_{118}	Cl	CF_3	NH	F	-11.1	-7.4
19	PK_{39}	PK_{119}	Cl	CF_3	NH	Cl	-10.6	-6.9
20	PK_{40}	PK_{120}	Cl	CF_3	NH	Br	NA*	NA*
21	PK_{45}	PK_{125}	CN	CF_3	NH	H	-11.3	-8.3
22	PK_{46}	PK_{126}	CN	CF_3	NH	F	-11.7	-7.2
23	PK_{47}	PK_{127}	CN	CF_3	NH	Cl	-11.6	-6.5
24	PK_{48}	PK_{128}	CN	CF_3	NH	Br	NA*	NA*

25	PK_{53}	PK_{133}	NO_2	CF_3	NH	H	-10.6	-8
26	PK_{54}	PK_{134}	NO_2	CF_3	NH	F	-10.9	-6.7
27	PK_{55}	PK_{135}	NO_2	CF_3	NH	Cl	-11	-6.2
28	PK_{56}	PK_{136}	NO_2	CF_3	NH	Br	NA*	NA*
29	PK_{61}	PK_{141}	NO_2	CF_3	NH	H	NA*	-7.5
30	PK_{62}	PK_{142}	NO_2	CF_3	NH	F	-10.3	-6.4
31	PK_{63}	PK_{143}	NO_2	CF_3	NH	Cl	-10.1	-5.9
32	PK_{64}	PK_{144}	NO_2	CF_3	NH	Br	NA*	NA*
33	PK_{69}	PK_{149}	CF_3	F	NH	H	-10.2	-7.5
34	PK_{70}	PK_{150}	CF_3	F	NH	F	-10.8	-6.3
35	PK_{71}	PK_{151}	CF_3	F	NH	Cl	-10.5	-5.9
36	PK_{72}	PK_{152}	CF_3	F	NH	Br	NA*	NA*
37	PK_{77}	PK_{157}	CH_3O	CH_3O	NH	H	-9.1	-6.8
38	PK_{78}	PK_{158}	CH_3O	CH_3O	NH	F	-9.4	-6.4
39	PK_{79}	PK_{159}	CH_3O	CH_3O	NH	Cl	-9.4	-5.9
40	PK_{80}	PK_{160}	CH_3O	CH_3O	NH	Br	NA*	NA*
41			*R*-Bicalutamide				-10.9	

Most potentially active compounds on the basis of their affinity score were identified. The affect of geometrical isomers: *cis* and *trans* was studied and *cis* geometrical isomers were found to be

more potent as compared to trans isomers. Geometry has profound influence on binding affinity as observed that *cis* isomers (adopt perfect bent conformation) are found better than their corresponding *trans* isomers as shown below in figure 32 and 33. The close contacts have shown for the most potent compounds or ligands in figure 32 and 33. In figure 34 and 35 the ribbon structure of protein depicting the docked conformation of ligand onto the active site of protein.

Ring A- CN forms H bonds with Q711, M745 & R752 whereas CF_3 situated in hydrophobic region

Ring B– adopt a bent conformation and disrupt the AF2 region

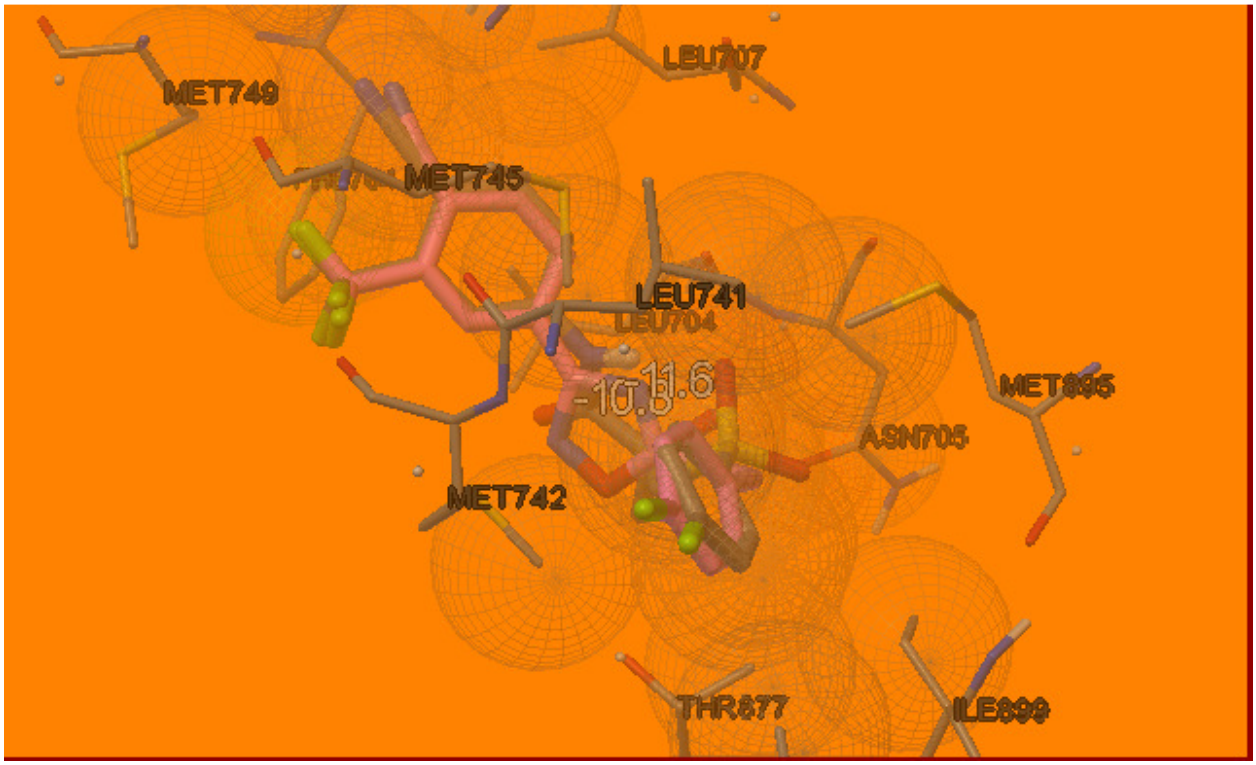


Figure 32: Overlay of close contacts of isomers: (pk42) with neighboring amino acid residues.

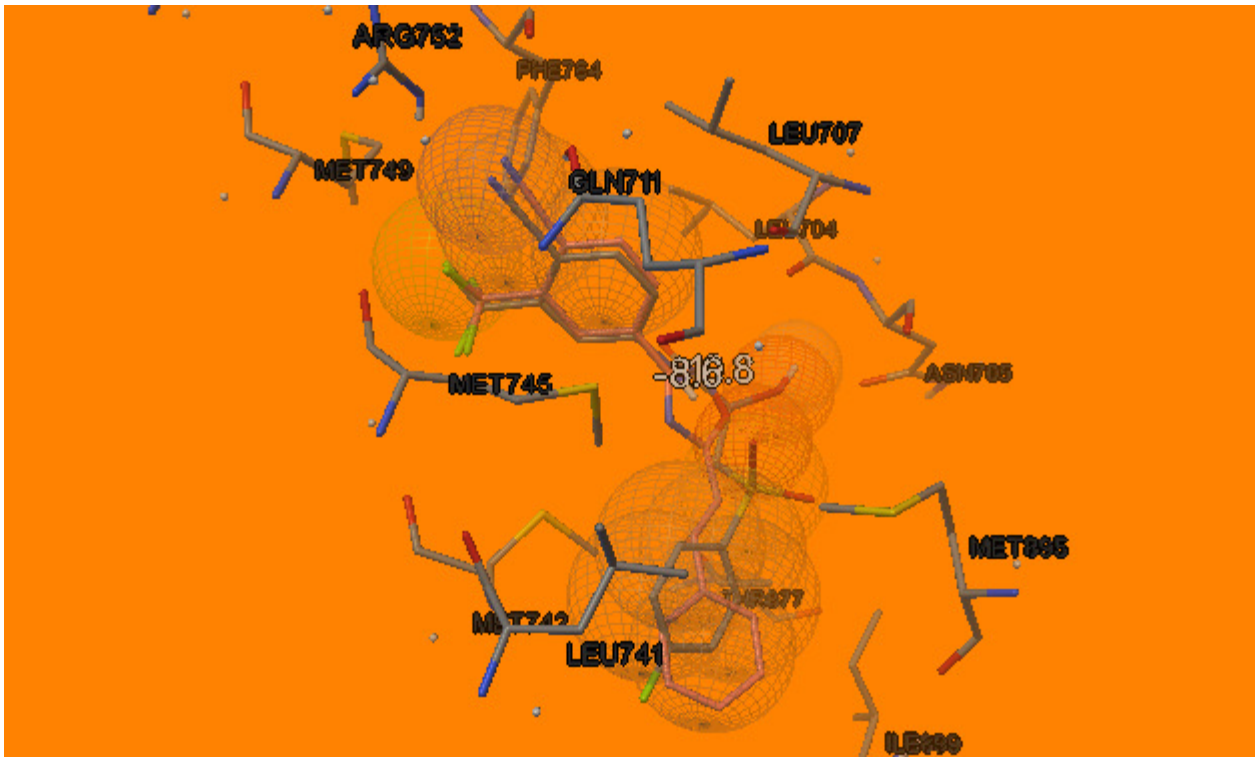


Figure 33: Overlay of close contacts of isomers: (pk122) with neighboring amino acid residues.

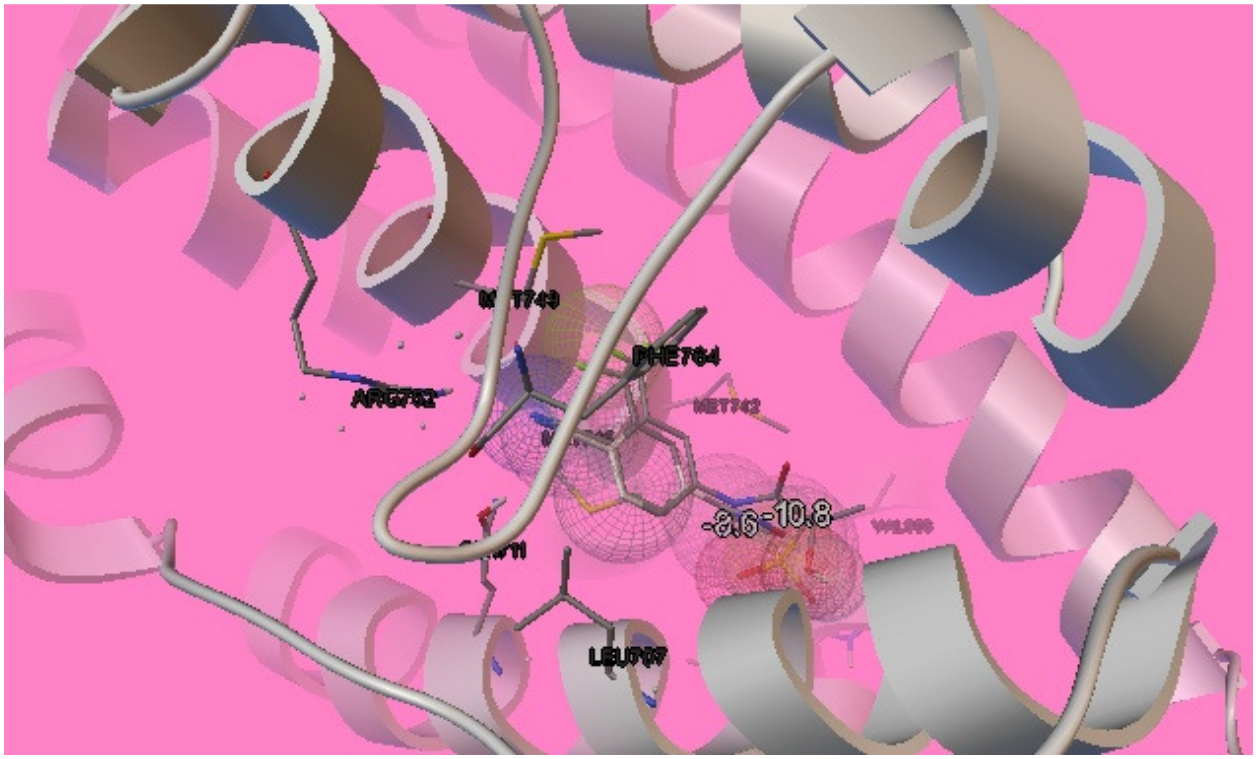


Figure 34. Visualization of active binding sites of protein with bound ligand pk122.

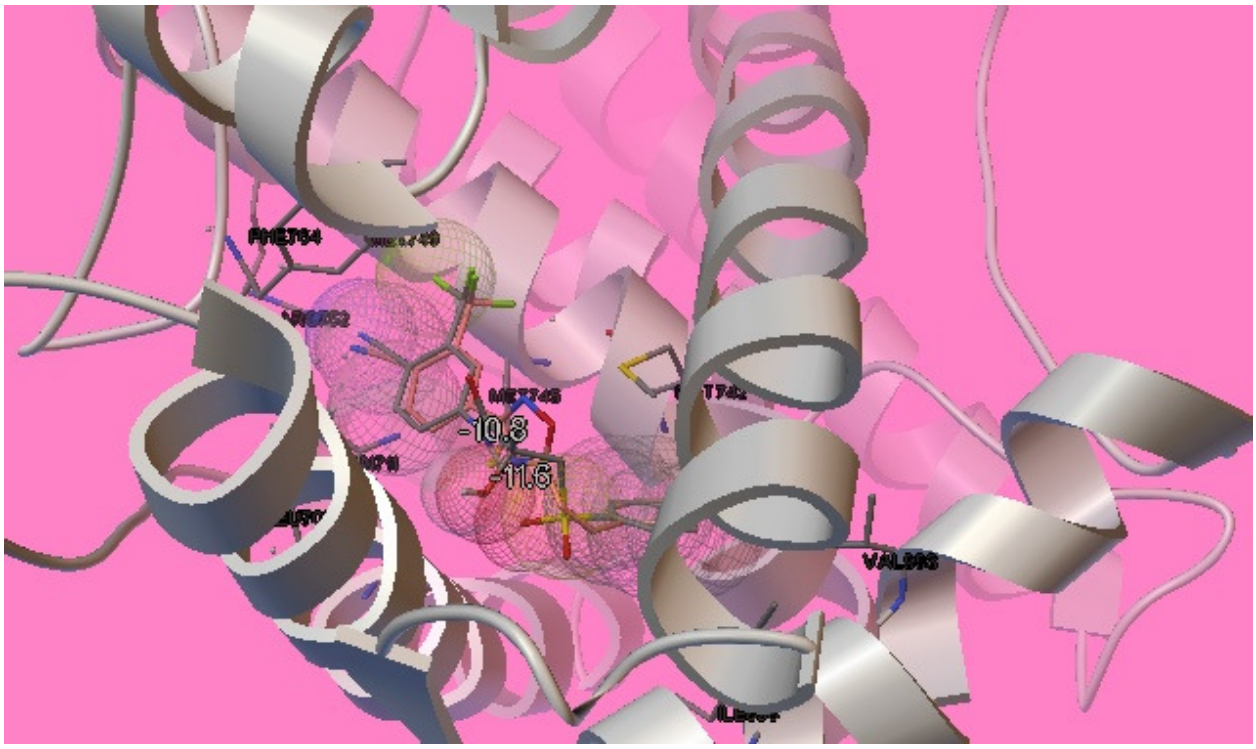


Figure 35. Visualization of active binding sites of protein with bound ligand pk42.

Taking in account of availability , cost and feasibility of reaction, some of the oxadiazole were chosen to synthesize and gain in-sight of their anti-androgen activity or efficacy (table 15). Here it is needless to mention that although the activity was shown by cis isomers are better then trans but cis intermediate is unstable and difficult to synthesize . so only trans isomers were chosen for synthetic purpose. Here we also chosen triazole to just get to know the activity for comparison although their binding affinity is lower than corresponding oxadiazole (table 15).

Table 15:Binding affinities of potent and feasible compounds:

S.no	Code	R_1	R_2	X	R_3	Affinity score
1	Pk161	NO_2	H	O	Cl	-9.6
2	Pk89	F	Cl	O	H	-8.5
4	Pk169	Cl	H	O	H	-8.3
5	Pk81	H	H	O	H	-8.0
6	Pk163	Cl	H	O	H	-8.0

7	Pk90	F	Cl	O	Cl	-7.9
9	Pk167	Cl	H	O	Cl	-7.6
10	Pk170	NO_2	H	O	F	-6.3
10	Pk170	Cl	H	NH	Cl	-8.5
12	Pk93	F	Cl	NH	F	-7.8

The results obtained from molecular docking of designed analogues indicate the high binding affinity of the compounds towards the androgen receptor. To gain more insights of the binding site, an in-depth analysis was carried out, for chosen molecule pk161 (figure36-38). Ribbon structure of protein depicted with the docked conformation of ligand onto the active site of protein.

Ring A: NO_2 forms hydrogen bond with Gln711, Arg757, Phe764, Met745 whereas Cl situated in hydrophobic region.

Ring B: adopts bent conformation and disrupts AF_2 region.

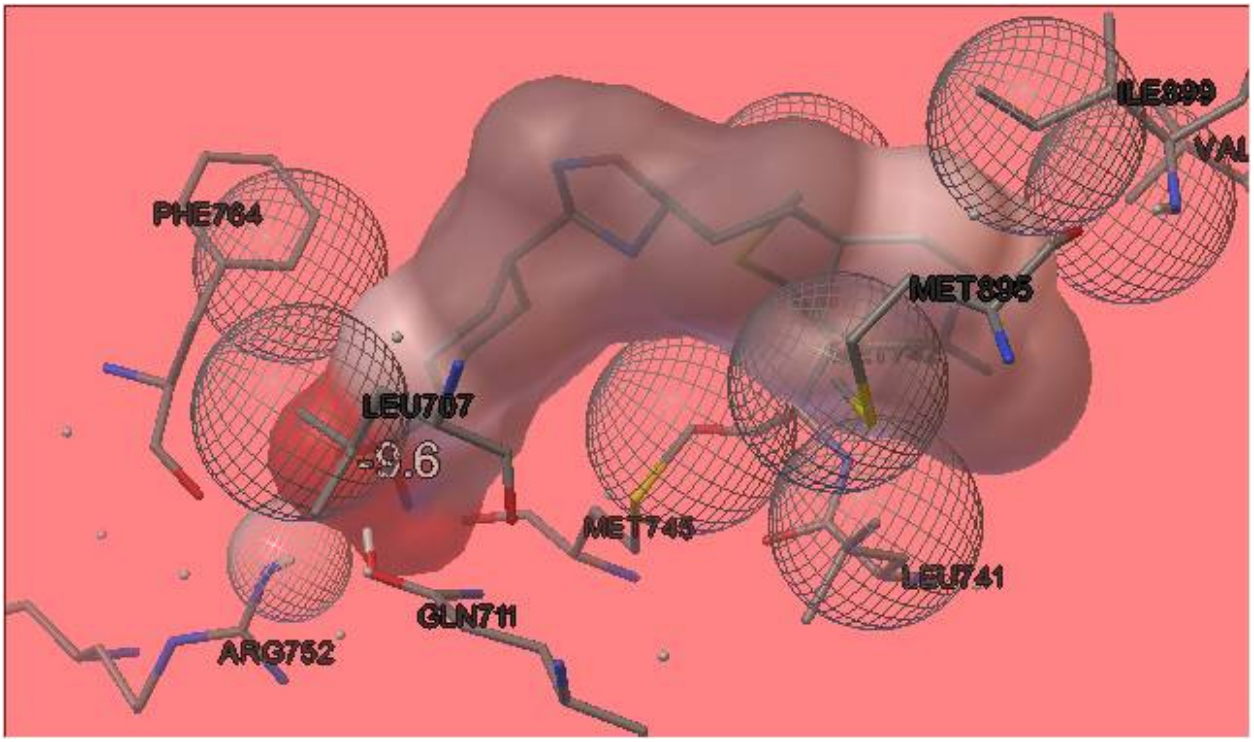


Figure 36: Overlay of close contacts of pk161 with neighboring amino acid residues

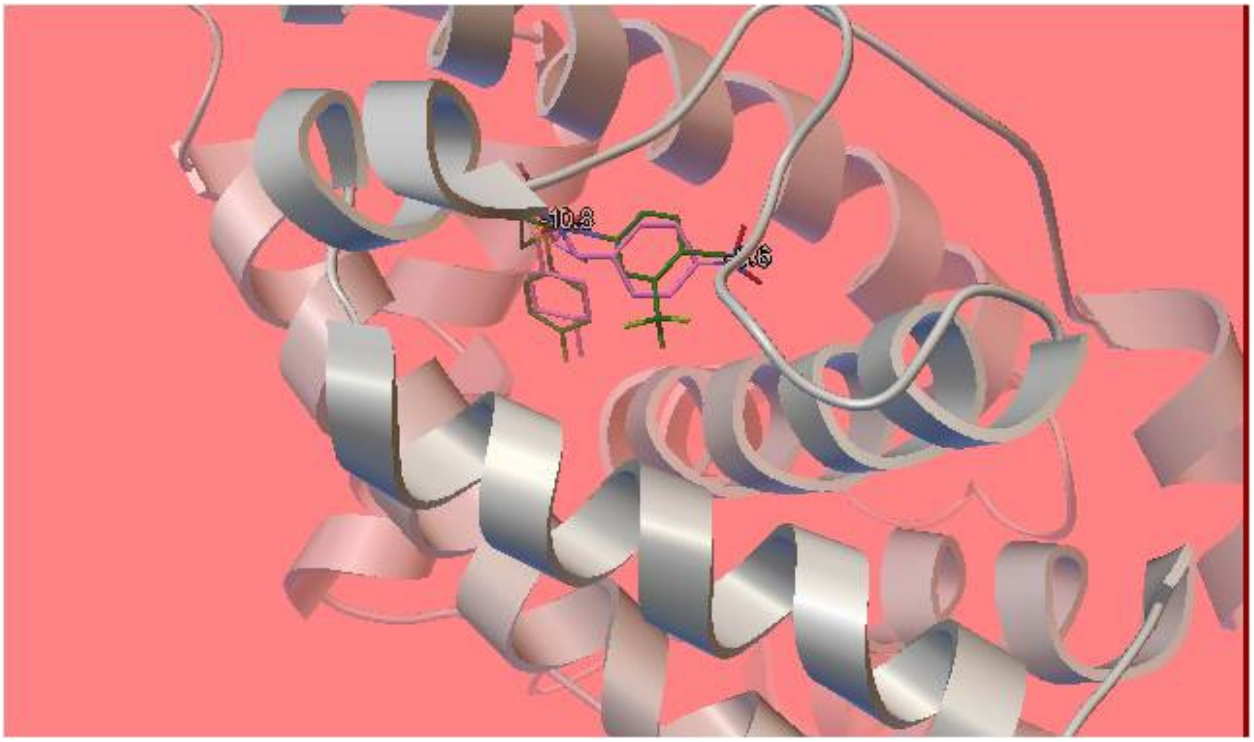


Figure 37. Visualization of active binding sites of protein with bound ligand pk161

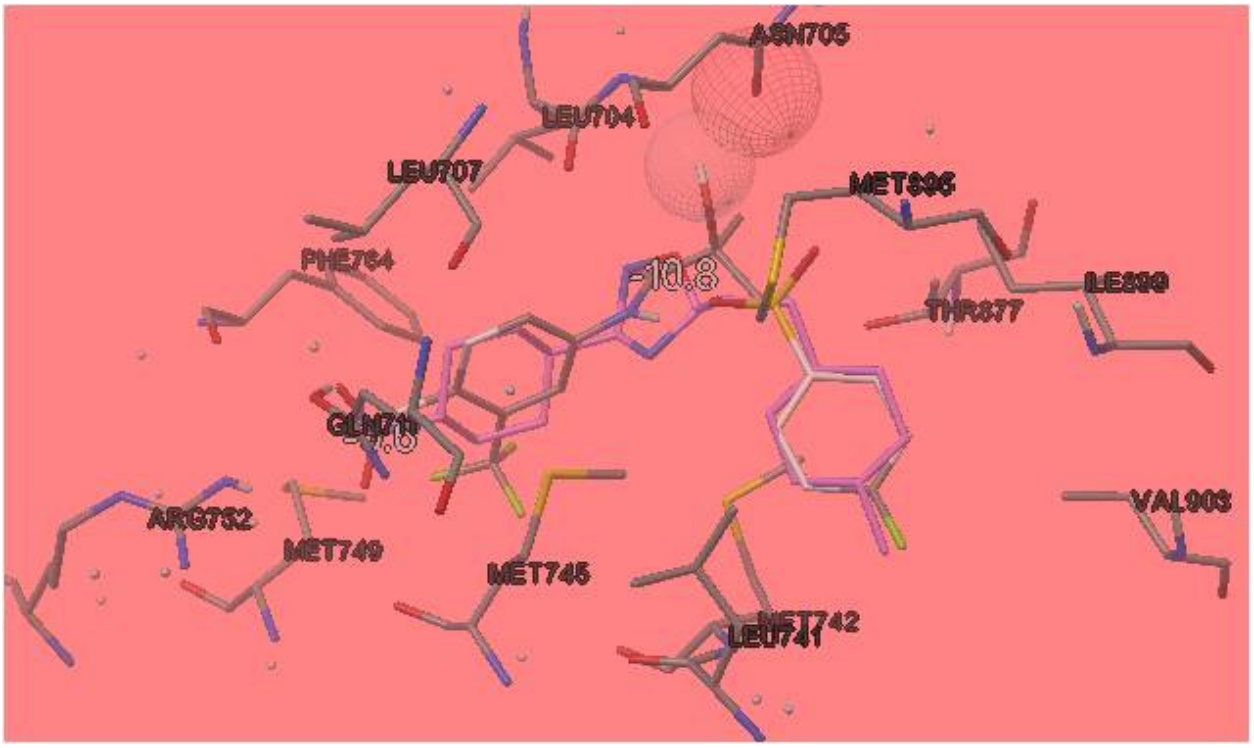
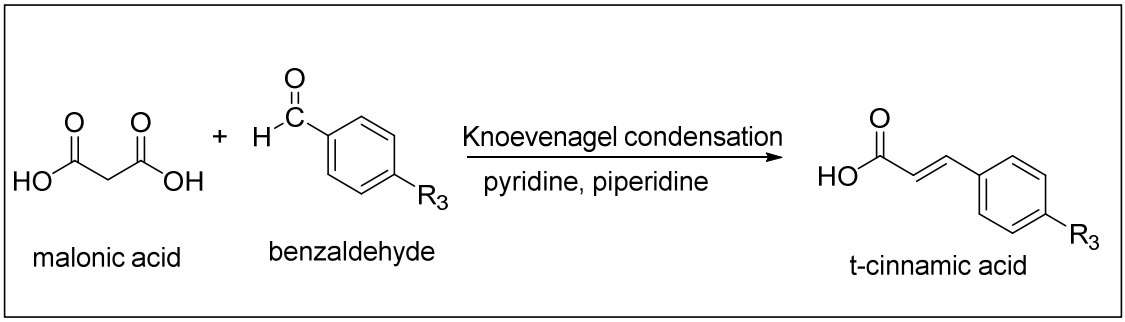


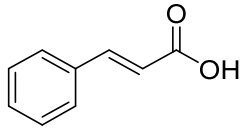
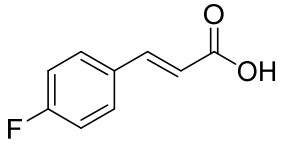
Figure 38. Overlay of *trans* isomer pk161 with *R*-Bicalutamide at AR LBD.

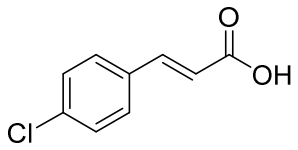
6.2 **CHEMISTRY AND SYNTHESIS:** Synthetic routes to obtain to obtain the designed compounds and their intermediates are described as follow:

6.2.1 Preparation of trans-cinnamic acid: Trans-cinnamic acid and their derivatives were prepared by dissolving malonic acid in pyridine in RBF fitted with reflux condenser. Then added benzaldehyde and piperidine to the mixture. The mixture was heated at 100ºC under reflux (effervescence of CO_2 produced). The solution was then cooled down by ice bath. Then added 4mL conc. HCl slowly into the solution. Crude product was then obtained by vacuum filtration. (the crude product can be washed by ethanol). Thereafter the crude cinnamic acid was recrystallized fron ethanol/water (3:1). All derivatives of cinnamic acid were obtained in good yield and characterized by IR (Table 16).

Table 16: preparation of cinnamic acid derivatives



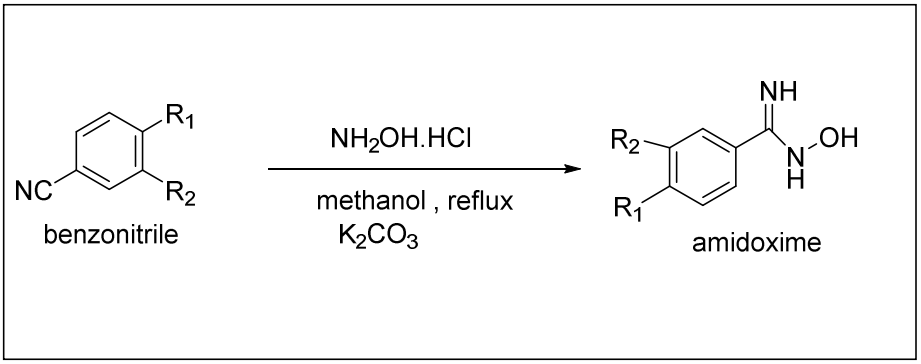
Entry	R^3	Compound	Yield(%)	M.P (˚C)	Rf*
1.	H		59	128	0.51
2.	F		82	204	0.25

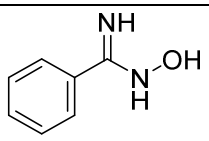
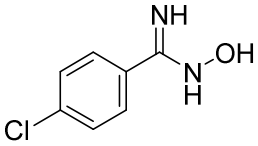
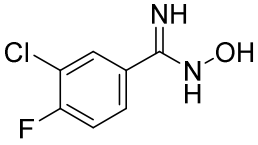
3.	Cl		93	243	0.27

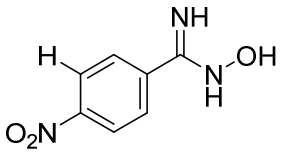
*Rf = retention factor, eluent: EtoAc : hexane (1:1)

6.2.2 Preparation of N-hydroxy benzimidamide (amidoxime) : Amidoxime and their derivatives were synthesized by adding benzonitrile and potassium carbonate in methanol. Reaction mixture was cooled and hydroxylamine hydrochloride was added portion wise. After completion of the addition it was refluxed for 6hr. The solvent evaporated and excess water was added to afford the precipitate of the desired product which was filtered and dried. All amidoximes were obtained in good yield and characterized by IR. (Table 17).

Table 17: synthesis of amidoxime derivatives



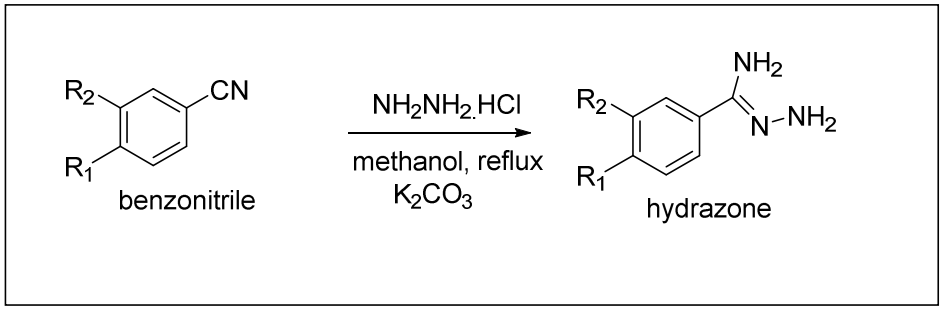
Entry	R_1	R_2	Compound	Yield(%)	M.P(ºC)	Rf*
1.	H	H		59	89-93	0.86
2.	Cl	H		66	122-124	0.21
3.	F	Cl		69	140-142	0.28

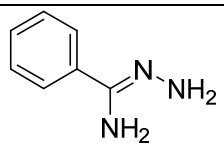
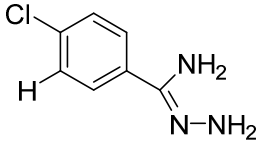
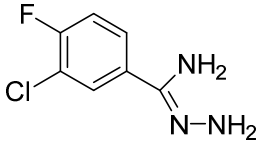
4.	NO_2	H		60	131-133	0.33

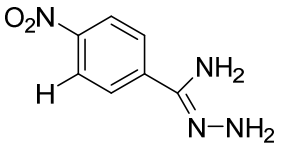
*Rf = retention factor, eluent: EtoAc : hexane (2:8)

6.2.3 Preparation of hydrazone: Hydrazone and their derivatives were synthesized by adding benzonitrile and potassium carbonate in methanol. Reaction mixture was cooled and hydrazine hydrochloride was added portion wise. After completion of the addition it was refluxed for 6hr. The solvent evaporated and excess water was added to afford the precipitate of the desired product which was filtered and dried to afford them in good yield and characterized by IR (Table 18).

Table 18: preparation of hydrazone



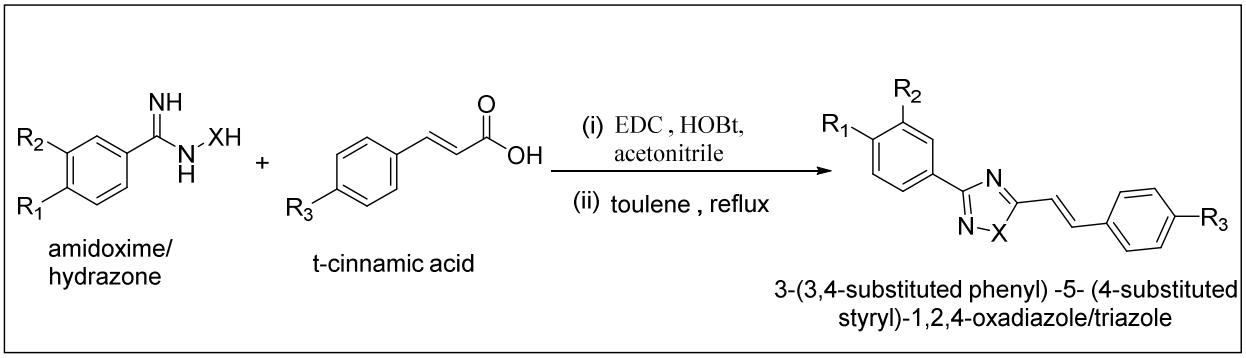
Entry	R_1	R_2	Compound	Yield (%)	M.P(ºC)	Rf*
1	H	H		75	98	0.63
2	Cl	H		61	120	0.34
3	F	Cl		66	135	0.39

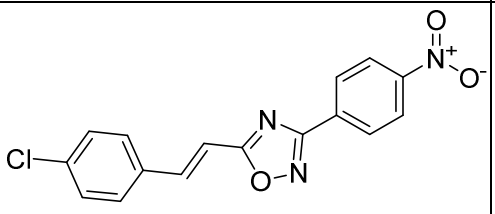
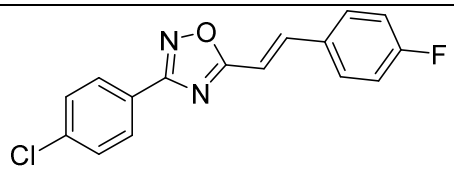
4	NO_2	H		62	141	0.21

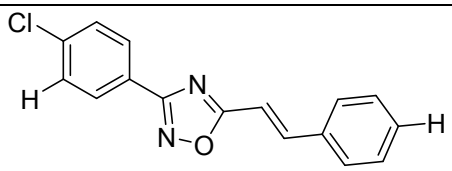
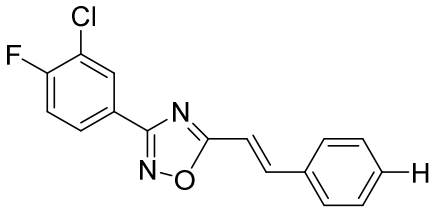
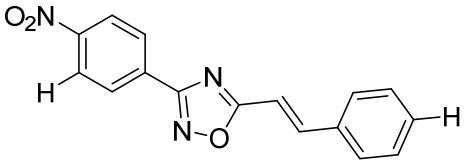
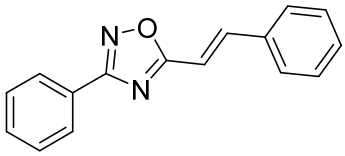
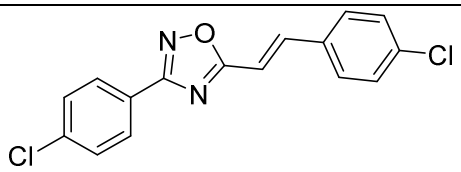
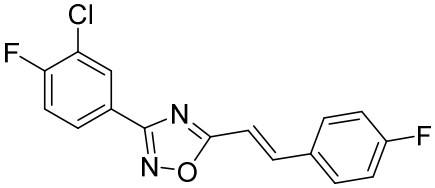
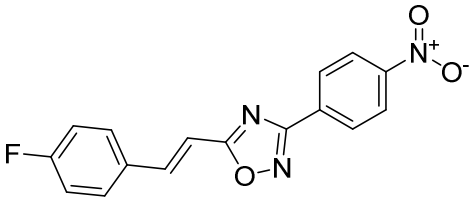
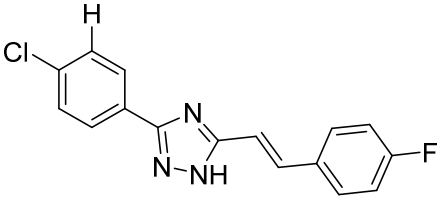
*Rf = retention factor, eluent: EtoAc : hexane (2:8)

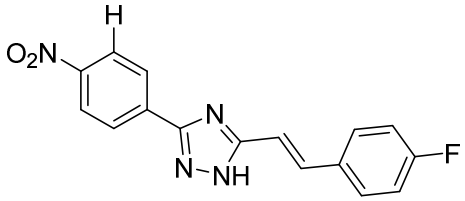
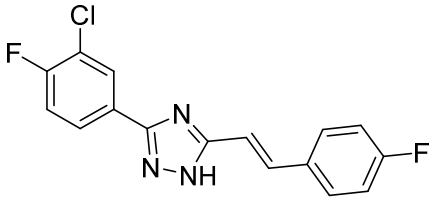
6.2.4 Preparation of 1,2,4,-oxadiazole/triazole derivative: 1,2,4,-oxadiazole/triazole were prepared by mixing cinnamic acid with cyclizing agents EDC and HOBT in acetonitrile. Therefore crude intermediate was refluxed in toluene for 2hr which afforded crude oxadiazole. The crude was purified by crystallization or column chromatography and characterized by IR, NMR and MS. (Table 19)

Table 19: preparation of oxadiazole/ triazole



Entry	code	R1	R2	R3	Compound	Yield	M.P (ºC)	RF*
1.	pk161	NO_2	H	Cl		80	180	0.36
2.	pk168	Cl	H	F		56	172	0.21

3	Pk169	Cl	H	H		82	163	0.24
4.	pk89	F	Cl	H		84	NA	0.21
5.	pk163	NO_2	H	H		78	172	0.32
6.	pk81	H	H	H		67	NA	0.22
7.	pk167	Cl	H	Cl		68	183	0.50
8.	pk90	F	Cl	F		83	NA	0.66
9.	pk162	NO_2	H	F		79	192	0.61
10.	pk170	Cl	H	F		84	168	0.66

11.	pk165	NO_2	H	F		73	180	0.43
12.	pk93	F	Cl	F		89	183	0.21

*Rf = Retention factor, eluent : EtOAc:hexane (2:8), NA= not determined

6.3 Biological Evaluation : The all synthesized compounds were identified by spectroscopy and will be submitted to the laboratory of Deptartement of Pharmacology and Toxicology, NIPER , SAS Nagar for the cytotoxicity assay on LNCap and DU145 cell lines.

CHAPTER 7: EXPERIMENTAL WORK

Table 20: LIST OF CHEMICALS

S.No.	Chemical name	Company name
1	Acetonitrile	Qualikem
2	Benzaldehyde	Qualigens
3	Benzonitrile	Loba chemicals
4	Cinnamic acid	CDH
5	Chlorobenzaldehyde	Qualigens
6	Chlorobenzonitrile	Loba chemicals
7	1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide (EDC)	Spectrochem
8	Florobenzaldehyde	Qualigens
9	Florobenzonitrile	Loba chemicals
10	Hydrochloric acid	Rankem
11	Hydroxylamine.HCl	Thomas baker
12	Hydrazine.HCl	Thomas baker
13	Malonic acid	Loba chemicals
14	Methanol	Loba chemicals
15	N-hydroxybenzotriazole	Spectrochem
16	Piperidine	CDH
17	Potassium carbonate	CDH
18	Pyridine	CDH
19	Toluene	Rankem

Table 21: LIST OF INSTRUMENTS

S.No.	Instruments	Company name
1	FT-IR spectrophotometer	Shimadzu
2	Heating mentle	Navyug scientific works
3	Hot air oven	Navyug scientific works
4	Magnetic stirrer	Remi sales
5	Mass spectrophotometer	WATERS, Q-TOF micromas
6	NMR spectrometer	Brucker Avance
7	Refrigerator	Kelvinator
8	Rotatory evaporator	Popular traders
9	U.V chamber	Popular traders

Chemical synthesis:

The ^{1}H-NMR spectra were recorded at 400 MHz on a Bruker Avance 400 (400MHz) spectrometer in $CDCl_3$ using TMS as an internal standard. The chemical shifts (δ) for ^{1}H are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; m, multiplet. The reactions were monitored by TLC (merck). Evaporation of solvents was performed under reduced pressure using rotator evaporator commercial grade reagents and solvents were used without further purification.

Synthesis of trans-cinnamic acid:

In a typical experiment, 2g of malonic acid was taken and dissolved in 2.04 ml pyridine in RBF fitted with reflux condenser. 1.7 ml benzaldehyde and 0.036 ml piperidine was added to the reaction mixture. The mixture is heated at 100ºC under reflux for 1 hour (CO_2 produced). It was then cooled down by ice bath and 4 mL conc. HCl was added slowly into the solution. Subsequently the solution was filtered by vacuum and crude product was obtained. The crude cinnamic acid was recrystallized from ethanol/water (3:1).

Cinnamic acid (table 16, entry 1): white powder solid, 59% yield, M.P = 128ºC, Rf = 0.51 (1:1 EtOAc-hexane) , IR (cm^{-1}) = 1627 (C=C); 1693 (C=O); 2827 (C-H); 3066 (O-H).

(E)-3-(4-fluoropnhhenyl)acrylic acid (table 16, entry 2): white crystal, solid , yield = 93%, M.P = 243ºC , Rf = 0.51 (1:1 EtOAc-hexane) , IR (cm^{-1}) = 1627 (C=C), 1681 (C=O), 2837 (C-H), 3037 (O-H).

(E)-3-(4-chlorophenyl)acrylic acid (table 16, entry 3): White crystal, solid, yield = 82%, M.P = 204ºC , Rf = 0.27 (1:1 EtOAc-hexane), IR(cm^{-1}) = 1626 (C=C) , 1683 (C=O) , 2852 (C-H) , 2929 (O-H).

General procedure for the synthesis of N-hydroxy benzimidamide (amidoxime) : To a solution of benzonitrile (1g) in methanol (50ml) was added potassium carbonate (2.0 g). Reaction mixture was cooled and hydroxylamine hydrochloride (1.4g) was added in portion wise. After completion of the addition it was refluxed for 6hr. The solvent was evaporated and excess of water was added to afford the precipitate of the desired product which was filtered and dried.

N-hydroxy benzimidamide (table 17, entry 1): brown color solid, 59% yield, Rf = 0.25 (1:1 EtOAc-hexane), M.P = 89-93 ºC, IR (cm^{-1})= 1651 cm^{-1} (C=N), 1660 cm^{-1} (C=C), 2359 cm^{-1} (C-H), 3000cm^{-1} (N-H), 3400 cm^{-1} (O-H).

4-chloro-N-hydroxybenzimidamide (table 17, entry 2): light green powder, 66% yield, Rf = 0.65 (1:1 EtOAc-hexane), M.P =122-124ºC, IR (cm^{-1}) = 1591 cm^{-1} (C=N), 1656 cm^{-1} (C=C), 2868 cm^{-1} (C-H), 3400 cm^{-1} (O-H), 3500 cm^{-1} (N-H).

4-Nitro-N-hydroxybenzimidamide (table 17, entry 4): yellow powder, 60% yield, Rf = 0.62 (1:1 EtOAc-hexane), M.P = 139-141ºC, IR (cm^{-1}) = 1660 cm^{-1} (C=C), 1600 cm^{-1} (C=N), 2843 cm^{-1} (C-H), 3358 cm^{-1} (N-H), 3464 cm^{-1} (O-H).

3-chloro-4-floro-N-hydroxybenzimidamide (table 17, entry 3) : white crystals, 69% yield, Rf = 0.33 (1:1 EtOAc-hexane), M.P = 134-135ºC, IR (cm^{-1})=1660 cm^{-1} (C=N), 1703 cm^{-1} (C=C), 3470 cm^{-1} (O-H), 3344 cm^{-1} (N-H), 2800 cm^{-1} (C-H)

General procedure for the synthesis of hydrazone ((Z)-benzohydrazonamide): To a solution of benzonitrile (1g) in methanol (50ml) was added in potassium carbonate (2.0 g).

Reaction mixture was cooled and hydrazine hydrochloride (1.4g) was added portion wise. After completion of the addition it was refluxed for 6hr. The solvent was evaporated and excess of water was added to afford the precipitate of the desired product which was filtered and dried.

(Z)-benzohydrazonamide (table 18, entry 1) : purple powder solid, 81% yield, Rf = 0.34 (1:1 EtOAc-hexane), M.P = 90-110ºC , IR(cm^{-1}) = 1614 cm^{-1} (C=N), 1660 cm^{-1} (C=C), 2360 cm^{-1} (C-H), 3371 cm^{-1} (N-H).

(Z)-4-chloro-3-fluorobenzohydrazonamide (table 18, entry 3) : purple powder solid, 81% yield, Rf = 0.41(1:1 EtOAc-hexane), M.P = 140-142ºC , IR(cm^{-1}) = 1597 cm^{-1} (C=N), 1600 cm^{-1} (C=C), 2360 cm^{-1} (C-H), 3363 cm^{-1} (N-H).

(Z)-4-chlorobenzohydrazonamide (table 18, entry 2): light green solid, 72% yield, Rf = 0.52(1:1 EtOAc-hexane), M.P = 122-124ºC, IR (cm^{-1}) = 1600 cm^{-1} (C=N), 1654 cm^{-1} (C=C), 2360 cm^{-1} (C-H) , 3377 cm^{-1} (N-H).

(Z)-4-Nitrobenzohydrazonamide (table 18, entry 4) : yellow solid, 76% yield, Rf = 0.66 (1:1 EtOAc-hexane), M.P = 131-133ºC, IR (cm^{-1}) = 1600 cm^{-1} (C=N), 1627 cm^{-1} (C=C), 2360 cm^{-1} (C-H), 3371 cm^{-1} (N-H).

General procedure for the synthesis of (E)-3-phenyl-5-styryl-1,2,4-oxadiazole/ triazole : amidoxime/hydrazone (2g), t-cinnamic acid (2.17g), EDC (2.28g), HOBt (10mg), Acetonitrile (5ml) was stirred for 1 hour and then evaporated acetonitrile on rotavapour. toluene (10ml) was added later and refluxed for 3 hour. The reaction mixture was then concentrated, diluted with water and extraction was carried out in ethyl acetate. The organic layer was separated and dried and the solvent was evaporated to obtain the crude product. It was purified by column chromatography on silica gel (60-120) using EtOAc-hexane mixture(2:8) as eluent to afford the desired product.

(**E)-5-(4-chlorostyryl)-3-(4-nitrophenyl)-1,2,4-oxadiazole (pk 161) :** light yellow solid, M.P = 178-180ºC , yield 80% ; R_f 0.3(2:8 EtOAc-hexane); IR (cm^{-1}) = 1633 cm^{-1} (C=N) , 1693 cm^{-1} (C=C) , 2854 cm^{-1}(C-H) 1287 cm^{-1} (N-O), ^{1}H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: 6.49 (d, 1H, J = 16 Hz, CH_2=CH_2), 7.40-7.70(m, 6H, Ar-H); 7.88 (m, 1H, Ar-H); 8.12-8.39 (m, 3H, Ar-H);

(E)-3-(4-chlorophenyl)-5-(4-fluorostyryl)-1,2,4-oxadiazole (pk 168) : brown solid, M.P = 171-172ºC , yield 56%, Rf 0.21 (2:8 EtOAc-hexane); IR (cm^{-1}) = 1629 (C=N) , 1687 (C=C) , 2360 (C-H), 1300 (N-O) , ^{1}H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.39 (d, 1H, J = 16 Hz, CH_2=CH_2), 7.10-7.14(m, 3H, Ar-H); 7.41(d, 1H, J=8Hz, Ar-H); 7.55(d, 1H, J=16Hz, CH_2=CH_2); 7.58-7.62 (m, 3H, Ar-H); 7.9 (d, H, J=8Hz, Ar-H).

(E)-3-(4-chlorophenyl)-5-styryl-1,2,4-oxadiazole (pk 169) : brown solid, M.P = 161-163ºC, yield 82%, Rf 0.24 (2:8 EtOAc-hexane); IR (cm^{-1}) = 1629 cm^{-1} (C=N) , 1699 cm^{-1} (C=C) , 3309 cm^{-1} (C-H), 1309 cm^{-1} (N-O).

(E)-3-(4-fluoro-3-chlorophenyl)-5-styryl-1,2,4-oxadiazole (pk89): brown gummy, yield 84% , Rf 0.21(1:10 EtOAc-hexane); IR (cm^{-1}) = 1633 cm^{-1} (C=N), 1681 cm^{-1} (C=C), 2360 cm^{-1} (C-H), 1273 cm^{-1} (N-O); ^{1}H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.48 (d, 1H, J = 16 Hz, CH_2=CH_2), 7.40-7.44(m, 4H, Ar-H); 7.58(d, 1H, J=16Hz, CH_2=CH_2); 7.62-7.64 (m, 2H, Ar-H); 7.89-7.92(m, 1H, Ar-H), 8.07-8.09 (m, 1H, Ar-H)

(E)-3-(4-nitrophenyl)-5-styryl-1,2,4-oxadiazole (pk163): brown solid, M.P = 171-172ºC , yield 78%, Rf 0.32 (2:8 EtOAc-hexane); IR (cm^{-1}) = 1628 (C=N) , 1691 (C=C) , 3300 (C-H), 1298 (N-O)

(E)-3-phenyl-5-styryl-1,2,4-oxadiazole (pk 81) : dark brown paste, yield 67% , Rf 0.22 (2:8 EtOAc-hexane) , IR (cm^{-1}) = 1662 cm^{-1} (C=C), 1637 cm^{-1} (C=N), 2881 cm^{-1} (C-H), 3348 cm^{-1} (N-H), 3470 cm^{-1} (O-H); ^{1}H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.44(d, 1H, J = 16

Hz, $CH_2=CH_2$); 4.06(d, 1H, 16Hz, $CH_2=CH_2$); 7.31-7.36(m, 3H, Ar-H); 7.38-7.43(m, 3H, Ar-H), 7.47-7.52(m, 4H, Ar-H)

(E)-3-(4-chlorophenyl)-5-(4-chlorostyryl)-1,2,4-oxadiazole (pk 167): pale yellow color solid, M.P = 181-183ºC, yield 68% , Rf 0.50(2:8 EtOAc-hexane) , IR (cm^{-1}) = 1631 cm^{-1} (C=N), 1691 cm^{-1} (C=C), 2823 cm^{-1} (C-H), 1282 cm^{-1} (N-O); ^1H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.51 (d, 1H, J = 16 Hz, $CH_2=CH_2$); 7.39-7.48 (m, 4H, Ar-H); 7.54 (d, 1H, J = 16Hz, $CH_2=CH_2$); 7.64-7.68 (m, 3H, Ar-H); 7.83-7.91(m, 1H, Ar-H)

(E)-3-(4-fluoro -3-chlorophenyl)-5-(4-fluorostyryl)-1,2,4-oxadiazole (pk 90) : Pale yellow solid, yield 83% , Rf 0.66(2:8 EtOAc-hexane) , IR (cm^{-1}) = 1600 (C=N) , 1685 (C=C) , 2378 (C-H), 1240 (N-O)

(E)-5-(4-fluorostyryl)-3-(4-nitrophenyl)-1,2,4-oxadiazole (pk 162) : dark brown gummy, M.P = 191-192ºC, yield 79% , Rf 0.61(1:10 EtOAc-hexane) , IR (cm^{-1}) = 1683 cm^{-1} (C=N), 1691 cm^{-1} (C=C), 2362 cm^{-1} (C-H), 1261 cm^{-1} (N-O); ^1H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.39(d, 1H, J = 16Hz, $CH_2=CH_2$); 7.13(m, 3H, Ar-H); 7.55(d, 1H, J = 16Hz, $CH_2=CH_2$); 7.60-7.63 (m, 4H, Ar-H); 7.95-8.04 (m, 1H, Ar-H)

(E)-5-(4-fluorostyryl)-3-(4-nitrophenyl)-1H-1,2,4-triazole (pk 170): purple powder, M.P = 167-168ºC, yield 84%, Rf 0.66 (1:10 EtOAc-hexane), IR (cm^{-1}) = 1622 cm^{-1} (C=N), 1699 cm^{-1} (C=C), 2928 cm^{-1} (C-H), 3034 cm^{-1} (N-H). ^1H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.42 (d, 1H, J= 16Hz, $CH_2=CH_2$); 7.08-7.17 (m, 3H, Ar-H); 7.54- 7.58 (d, 1H, J=16Hz, $CH_2=CH_2$); 7.64-7.67 (m, 4H, Ar-H); 8.10 (d, 1H, J= 8Hz, Ar-H); 8.38 (d, 1H, J= 8Hz, Ar-H).

(E)-3-(4-chlorophenyl)-5-(4-fluorostyryl)-1H-1,2,4-triazole (pk 165): yellowish green gummy, M.P = 179-180ºC, yield 73%, Rf 0.43(1:10 EtOAc-hexane), IR (cm^{-1}) = 1622 cm^{-1} (C=N), 1649 cm^{-1} (C=C) , 2854 cm^{-1} (C-H), 3078 cm^{-1} (N-H). ^1H NMR [(400 MHz, $CDCl_3$ + $(CD_3)_2SO$)]: δ 6.40 (d, 1H, J= 16Hz, $CH_2=CH_2$); 7.02 (d, 1H, J= 16Hz, $CH_2=CH_2$); 7.10-7.13

(m, 3H, Ar-H); 7.40-7.45 (m, 1H, Ar-H); 7.51-7.53 (m, 1H, Ar-H); 7.61-7.64 (m, 2H, Ar-H); 7.86-7.90 (m, 1H, Ar-H); 8.05-8.11 (m, 1H, Ar-H).

(E)-3-(3-chloro-4-fluorophenyl)-5-(4-fluorostyryl)-1H-1,2,4-tiazole (pk 93): pale brown gummy, M.P = 181-183ºC, yield 89%, Rf 0.21(1:10 EtOAc-hexane) , IR (cm^{-1}) = 1600 cm^{-1} (C=N) , 1626 cm^{-1} (C=C), 2928 cm^{-1} (C-H), 3443 cm^{-1} (N-H), 3475 cm^{-1} (O-H).

CHAPTER 8: CONCLUSION AND SUMMARY

Progression of prostate cancer occurs due to the over expression of androgen receptor. Though it is not limited to only prostate gland it can metastasize beyond the prostate gland also and start affecting other part of the body at that stage it becomes more complicated to treat. There are several ways to cure the prostate cancer but most commonly used is chemotherapy which mainly involves two classes of drugs in treatment that are steroidal based therapy and non steroidal based therapy. But due to the drawbacks in steroidal derivatives non-steroidal therapy is preferred. Nonsteroidal antiandrogens also has limited application due to resistance and severe toxicity. Herein we design the novel oxadiazole and triazole, by hoping to overcome these limitations. . Total 160 compounds were designed and studied by molecular docking through autodoock vina software. Oxadiazoles and triazoles are found to be potent as androgen receptor modulator. The potency is being affected by the geometry of these novel compounds. *Cis* isomers showed better binding affinity than the *trans* isomers. Among these compounds, 12 most feasible and potent derivatives were synthesized because of their cost effectiveness and availability of starting materials. These active compounds will be submitted for the cytotoxicity evaluation at Pharmacology & Toxicology Laboratory, NIPER SAS Nagar.

CHAPTER 9: REFERENCES

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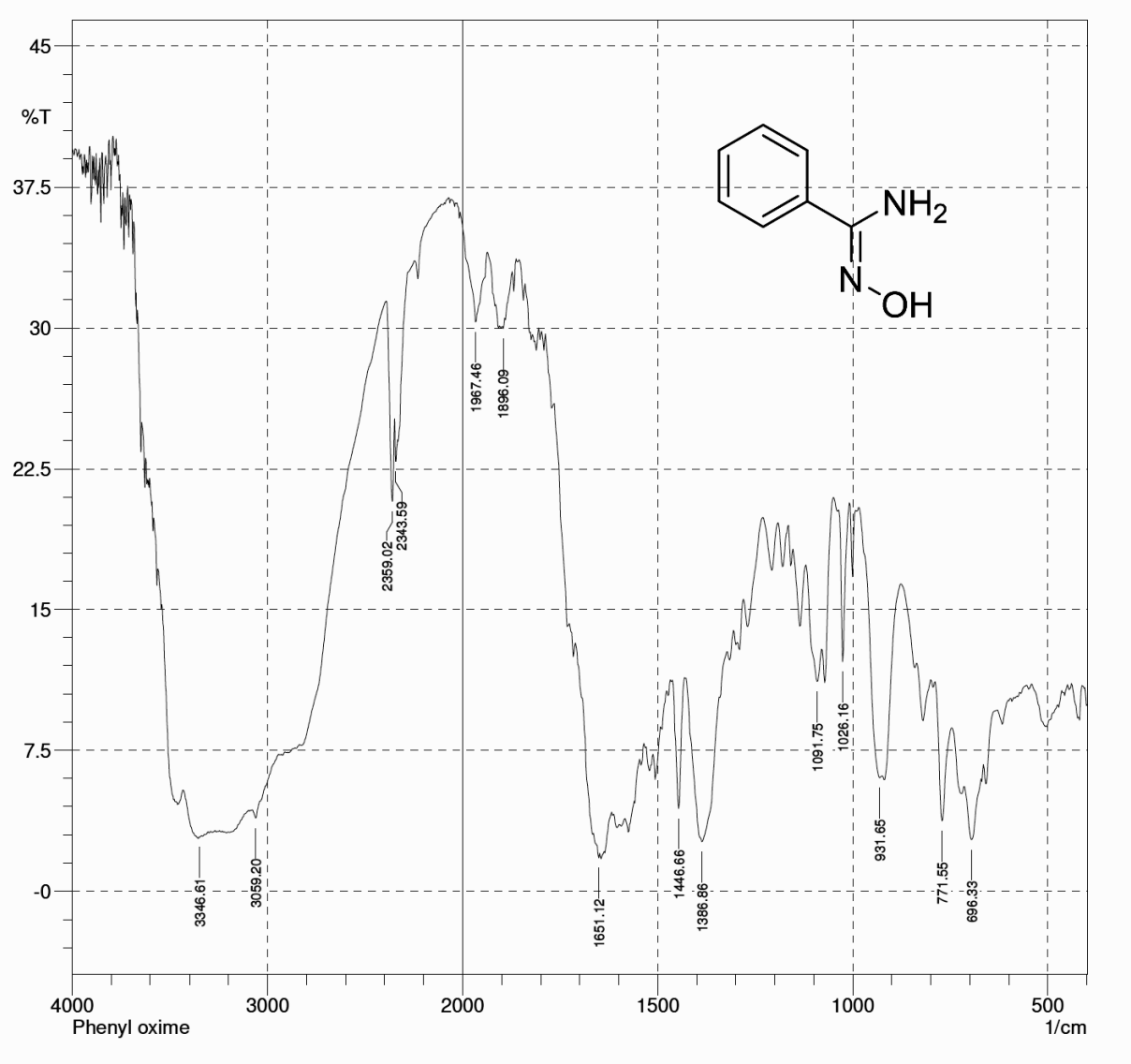
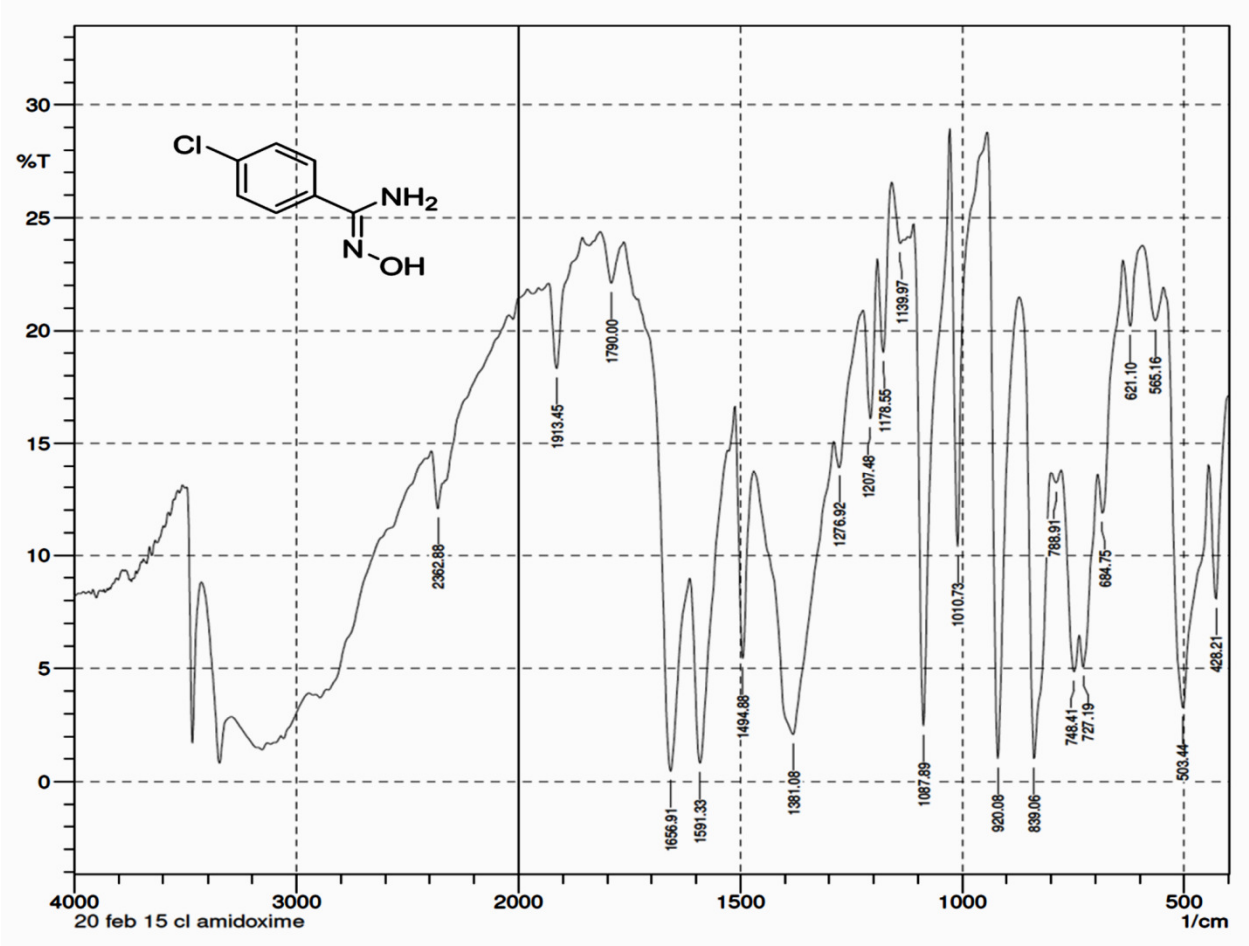
CHAPTER 10 : SUPPLEMENTARY DATA

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920.08
839.06
788.91
748.41
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621.10
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503.44
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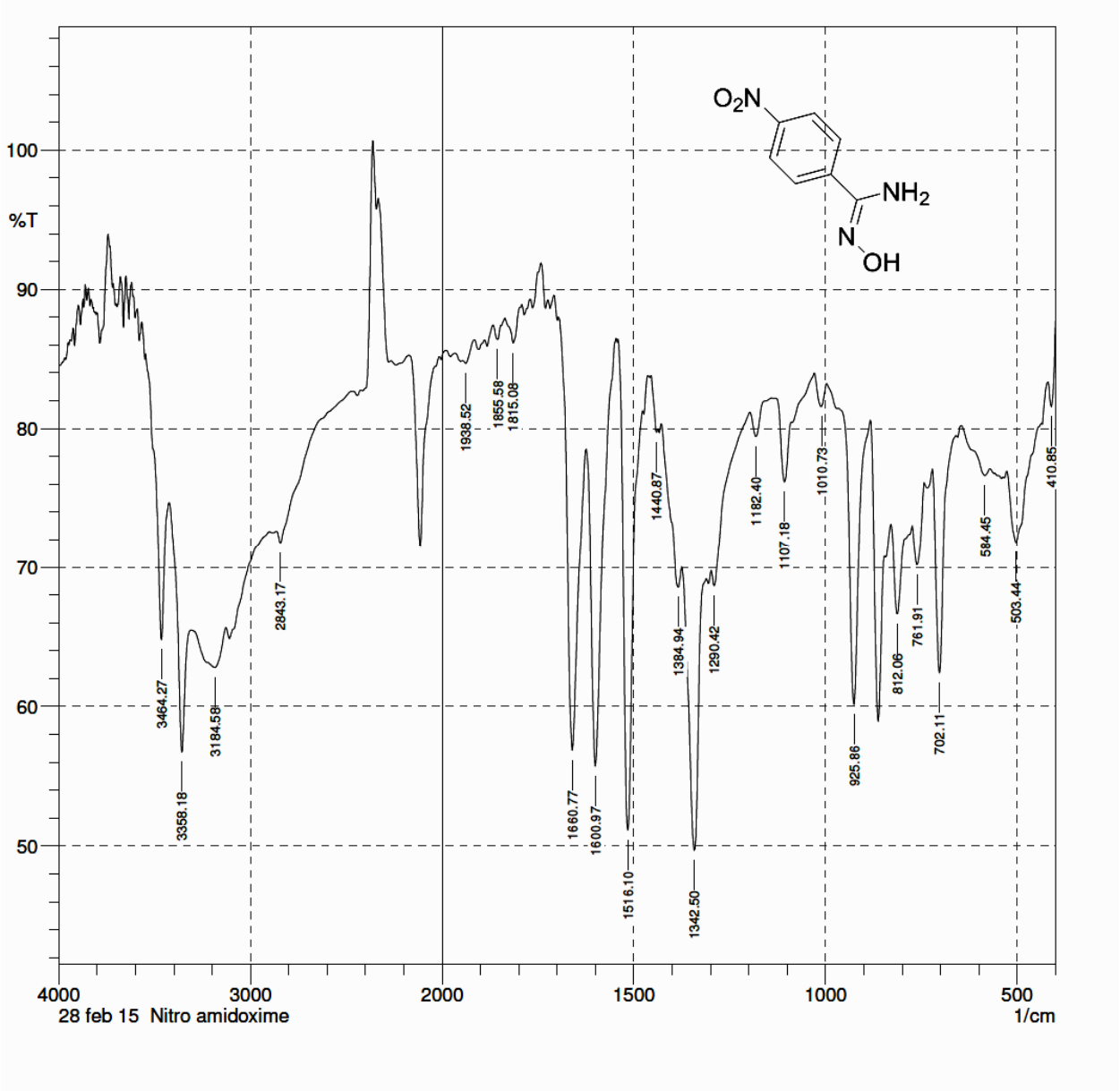
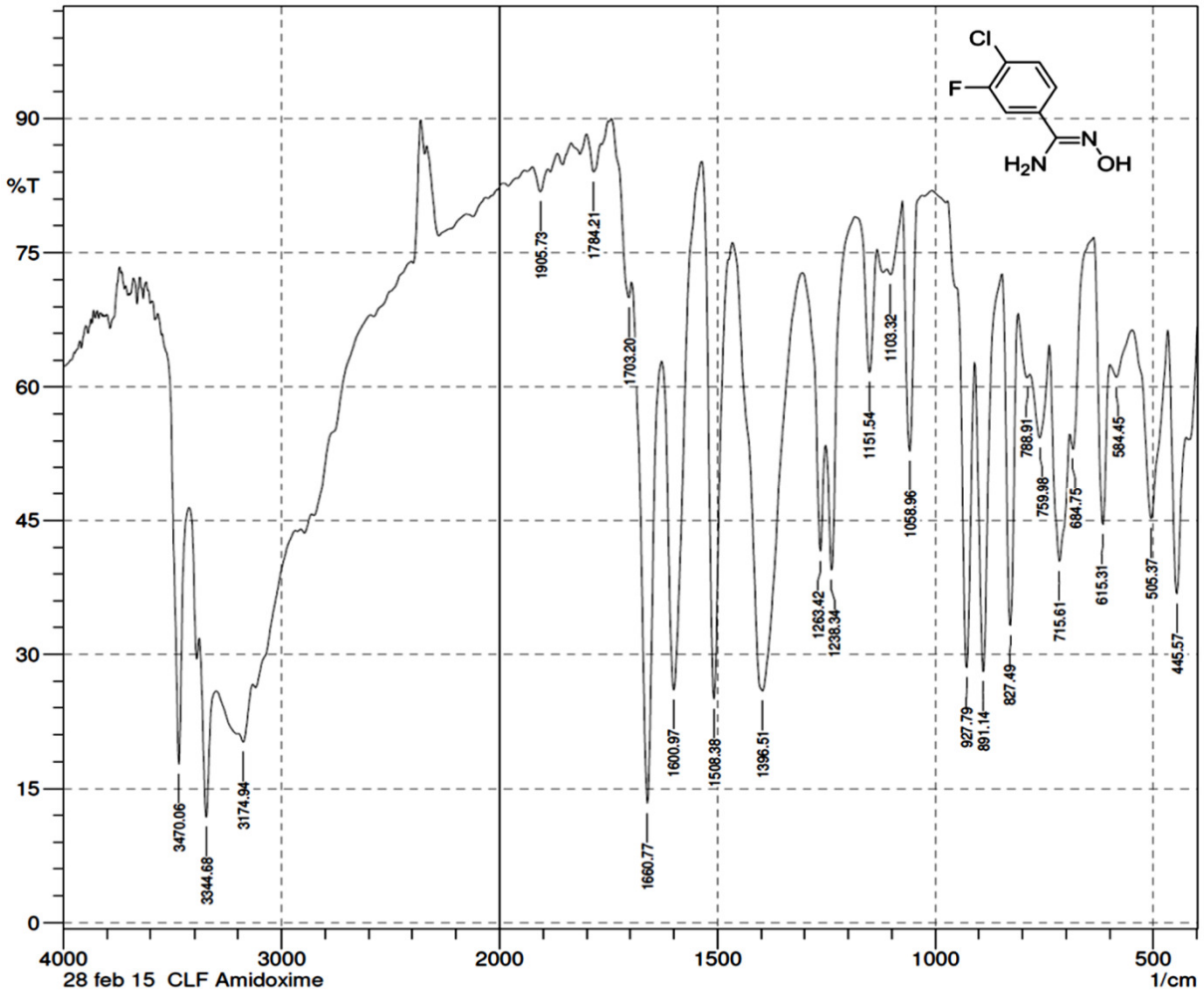
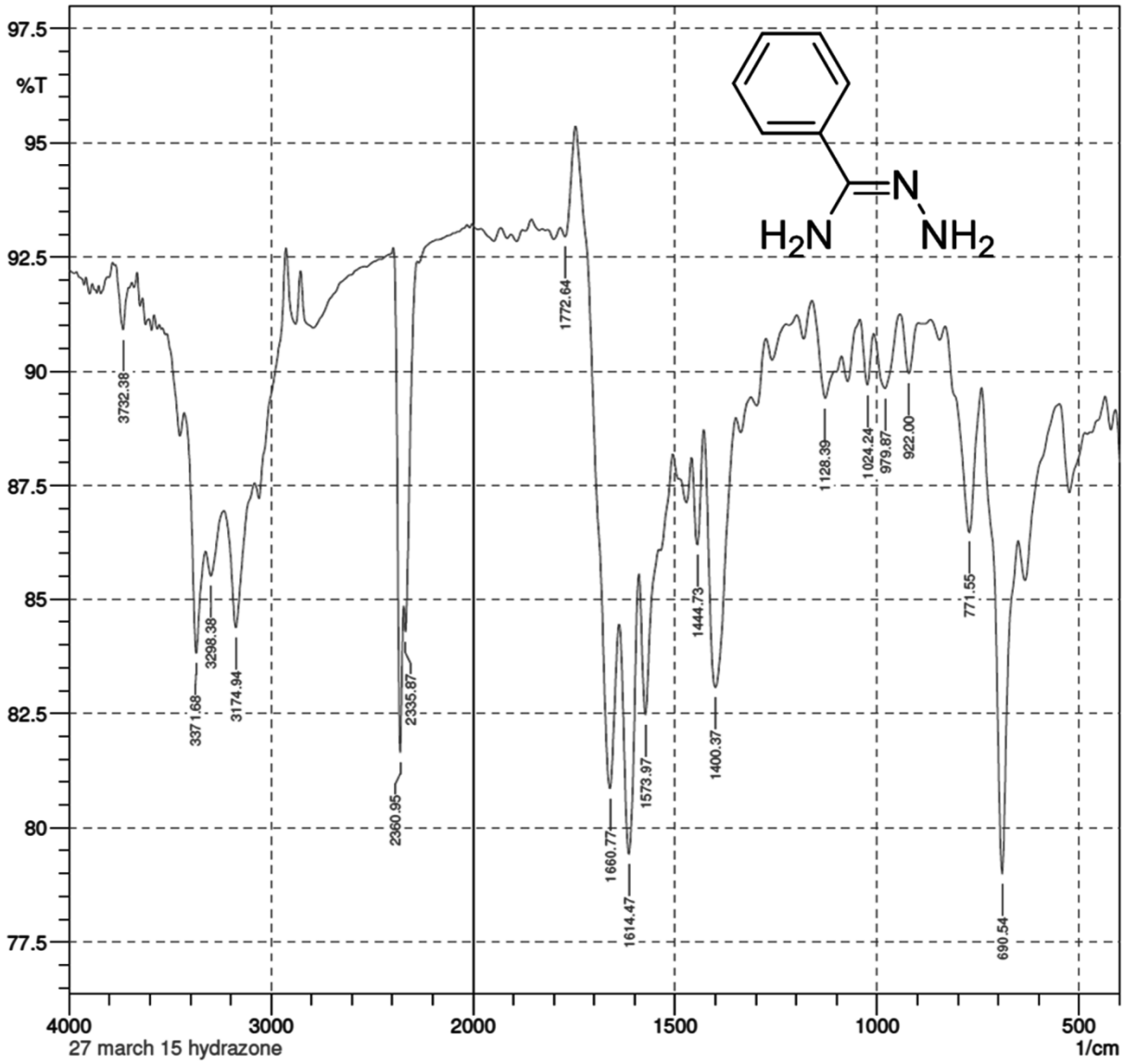
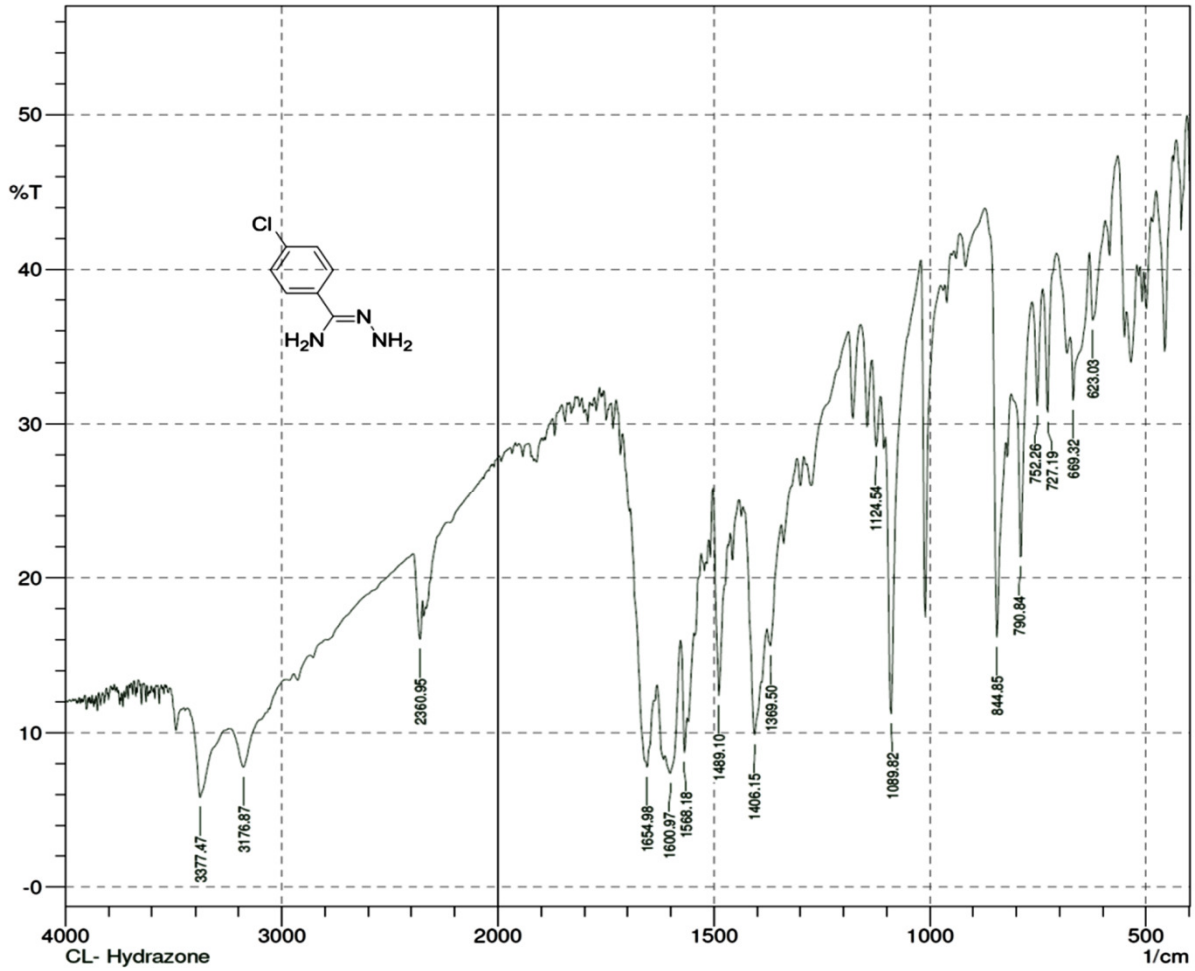
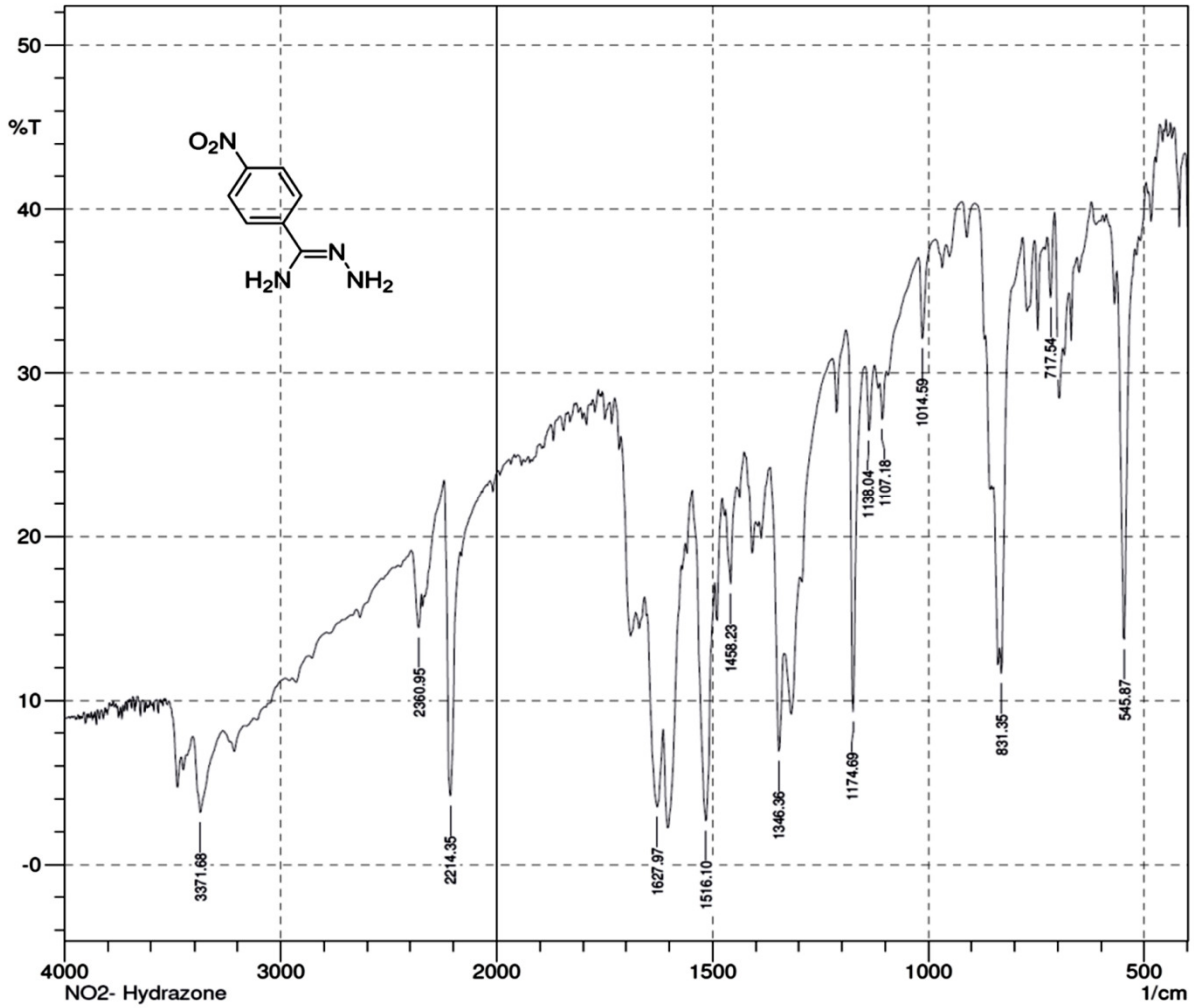
1/cm
20 feb 15 cl amidoxime

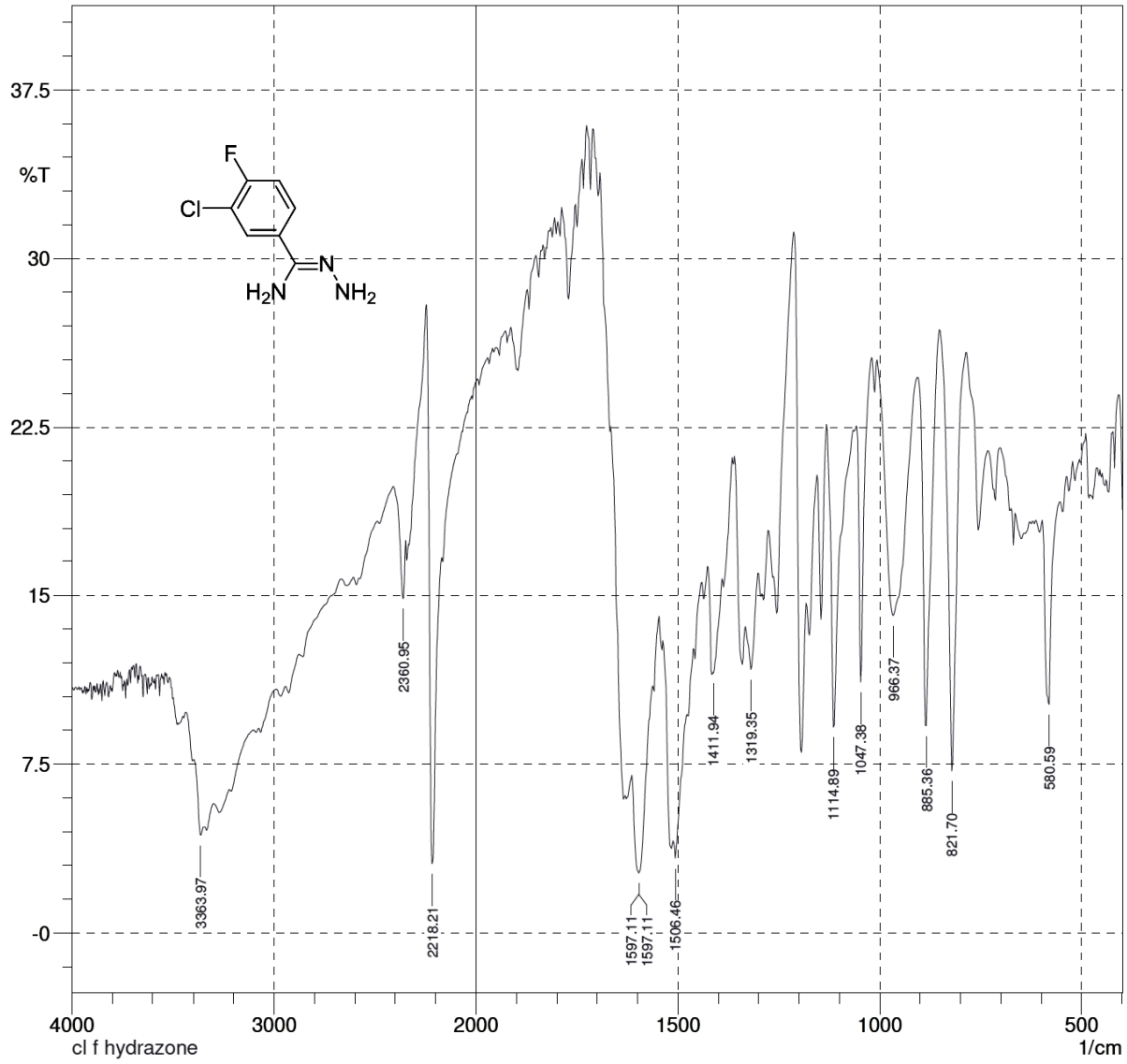

O2N
NH2
N
OH
100
%T
90
80
70
60
50
3464.27
3358.18
3184.58
2843.17
1938.52
1855.58
1815.08
1660.77
1600.97
1516.10
1440.87
1384.94
1342.50
1290.42
1182.40
1107.18
1010.73
925.86
812.06
761.91
702.11
584.45
503.44
410.85
4000
3000
2000
1500
1000
500
1/cm
28 feb 15 Nitro amidoxime

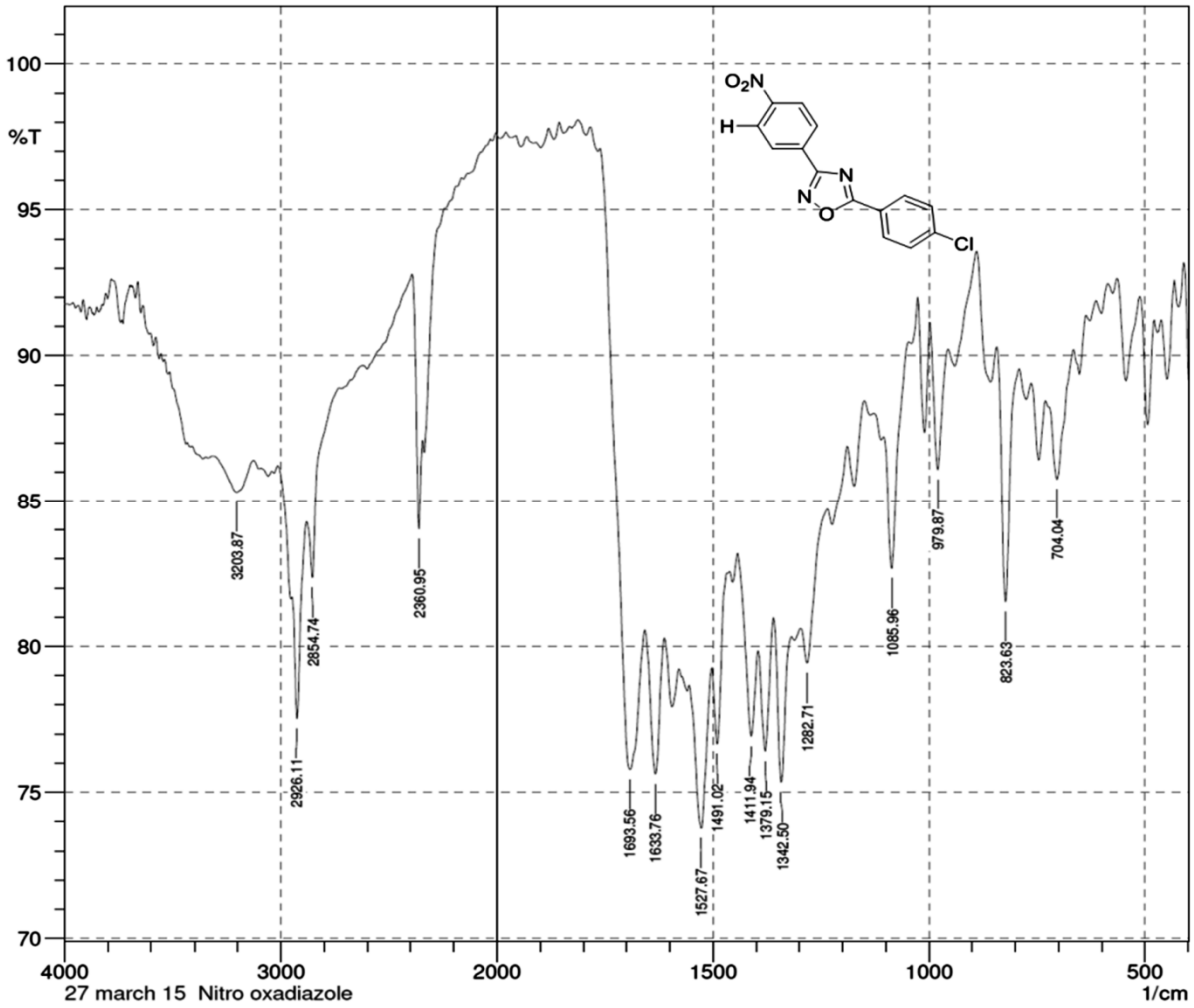

Cl
F
H2N
N
OH
%T
90
75
60
45
30
15
0
4000
3000
2000
1500
1000
500
1/cm
28 feb 15 CLF Amidoxime
3470.06
3344.68
3174.94
1905.73
1784.21
1703.20
1660.77
1600.97
1508.38
1396.51
1263.42
1238.34
1151.54
1103.32
1058.96
927.79
891.14
827.49
788.91
759.98
715.61
684.75
615.31
584.45
505.37
445.57

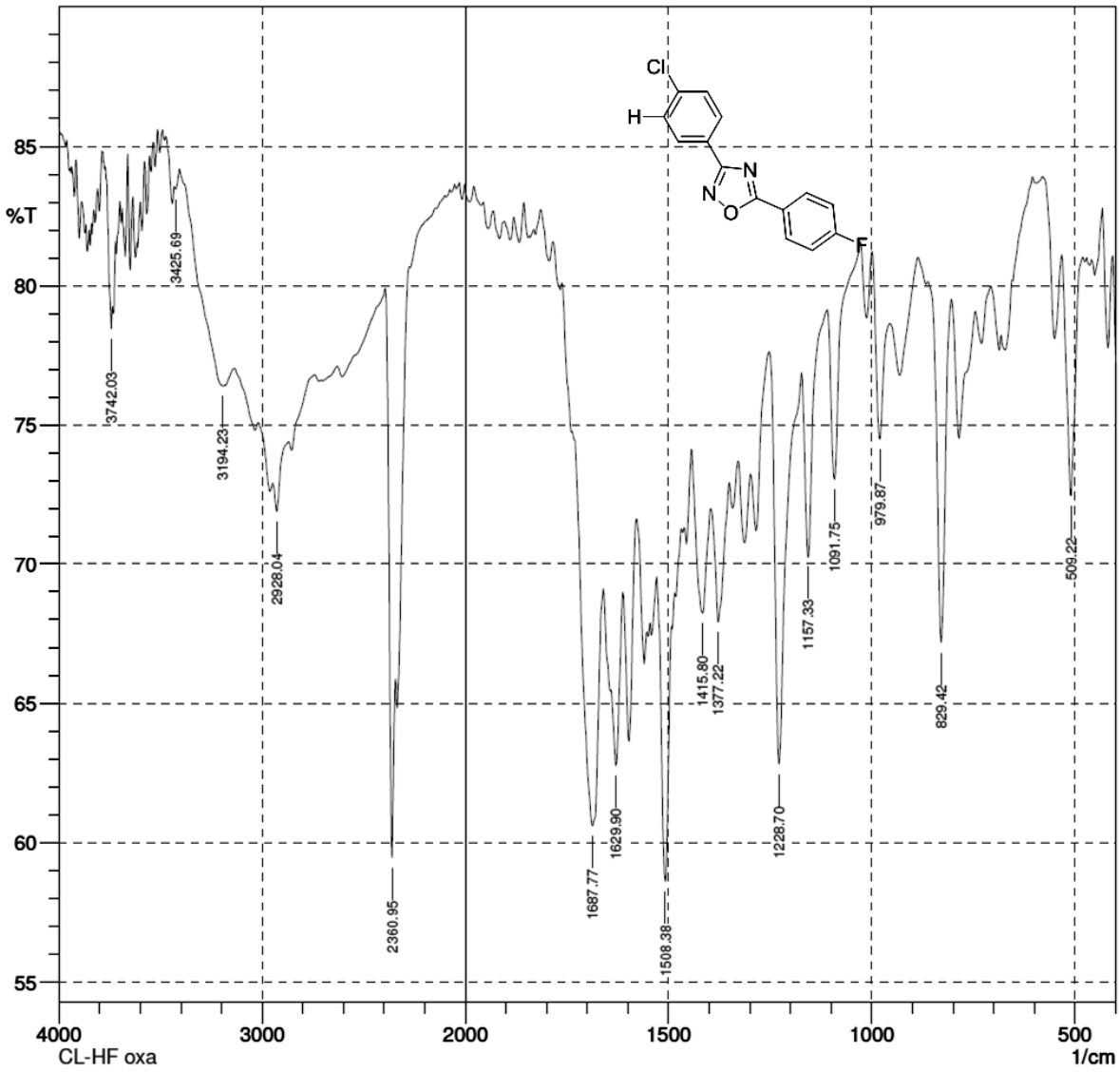

97.5
%T
95
92.5
90
87.5
85
82.5
80
77.5
4000
3000
2000
1500
1000
500
1/cm
27 march 15 hydrazone
3732.38
3371.68
3298.38
3174.94
2360.95
2335.87
1772.64
1660.77
1614.47
1573.97
1444.73
1400.37
1128.39
1024.24
979.87
922.00
771.55
690.54
N
H2N
NH2

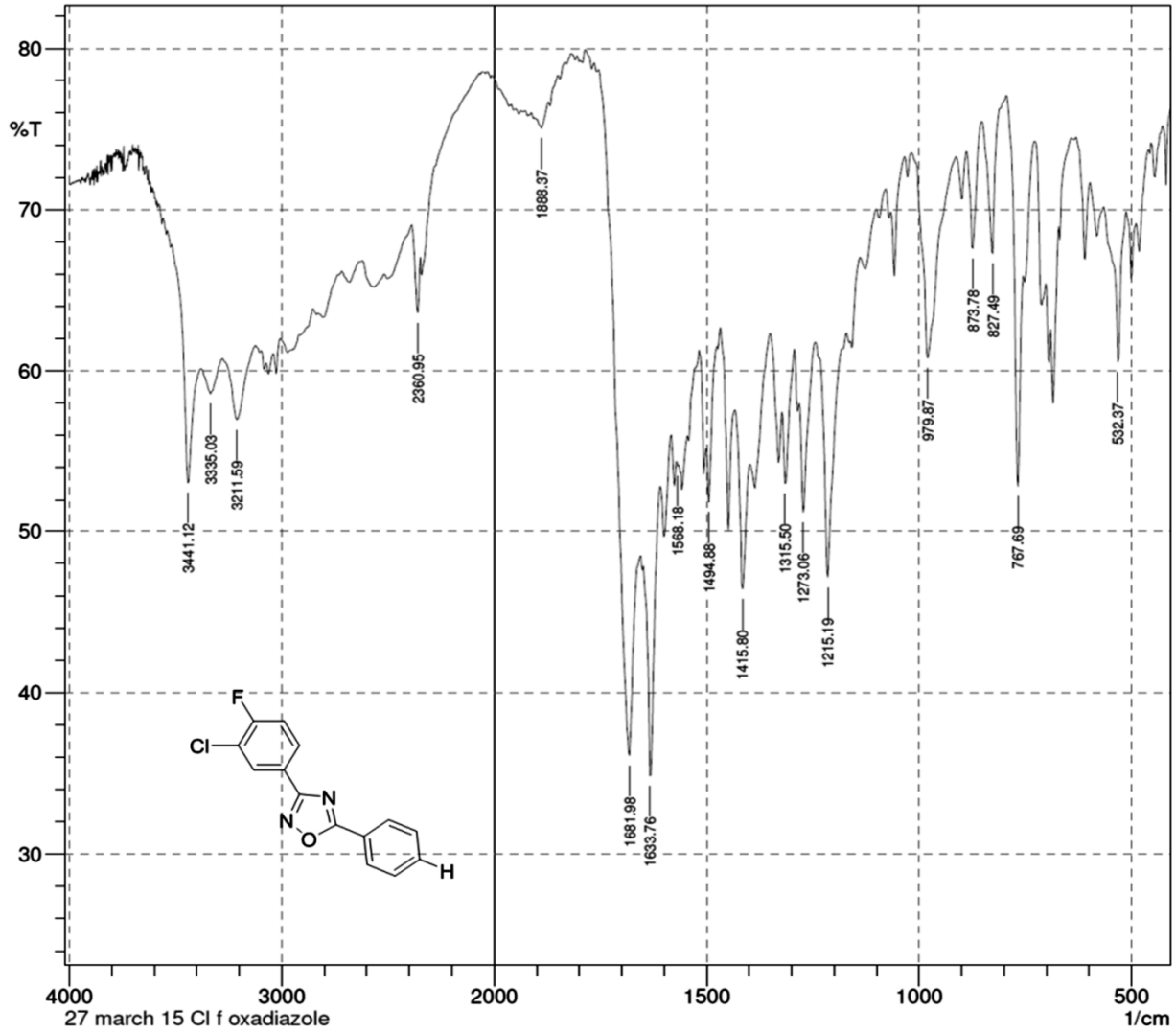

%T
50
40
30
20
10
-0
Cl
N
H2N
NH2
3377.47
3176.87
2360.95
1654.98
1600.97
1568.18
1489.10
1406.15
1369.50
1124.54
1089.82
844.85
790.84
752.26
727.19
669.32
623.03
4000
3000
2000
1500
1000
500
1/cm
CL- Hydrazone

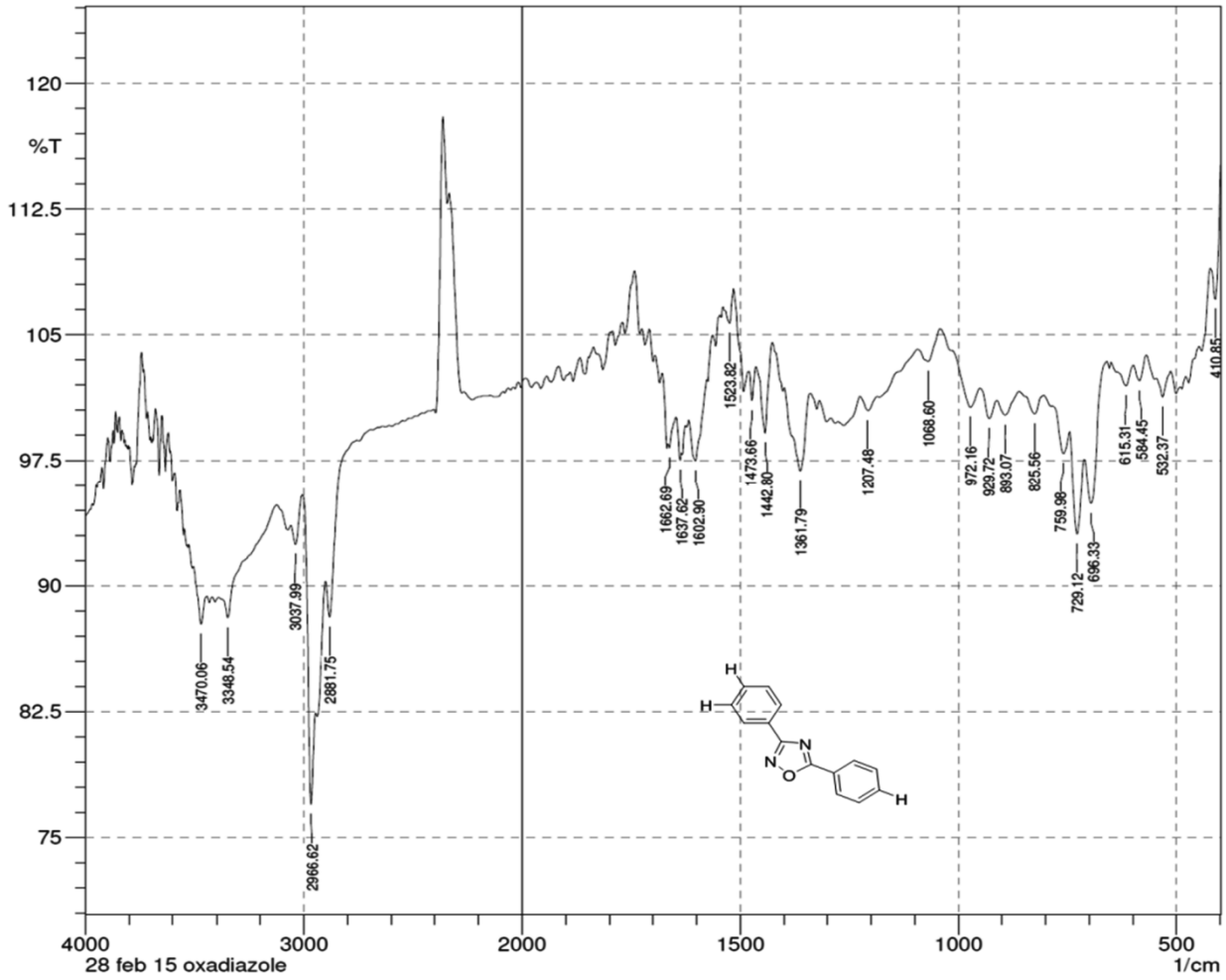

%T
50
40
30
20
10
-0
O2N
H2N
N
NH2
3371.68
2360.95
2214.35
1627.97
1516.10
1458.23
1346.36
1174.69
1138.04
1107.18
1014.59
831.35
717.54
545.87
4000
3000
2000
1500
1000
500
1/cm
NO2- Hydrazone

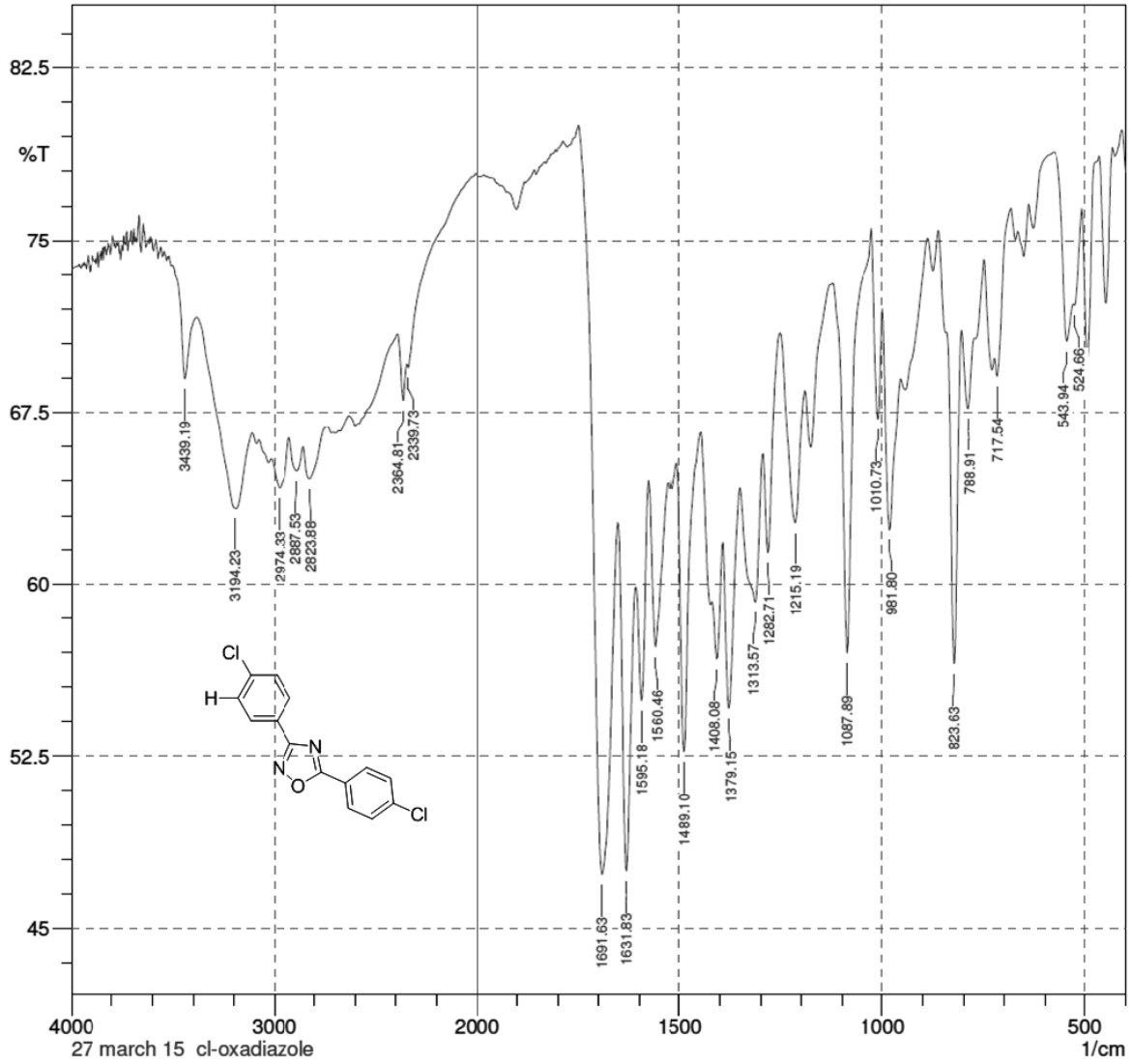

37.5
%T
30
22.5
15
7.5
-0
F
Cl
N
H2N
NH2
3363.97
2360.95
2218.21
1597.11
1597.11
1506.46
1411.94
1319.35
1114.89
1047.38
966.37
885.36
821.70
580.59
4000
3000
2000
1500
1000
500
1/cm
cl f hydrazone

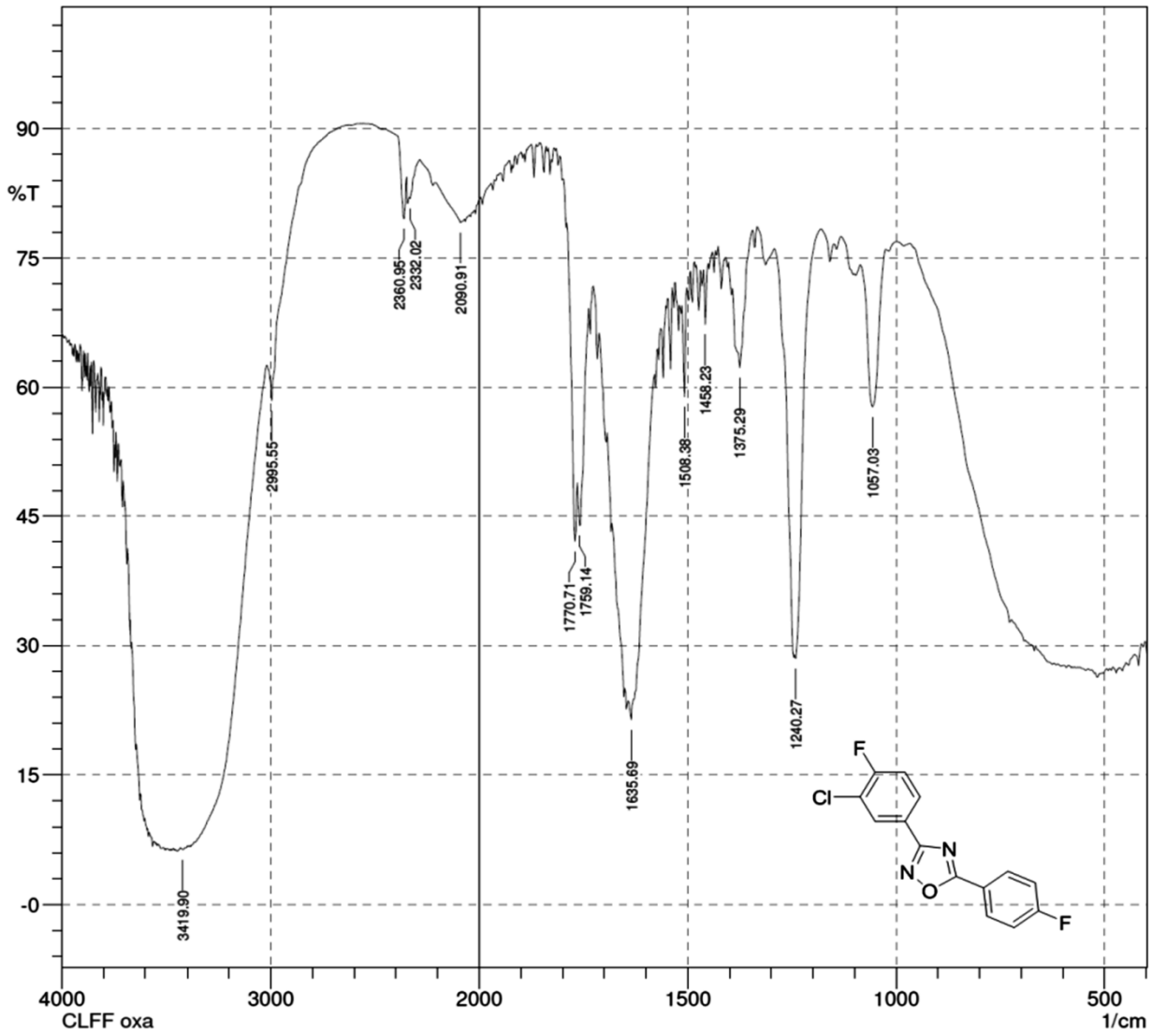

100
%T
95
90
85
80
75
70
4000
3000
2000
1500
1000
500
1/cm
27 march 15 Nitro oxadiazole
3203.87
2926.11
2854.74
2360.95
1693.56
1633.76
1527.67
1491.02
1411.94
1379.15
1342.50
1282.71
1085.96
979.87
823.63
704.04
O2N
H
N
N
O
Cl

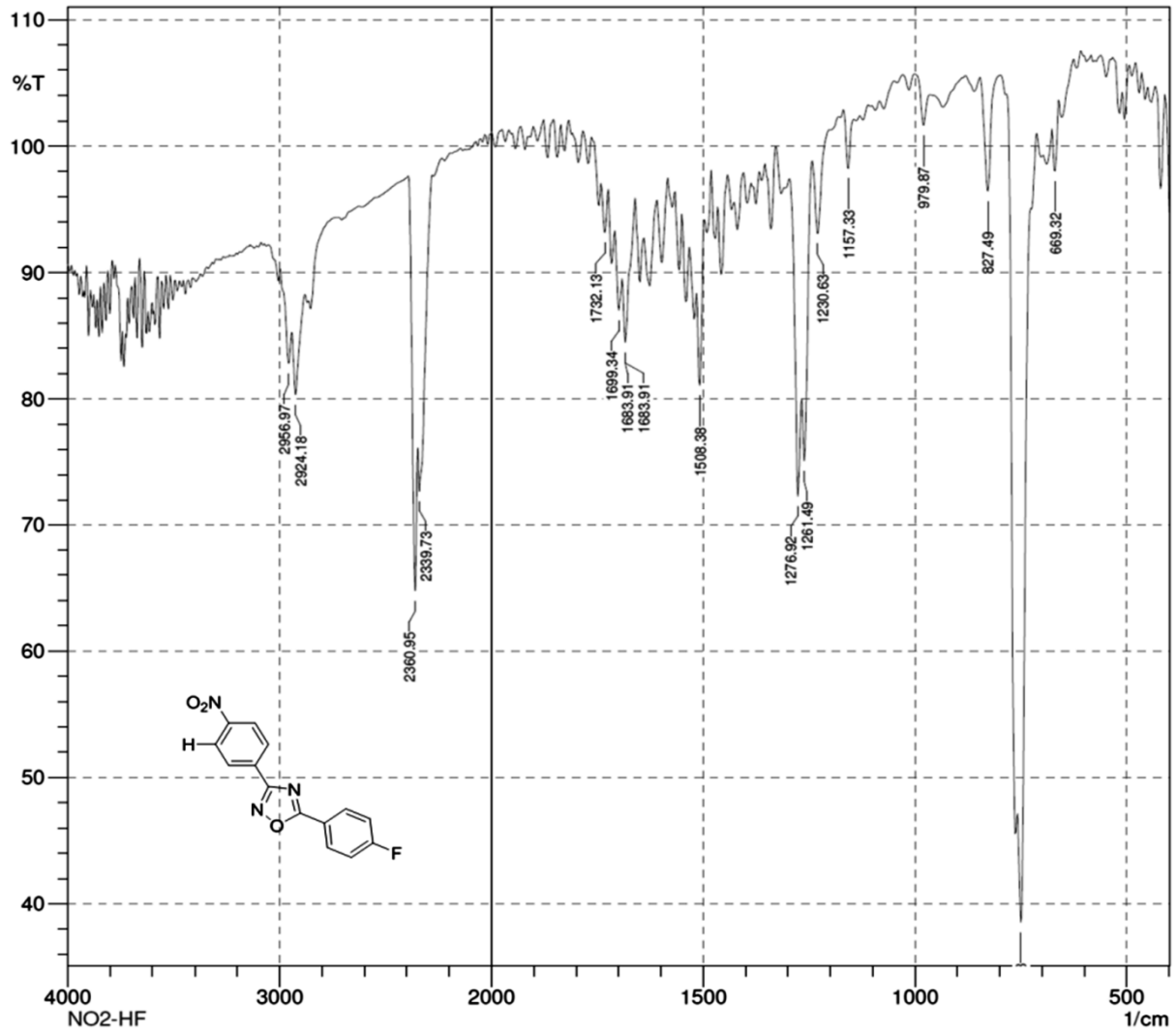

Cl
H
N
N
O
F
%T
85
80
75
70
65
60
55
3742.03
3425.69
3194.23
2928.04
2360.95
1687.77
1629.90
1508.38
1415.80
1377.22
1228.70
1157.33
1091.75
979.87
829.42
509.22
4000
3000
2000
1500
1000
500
1/cm
CL-HF oxa

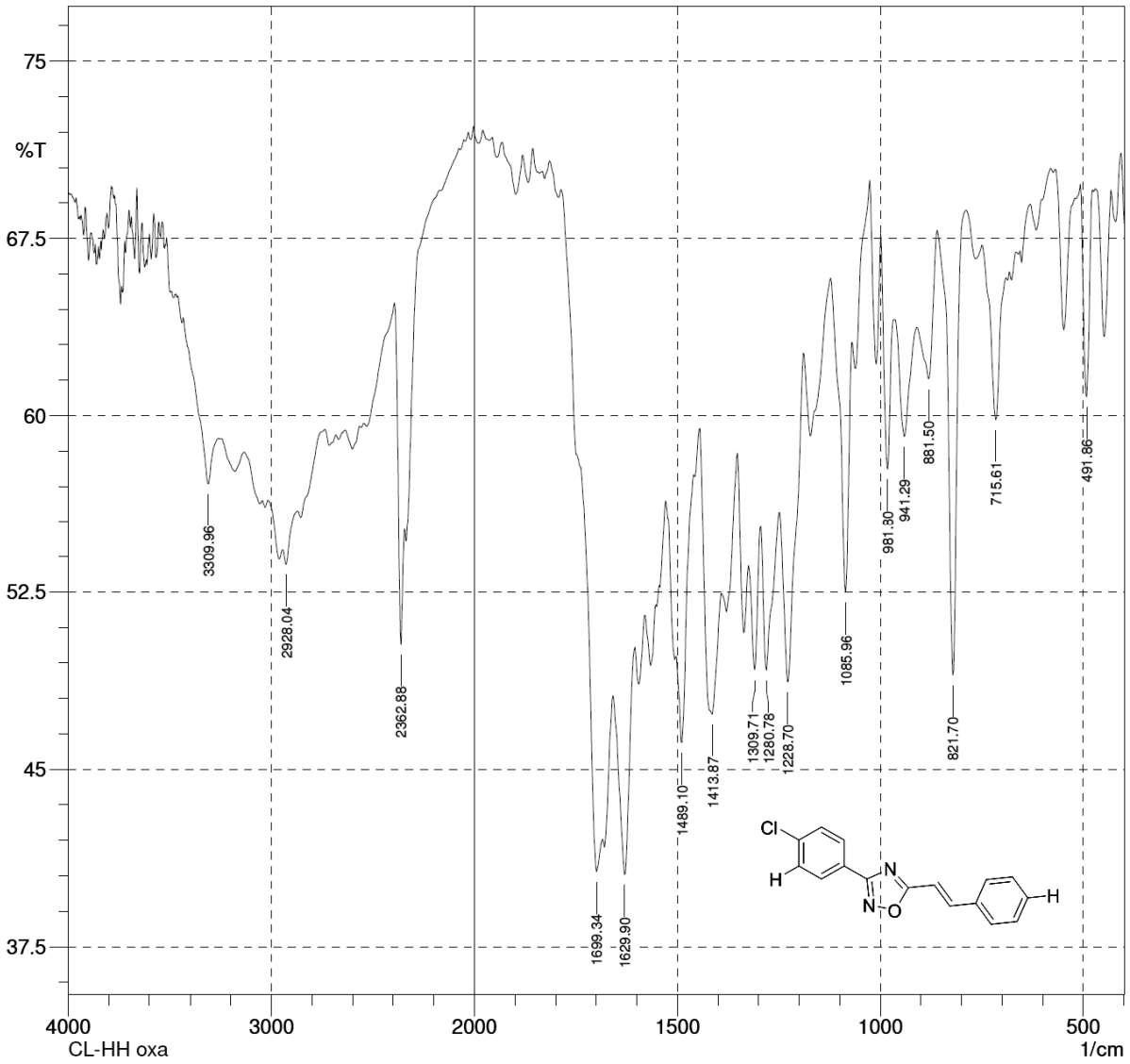

%T
80
70
60
50
40
30
4000
3000
2000
1500
1000
500
1/cm
3441.12
3335.03
3211.59
2360.95
1888.37
1681.98
1633.76
1568.18
1494.88
1415.80
1315.50
1273.06
1215.19
979.87
873.78
827.49
767.69
532.37
F
Cl
N
N
O
H
27 march 15 Cl f oxadiazole


120
%T
112.5
105
97.5
90
82.5
75
4000
3000
2000
1500
1000
500
1/cm
28 feb 15 oxadiazole
3470.06
3348.54
3037.99
2966.62
2881.75
1662.69
1637.62
1602.90
1523.82
1473.66
1442.80
1361.79
1207.48
1068.60
972.16
929.72
893.07
825.56
759.98
729.12
696.33
615.31
584.45
532.37
410.85
H
H
N
N
O
H


82.5
%T
75
67.5
60
52.5
45
3439.19
3194.23
2974.33
2887.53
2823.88
2364.81
2339.73
1691.63
1631.83
1595.18
1560.46
1489.10
1408.08
1379.15
1313.57
1282.71
1215.19
1087.89
1010.73
981.80
823.63
788.91
717.54
543.94
524.66
Cl
H
N
N
O
Cl
4000
3000
2000
1500
1000
500
1/cm
27 march 15 cl-oxadiazole


90
%T
75
60
45
30
15
-0
2360.95
2332.02
2090.91
2995.55
1770.71
1759.14
1508.38
1458.23
1375.29
1057.03
1240.27
1635.69
3419.90
F
Cl
N
N
O
F
4000
3000
2000
1500
1000
500
CLFF oxa
1/cm


110
%T
100
90
80
70
60
50
40
4000
3000
2000
1500
1000
500
1/cm
NO2-HF
2956.97
2924.18
2360.95
2339.73
1732.13
1699.34
1683.91
1683.91
1508.38
1276.92
1261.49
1230.63
1157.33
979.87
827.49
669.32
O2N
H
N
N
O
F


75
%T
67.5
60
52.5
45
37.5
3309.96
2928.04
2362.88
1699.34
1629.90
1489.10
1413.87
1309.71
1280.78
1228.70
1085.96
981.30
941.29
881.50
821.70
715.61
491.86
Cl
H
N
N
O
H
4000
3000
2000
1500
1000
500
CL-HH oxa
1/cm