PROJECT REPORT

ON

SYNTHESIS AND CHARACTERISATION OF AZO DYES AND THEIR ANTI DIABETIC STUDY BY DOCKING

SUBMITTED BY

ANN RIVNA ROSE

Register No: AM20CHE002

In partial fulfillment for the award of the Post graduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
AND CENTER FOR RESEARCH

ST.TERESAS COLLEGE (AUTONOMOUS)
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DEPARTMENT OF CHEMISTRY

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

M.Sc. CHEMISTRY PROJECT REPORT

Name	:	**ANN RIVNAROSE**
Register Number	:	**AM20CHE002**
Year of Work	:	**2020-2022**

This is to certify that the project SYNTHESIS AND CHARACTERISATION OF AZODYES AND THEIR ANTI DIABETIC STUDY BY DOCKING is the work done by.

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Date: 9/6/2022

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CERTIFICATE

This is to certify that the project work entitled "SYNTHESIS AND CHARACTERISATION OF AZO DYES AND THEIR ANTIDIABETIC STUDYBY DOCKING" is the work done by **ANN RIVNAROSE** under the guidance of **Dr. MARIA LINSHA P.L**, Guest Lecturer Department of Chemistry **and Centre for Research, St. Teresa's College, Ernakulam in partial** fulfillment of **the award of the Degree of Master of Science in Chemistry at St. Teresa's College,** Ernakulam affiliated to Mahatma Gandhi University, Kottayam

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Dr. Maria Linsha P.L
Project Guide

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DECLARATION

I hereby declare that the project work entitled "**SYNTHESIS AND CHARACTERIZATION OF AZO DYES AND THEIR ANTIDIABETIC STUDY BY DOCKING**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by me under the guidance of **Dr MARIA LINSHA P.L**Guest Lecturer, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam (Internal Guide).This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

ANN RIVNA ROSE

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Primarily, I thank God almighty for being with us throughout all the days and helping us complete the project successfully.

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ANN RIVNA ROSE

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Chapter 1

Introduction

Abstract. The aromatic azo groups are highly colored and are often used as dyes. Aromatic Azo compounds are formed by a coupling reaction between a diazonium salt and a coupling agent. Azo dyes form a major class of chemically related compounds that are ubiquitous in foods, plastics, textiles, pharmaceuticals, paints, printing inks, and cosmetics.

The combination of aromatic substituted amines with coupling agents such as phenol derivatives could yield variety azo compounds having versatile biological properties.

In the present study a series of aromatic azo compounds are synthesized by diazo coupling reaction between aniline and para nitroaniline with resorcinol and naphthol . The dyes are characterized using U.V Visible spectroscopy .Optimization of synthesized azo compounds are performed by DFT calculation method. The combination of aromatic substituted amines with coupling agents could yield variety of azo compounds having versatile pharmacological properties including antioxidant, antidiabetic etc. As a pharmaceutical application we studied the antidiabetic properties of six azo dyes using molecular docking with α glucosidase .To find out the better α-glucosidase inhibitor we compared the binding energies of six azo dyes with that of standard acarbose drug

1.1 Azo Dyes

Any of a large class of synthetic dyes whose molecules contain two adjacent nitrogen atoms between carbon atoms. Azo dyes are the most important synthetic colorants which have been widely used in textile, printing, paper manufacturing[1] account for more than 60 % of total dyes [2, 3]. Approximately 70 % of all the dyes used in industry are azo dyes [4, 5]. These compounds are characterized by the functional group (-N¼N-) uniting two symmetrical and/or asymmetrical identical or non-azo alkyl or aryl radicals[6] As well as their harmful effects of azo dyes on humans and aquatic life, have aroused urgent calls for the treatment of

effluents containing azo dyes to eliminate them or convert them into useful and safe products. Most azo dyes are synthesized by diazotization of an aromatic primary amine, followed by coupling with one or more electron-rich nucleophiles such as amino and hydroxy group. The azo group may be

bonded to benzene rings, naphthalene, aromatic heterocycles or to enolizable aliphatic groups [7]. These are essential to give the color of the dye, with their shades of different intensities. In general, the chemical structure of an azo dye is represented by a backbone, the auxochrome groups, the chromophoric groups and the solubilizing groups. The color of the azo dyes is determined by the azo bonds and their associated chromophores and auxochromes,

1.11 Aromatic azo compounds

Azo compounds are derivatives of diazine, in which both hydrogens are substituted with hydrocarbyl groups. The -N=N- linkage in any organic molecules in which the ends of the nitrogen atoms were connected by o aromatic groups are known as aromatic azo dyes. They are a predominant class of colorants used in tattooing cosmetics, foods, and consumer products. In addition to this commercial application aromatic

. In aromatic azo compounds, the R groups are arene rings; the structures of these are stable than if the R groups are alkyl groups. This is because the –N=N– group becomes part of an extended delocalized system involving the arene groups. The aromatic azo groups are highly coloured and are often used as dyes. Aromatic azo compounds are formed by a coupling reaction between a diazonium salt and a coupling

1.12 Diazonium salt

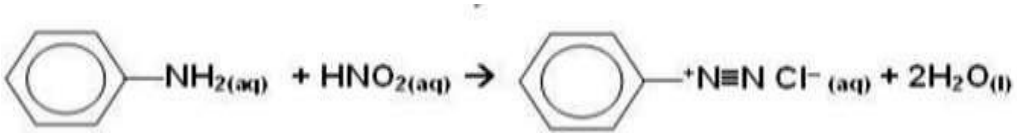
The diazonium salts are very unstable; the only relatively stable diazonium salts are the aromatic ones, and these are not particularly stable [8-9].This is because the presence of the benzene ring with its high electron density stabilizes the

±N≡N group. Benzene diazonium chloride is an example of a diazonium salt:

In a diazotization reaction- a cold solution of sodium nitrate is added to a solution of arylamine in concentrated acid (below 5°C). The acid firstly reacts with the sodium nitrate to form an unstable nitrous acid (nitric(iii)acid):

$$NaNO_2(aq) + HCl(aq) \rightarrow HNO_2(aq) + NaCl(aq)$$

The nitrous acid then reacts with the arylamine



1.13 Diazo coupling reactions

Diazo coupling is an **electrophilic substitution reaction** which occurs readily only with activated substrates. In a diazo coupling reaction, the diazonium salt reacts with another arene (the coupling agent). The diazonium salt acts as an electrophile, reacting with the benzene ring of the coupling agent. When the ice-cold solution of the diazonium salt is added to a solution containing the coupling agent, a coloured precipitate of an azo compound is formed; many of these compounds are dyes. The coupling agent always reacts in the two or four position of the benzene ring (where one position is the functional group). The color of the compound formed depends on the coupling agent that is being reacted with diazonium salt.

Many different azo compounds can be formed by coupling different diazonium salts with amines. Coupling' of diazonium salts with phenols yields azocompounds

containing the azo group -N=N-

1.14 Pharamacological properties

Aromatic azo phenol derivatives have been extensively studied in the recent years due to their broad spectrum pharmaceutical applicability mainly because of their simplistic ,cost effective and reproducible synthetic method. The combination of aromatic substituted amines with coupling agents such could yield variety of azo compound having versatile biological properties

phenolic compounds are simple and naturally occurring compound bearing an aromatic ring with one or more OH group received their considerable attention due to their biological function such as anticarcinogen antimicrobial anti diabetic etc. [11].

Hydroxytriazenes are a class of compounds containing alpha hydroxyl group relative to the diazo group, have versatile pharmacological properties including lipid-lowering, antidiabetic, antioxidant, anti-inflammatory, antimicrobial agents. Keeping in view the synthetic feasibility of azo derivatives and diverse therapeutic properties of phenolic compounds fused with azo moiety have gained immense attention in the field of pharmacological chemistry[11]

1.2 Type 2 Diabetics

Diabetes mellitus is a group of metabolic diseases in which the person has high blood glucose (blood sugar) level either due to inadequate insulin production or because the body's cells do not respond properly to insulin or both. The term "Diabetes Mellitus" describes a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat (dyslipidemia) and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Diabetes mellitus are of two types

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Type 1 diabetes: It is due to the body's malfunction to produce insulin in the body, and requires the person to inject insulin. This form was previously referred to as "Insulin- Dependent Diabetes Mellitus" (IDDM) or "Juvenile Diabetes".

Type 2 diabetes: It is due to insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as noninsulin-dependent diabetes mellitus(NIDDM) or "adult-onset diabetes"

Dependent Diabetes Mellitus" (IDDM) or "Juvenile Diabetes".

Type 2 diabetes: It is due to insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was previously referred to as noninsulin-dependent diabetes mellitus(NIDDM) or "adult-onset diabetes".

Type 2 diabetes can be prevented after following healthy life style such as healthy diet, proper exercise or maintaining healthy weight.

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The third main form, Gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may lead to type 2 DM.

Type two diabetics account for the 70- 80 % of all the diabetic patients worldwide and it leads to post prandial hyper glycaemia condition characterized by an excess of glucose in the bloodstream, often associated with diabetes mellitus.one of the critical strategies to prevent this condition is to inhibit the enzyme that is responsible for the carbohydrate enzyme eg. Alpha amylase and alpha glucosidase. Several alpha glucosidase inhibitors are being administrated in the treatment of type 2 diabetics such as acarbose, voglibose etc.

1.21 Alpha Glucosidase

The **α- glucosidase** is a class of hydrolase enzyme and is actively involved in the digestion of food carbohydrates. The a-glucosidase comprises 811 amino acids with the residual architecture of 35% helices, 25% b sheets and 38% coils. Glucosidase enzymes catalyze hydrolysis of starch to simple sugars. In humans, these enzymes aid digestion of dietary carbohydrates and starches to produce glucose for intestinal absorption, which in turn, leads to increase in blood glucose levels. Inhibiting the function of these enzymes in patients with type-2 diabetes may reduce hyperglycemia. In the human digestive system, multiple glucosidases (carbohydrate digesting enzymes) coordinate breakdown of dietary starches and other polysaccharides to release glucose. Two main classes of carbohydrate digesting enzymes are discussed herein: α-glucosidase mainly includes enzymes such as maltase, sucrase, isomerized maltase, and lactase, which are mainly distributed along the brush border of the intestinal epithelium and play an important role in the catabolism of sugar. The process is that polysaccharides is digested by oral saliva, pancreatic amylase into oligosaccharides containing a small number of glucose molecules, α-glucosidase then cut α-1,4 glycosidic bond inthese non-reducing end of the oligosaccharide, releasing glucose. Glucose is absorbed by the small intestine into the blood circulation, it becomes blood sugar. In the physiological state, the upper, middle and lower sections of small intestine all contain α- glucosidase, but the upper section is the main part of absorption.

1.22 Acarbose

Acarbose is a medicine used in the treatment of type 2 diabetes mellitus. It should be taken just before meals as it lowers the post-meal blood sugar levels. Acarbose inhibits enzymes (glycoside hydrolases) needed to digest carbohydrates, specifically, alpha- glucosidase enzymes in the brush border of the small intestines and pancreatic alpha- amylase. Alpha-glucosidases hydrolyses oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates. Less glucose is absorbed because the carbohydrates are

not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels

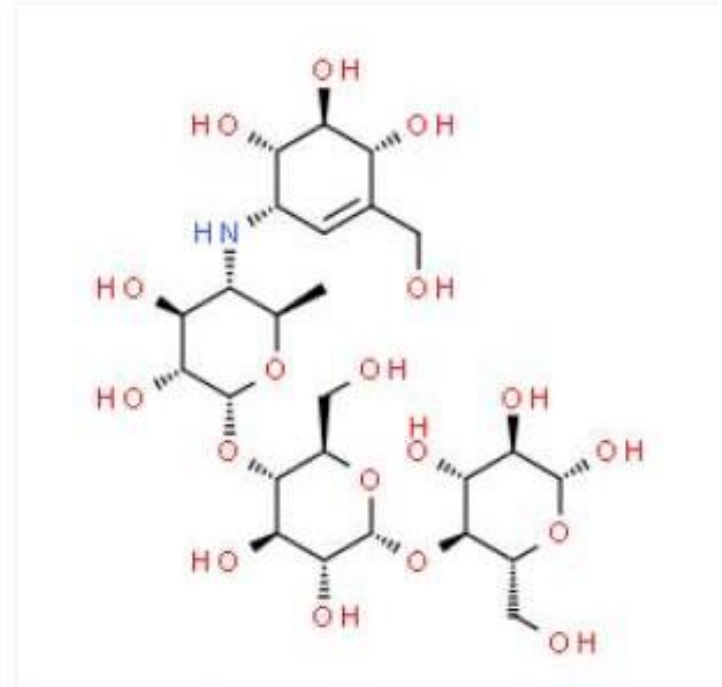

fig.1.22 a structure of acarbose

This prevents the long-term complications of diabetes mellitus. However, the prolonged use of these agents could result in various side effects such as vomiting, pomposity, diarrhea and flatulence.

1.3 UVSPECTROSCOPY

The UV and visible spectra of compounds are associated with electronic transitions between energy levels . The transitions are generally between a bonding or lone pair orbitals and non –bonding or antibonding orbital. The UV/VIS spectral wavelength of light absorbed versus the absorption intensity.

Beer's law: It states that the faction of the incident light absorbed is proportional to the number of the absorbing molecules in the light-path and will increase with increasing concentration or sample thickness.

Lambert's law: It states that the fraction of the monochromatic light absorbed by a homogenous medium is independent of the intensity of the incident light and each successive unit layer absorbs an equal fraction of light incident on it.

From these two laws, the following empirical expression, known as **Beer-Lambert law**, may be formulated.

Log (I_0/I)

$= \varepsilon cl = A$

Where,

$I_{0=}$ intensity of incident light; I = intensity of emergent light

ε =molar absorptivity; C = concentration of solute in moles/litre

L =path length; A =absorbance

UV-visible spectrophotometer

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis

spectrophotometer. It measures the intensity of light passing through a sample (I), and compares it to the intensity of another light beam of same intensity and wavelength that did not passes through the sample (I_0). The ratio $I/I_0$is called the transmittance. The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator, a prism to separate the different wavelengths of light, and a detector. The radiation sources are often Tungsten filament (300-2500 nm), Deuterium arc lamp (190-400 nm), Xenon arc lamp (160-2,000 nm), Light emitting diodes (LED) for the visible wavelengths. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge coupled device (CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning monochromator moves the diffraction grating to "step-through" each wavelength so that its intensity may be measured as a function of wavelength. Fixed monochromators are used with CCDs and photodiode arrays. As both of these devices consist of many detectors grouped into one or two-dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.

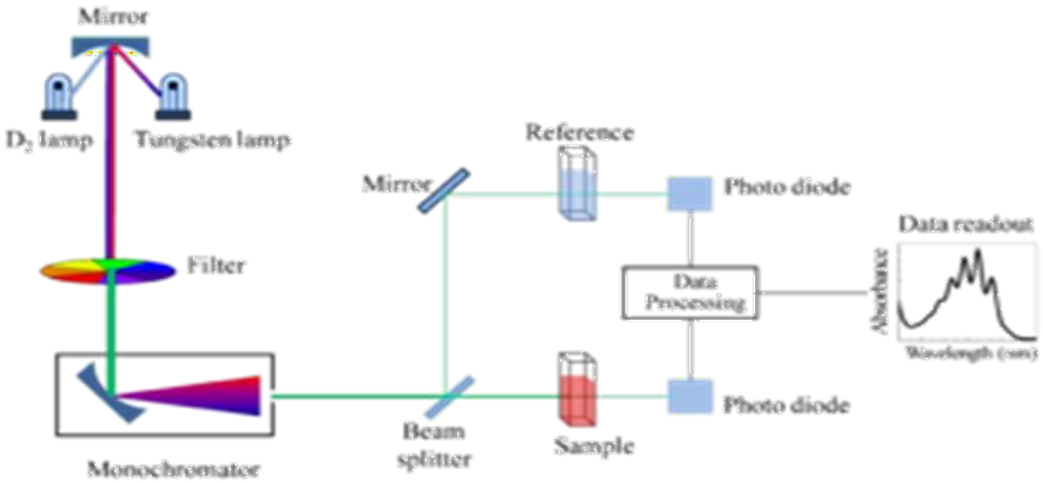

Fig 1.3 Schematic diagram of UV- visible spectrophotometer .

spectrophotometer can be either single beam or double beam. In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam.

1.4 Density Functional Theory method

Density functional calculations (often called density functional theory (DFT) calculations) are, like ab initio and SE calculations, based on the Schrodinger equation. However, unlike the other two methods, DFT does not calculate a wavefunction, but rather derives the electron distribution (electron density function)directly

Density functional theory is based on the **Hohenberg-Kohn theorems**, which state that, “The ground-state properties of an atom or molecule are determined by its electron density function, and that a trial electron density must give an energy greater than or equal to the true energy”. DFT is not variational - it can give an energy below the true energy.

In the Kohn-Sham approach the energy of a system is formulated as a deviation from the energy of an idealized system with non-interacting electrons The energy of the idealized system can be calculated exactly since its wavefunction (in the Kohn-Sham approach wavefunctions and orbitals were introduced as a mathematical convenience to get at the electron density)can be presented exactly by a slater determinant. The relatively small difference between the real energy and The energy of the idealized system contains the exchange correlation functional ,the only unknown item in the expression for the DFT energy the approximation of This functional is the main problem in DFT.

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. From the energy equation, by minimizing the energy with respect to the Kohn-Sham orbitals, the Kohn-Sham equations (***KS equations***) can be derived, analogously to the HF equations. The molecular orbitals of the KS equations are expanded with basis functions and matrix methods are used to iteratively find the energy, and to get a set of molecular orbitals, the KS orbitals, which are qualitatively similar to the orbitals of wave function theory.

The most popular current DFT method is the LSDA (Local Spin Density Approximation) gradient-corrected hybrid method which uses the B3LYP (Becke three parameter Lee-Yang-Parr) functional. For homolytic dissociation, correlated methods (e.g. B3LYP, pBP/DN* and MP2) are vastly better than HF-level calculations; these methods also tend to give fairly good activation barriers. DFT gives reasonable IR frequencies and intensities, comparable to those from MP2 calculations. Dipole moments from DFT appear to be more accurate than those from MP2. Time-dependent DFT (TDDFT) is the best method for calculating UV spectra reasonably quickly. DFT is said to be better than HF (but not as good as MP2) for calculating NMR spectra

1.41 Basis Sets

An approximate wavefunction (*e.*g. a Slater determinant) can be made up from MO's which are themselves approximated by atomic orbitals (LCAO). The AOs are in turn constructed from combinations of basis functions.

Basis functions ⟶ AO's ⟶ MO's ⟶ Wavefunction

The list of all basis functions used in a calculation is called basis set.

The basis function model all the possible ways that electrons behave in a molecule. We should include enough functions to model the orbital properly.

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A basis set is a set of mathematical functions (basis functions), linear combinations of which yield molecular orbitals. The functions are usually, but not invariably, centered on atomic nuclei. Approximating molecular orbitals as linear combinations of basis functions is usually called the LCAO or linear combination of atomic orbitals approach, although the functions are not necessarily conventional atomic orbitals: they can be any set of mathematical functions that are convenient to manipulate and which in linear combination give useful representations of MOs With this reservation, LCAO is a useful acronym. Physically, several (usually) basis functions describe the electron distribution around an atom and combining atomic basis functions yields the electron distribution in the molecule as a whole.

The electron distribution around an atom can be represented in several ways. Hydrogen-like functions based on solutions of the Schrodinger equation for the hydrogen atom, polynomial functions with adjustable parameters, Slater functions, and Gaussian functions have all been used. Of these, Slater functions (STOs) and Gaussian functions (GTOs) are mathematically the simplest, and it is these that are currently used as the basis functions in molecular calculations. Slater functions are used in semi-empirical calculations. Modern molecular *ab initio* programs employ Gaussian functions.

The GTF's have zero slope and no cusp at the nucleus. So GTF's have problems representing the proper behavior near the nucleus. GTF's fall off too rapidly away from the nucleus and the "tail" of the wave function is consequently represented poorly.

These problems can be solved by adding together several primitive Gaussians, called a **contraction**, with different exponents and coefficients into one basis function to approximate the shape

1.42Minimal Basis set

This basis set consists of one function each for the core orbitals and valence orbitals (whether occupied or not).

Hydrogen 1s = one basis function; Fluorine 1s + 2s +2px + 2py + 2pz = five basis functions

Carbon 1s + 2s +2px + 2py + 2pz = five basis functions. Unoccupied valence p orbital also counted.

Iron 1s, 2s, 2px, 2py, 2pz, 3s, 3px, 3py, 3pz, 3dxy, 3dxz, 3dyz, 3dx2, 3dy2, 3dz2, 4s, 4px,4py,4pz = 19 basis functions.

For example, “STO-3G” is short-hand for a minimal basis set in which each basis function is a contraction of three primitive Gaussians. The minimal basis sets are good for rough and. quick calculations, but are not very accurate.

1.43 Multi zeta Basis sets:

To overcome the deficiencies of the minimal basis set other basis sets have been developed.

- **Double zeta basis sets:** These have twice the number of functions for each orbital. Thus hydrogen would have two functions, carbon and oxygen 10 functions each.
- **Triple zeta basis sets** have thrice the number of functions compared to minimal basis set.
- **Split valence basis set** developed by Pople have single function for the core, the valence functions are split into double zeta or triple zeta type.

For example, “6-31G” basis set for fluorine: 1s orbital described by 6 primitive Gaussians contracted to one basis function, One set of 2s and 2p orbitals described by contraction of 3 primitive Gaussians, One set of 2s and 2p orbitals described by 1primitive Gaussian That is1

function for the core + 2 functions each for the valence 2s, 2px, 2py and 2pz orbitals. i.e. 9 functions after contraction.

6-311G means one basis function each for the core orbitals and three basis functions each for the valence orbitals with contractions of 6,3 ,1 and 1 primitives respectively.

- **Polarisation functions** are functions of higher angular momentum used to account for the polarization of atoms that occurs when forming chemical bonds. Usually p functions are used to polarises electrons, d functions to polarize p electrons and f functions to polarize d electrons.
  e.g. *p* functions for H or *d* functions for carbon

  The notation 6-31G(d) (or 6-31G*) implies a 6-31G basis set to which a set of polarization functions added to heavier atoms (non hydrogen atoms). The notation 6- 31G(d,p) (or 6-31G**) implies a 6-31G basis set to which a set of d polarization functions added to heavier atoms and a set of p functions on hydrogen atom.
- **Diffuse functions** are polarization functions which have a small exponent to describe the electron density away from the nucleus (eg for anions and weakly bonded molecules). They are indicated by a + symbol in the notation. Eg. 6-31++G(d) includes a set of polarization functions on heavy atoms and hydrogen. The choice of the basis set is dependent on the problem being considered and the availability of computational resources.

1.44 Guasian software

Gaussian is a computer program for computational chemistry initially released in 1970 by John Pople and his research group at Carnegie-Mellon University as Gaussian 70. It has been continuously updated since then. The name originates from Pople's use of Gaussian orbitals to speed up calculations compared to those using slater type orbitals a choice made to improve performance on the limited computing capacities often-current computer hardware for Hartree-Fock calculations. Originally available through the Quantum Chemistry Program Exchange, it has been developed and licensed by Gaussian, Inc. Gaussian quickly became a popular and widely used electronic structure program. Prof. Pople and his students and post-docs were among those who pushed the development of the package, to carry out cutting-edge research in quantum chemistry and other fields.

Gaussian 70,Gaussian76, Gaussian80, Gaussian 82, Gaussian 86,Gaussian 88,

Gaussian 90, Gaussian 92, Gaussian 92/DFT, Gaussian 94, Gaussian 98, Gaussian 03, Gaussian 09 and Gaussian 16 are different packages of Gaussian. Other programs named 'Gaussian XX' were placed among the holdings of the Quantum Chemistry Program Exchange. These were unofficial, unverified ports of the program to other computer platforms.

According to the most recent Gaussian manual, the package can do

- Molecular mechanics
-
- Semi-empirical quantum chemistry method calculations

  - Austin Model 1 (AM1), PM3, CNDO, INDO, MINDO/3,MNDO

- Self-consistent field (SCF methods)

  - Hartree – Fock method: restricted, unrestricted, and restricted open-shell

- Møller–Plesset perturbation theory (MP2, MP3, MP4, MP5).

- Built-in density functional theory (DFT)methods

  - B3LYP and other hybrid functionals

  - Exchange functional: PBE, MPW, PW91, Slater, X-alpha, Gill96,TPSS.

  - Correlation functionals: PBE, TPSS, VWN,[10]PW91, LYP, PL, P86,B95

- ONIOM (QM/MM method) up to three layers

- Complete active space (CAS) and multi-configurational self-consistent field calculations

1.5 Molecular Docking

Molecular docking is the process that involves placing molecules in appropriate configurations to interact with a receptor. Molecular docking is a natural process which occurs within seconds in a cell. In molecular modeling the term “molecular docking” refers to the study of how two or more molecular structures fit together. Over the years biochemists have developed numerous models to capture the key elements of the molecular recognition process. Although very simplified, these models have proven highly useful to the scientific community

The Lock and Key Theory as far back as 1890 Emil Fischer proposed a model called the "lock-and-key model" that explained how biological systems function. A substrate fits into the active site of a macromolecule, just like a key fits into a lock. Biological 'locks' have unique stereo chemical features that are necessary to their function. In 1958 Daniel Koshland introduced the "induced-fit theory". The basic idea is that in the recognition process, both ligand and target mutually adapt to each other through small conformational changes, until an optimal fit is achieved. In addition to small induced-fit adaptation, it has been observed that proteins can undergo much larger conformational changes. A recent model describes proteins as a pre-existing ensemble of conformational states. The plasticity of the protein allows it to switch from one state to another

Lock-and-key, induced-fit and the conformation ensemble model are not contradictory. Each one focuses on a particular aspect of the recognition process. The lock-and-key model introduces the principle of 3D complementarity, the induced-fit model explains how complementarity is achieved, and the ensemble model depicts the conformational complexity of proteins

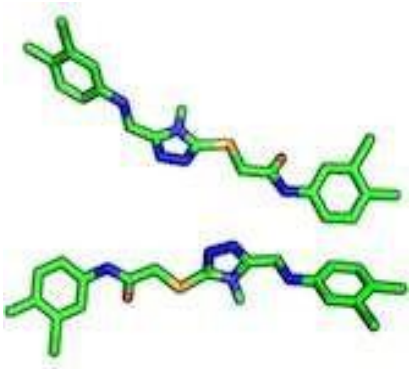
The difficulties in obtaining experimentally structural data of macromolecular complexes have triggered the development of computational predictive methods. Computational docking (also called in silico molecular docking or just docking) is a computational science aiming at predicting the optimal binding orientation and conformation of interacting molecules in space, and to estimate the stability of their complex. Molecular docking predicts whether or not the two molecules interact, the binding affinity and the 3D structure of the complex. Computational docking is an essential component in modern drug discovery. Over the last few decades, it has been routinely and successfully applied in most pharmaceutical and biotech companies for a large number of applications

Molecular docking classifies biomolecules into three categories: small molecules (also called 'ligands'), proteins, and nucleic acids .The most important types of docking systems are: protein ligand, protein-protein and nucleic acid-protein. The interactions between a small molecule and a protein are by far much better understood than those between a protein and a nucleic acids

Typically, the goals of molecular docking are the identification of a ligand that binds to a specific receptor binding site and the identification of its preferred, energetically most favorable, binding pose. Where the term “binding pose” considers the orientation of a ligand relative to its receptor as well as the ligand’s conformation. In order to accomplish this task, molecular docking tools will generate a set of different ligand binding poses and use a scoring function to estimate binding affinities for the generated ligand poses in order to determine the best binding mode.

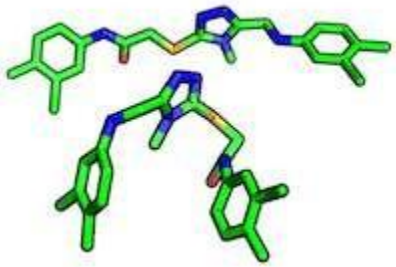
1.51 Ligand Orientation**:**

In contrast to “conformation”, the bond angles are the same between two/multiple ligands (as well as the chemical composition), but the orientation in space (transition, global rotation) is differs between two/multiple orientations.



1.52 Ligand Conformation:

Ligands/proteins can exist in different conformations. Usually, “conformation” refers to the same chemical composition but with altered bond-angles between two/multiple ligands or proteins.



1.53 Ligand Pose:

A ligand pose describes the binding-mode of a ligand in a protein binding site. Typically, this is considered to be a combination of orientation and conformation

Depending on the application, the a ligand of interest can either act as a receptor agonist - a molecule that triggers a response in a biological system upon binding - or a

receptor antagonist, which is a molecule that suppresses the agonist mediated response, respectively. In practice, often a large library of small drug-like compounds has to be screened in order to identify a promising lead compound. Thus, not only accuracy, but also computational efficiency is a major concern in the development of molecular docking software.

1.6 Computational docking

Computational docking is widely used for the study of protein– ligand interactions and for drug discovery and development. Typically, the process starts with a target of known structure, such as a crystallographic structure of an enzyme of medicinal interest. Docking is then used to predict the bound conformation and. binding free energy of small molecules to the target. Single docking experiments are useful for exploring the function of the target, and virtual screening, in which a large library of compounds are docked and ranked, may be used to identify new inhibitors for drug development.

.1.61 Auto Dock Vina Software

Auto Dock is a suite of free open-source software for the computational docking and virtual screening of small molecules to macromolecular receptors. The suite currently includes several complementary tools.

Auto Dock: a computational docking program based on an empirical free-energy force field and rapid Lamarckian genetic algorithm search method2,3. Raccoon2
an interactive graphical tool for virtual screening and analysis . Auto Dock Tools (ADT): an interactive graphical user interface (GUI) for coordinate preparation, docking and analysis5. Auto Ligand: a program for predicting optimal sites of ligand binding on receptors6. The Auto Dock suite, including source code, is freely available, and it has been widely used in research and drug discovery.

. Auto Dock Vina: a turnkey computational docking program that is based on a simple scoring function and rapid gradient optimization conformational search. Auto Dock Vina was developed more recently to fulfil the need for a turnkey docking method that does not require

extensive expert knowledge from users. It is highly optimized to perform docking experiments using well-tested default methods. Both methods are currently freely available. Auto Dock Vina is fast and effective for most system

Auto Dock Vina is an open-source program for doing molecular docking. It was originally designed and implemented by Dr. Oleg Trott in the Molecular Graphics Lab at The Scripps Research Institute. The latest version of Auto Dock Vina is v.1.2.0. Auto Dock Vina is one of the docking engines of the Auto Dock Suite.

1.7 Objectives of current work

- To synthesis and characterize aniline and p-nitro aniline derivatives of azo dyes.
- To optimize the structures of synthesized azo dyes by DFT method using Gaussian 09 software
- To find out the binding energy of α- Glucosidase –acarbose interactions (standard) by docking .
- To find out the binding energy of α- Glucosidase – azo dyes interactions by docking.
- To compare the binding energies of above interactions with standard and predict the potential α- Glucosidase inhibitor.

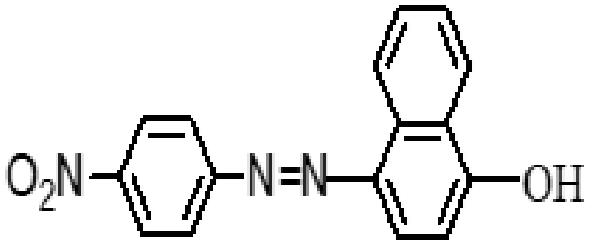
Chapter 2

Materials And Methods

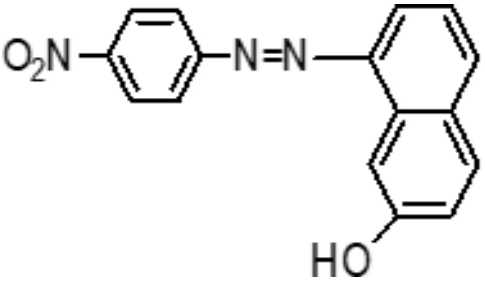
2.1 Experimental Section

The molecules chosen for the present study are

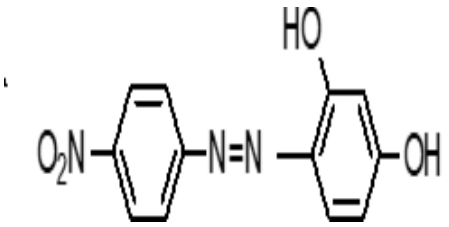
1. (E)-4-(nitrophenyl) diazenyl naphthalene-1-ol (NAA)



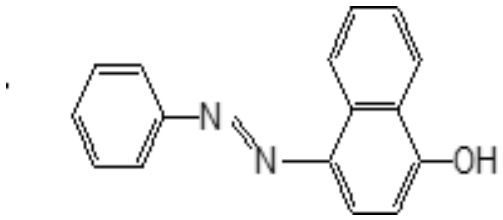
2. (E)-1-(nitrophenyl) diazenyl naphthalene-2-ol (NAB)



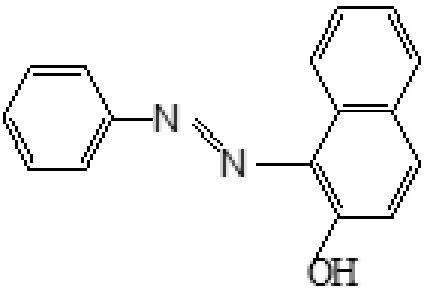
3.(E)-4-(nitrophenyl) diazenylbenzene1,3diol(NAR)



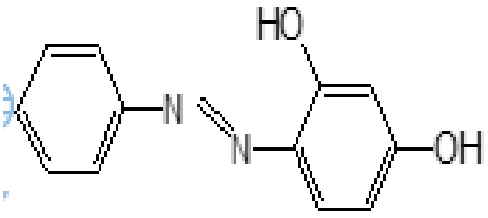
4.(E)-4-(phenyl)diazenylnaphthalene-1-ol (AA)



5.(E)-1-(phenyl)diazenylnaphthalene-2-ol (AB)



6.(E)-4-(phenyl) diazenylbenzene1,3diol (AR)



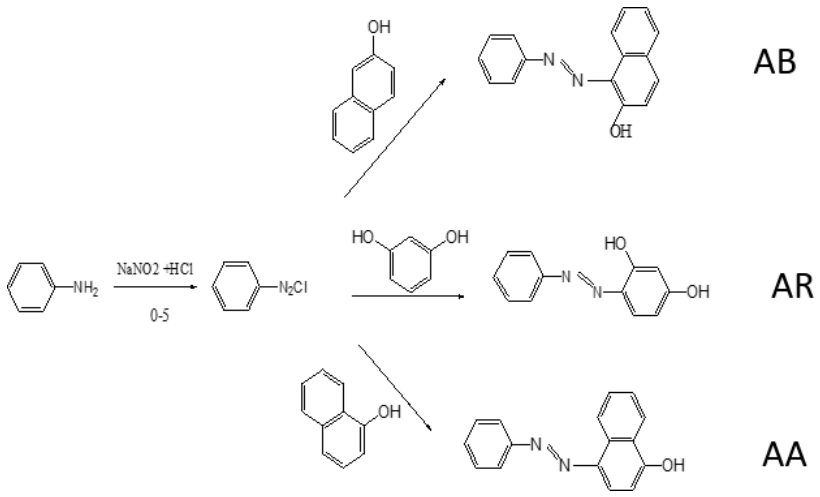
2.11 Chemicals

- Aniline
- P-nitroaniline
- Resorcinol
- 2-naphthol
- 1-naphthol

2.12 Synthesis of AA, AB, AR

The general procedure for the synthesis of aromatic azo compounds followed the traditional coupling approach i.e, coupling of aryl diazonium salt with electron rich aromatics such as naphthol and resorcinol

Synthesis is based on the following equation,



,

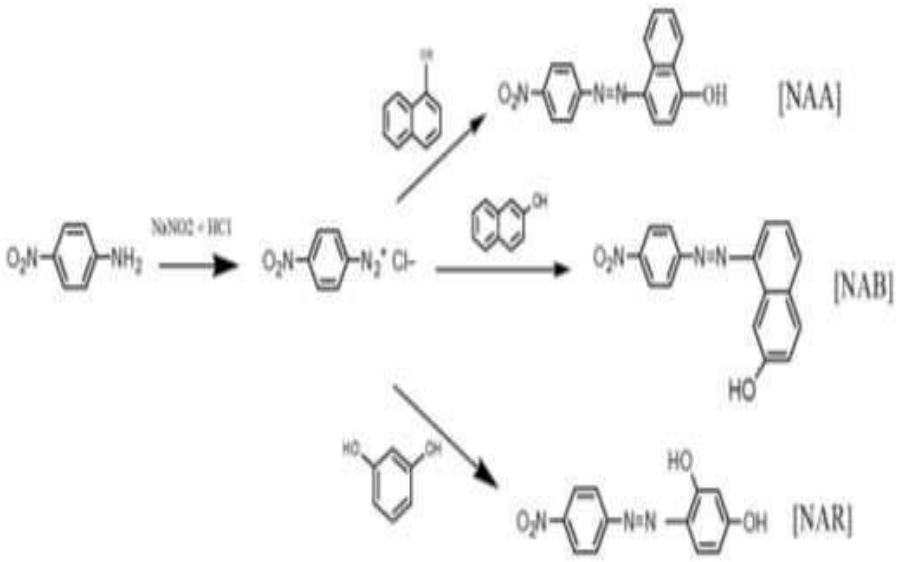
In a test tube, added 1.5 mL of water and 1.5 mL of conc. HCl and placed the test tube in an ice water bath. In a 25 mL RBF, add 0.7 ml of aniline (5 mmol), 0.38 g (5.5 mmol) of sodium nitrite ($NaNO_2$), 1.5 ml of water is added . Stirred the contents rapidly using a stirrer. Removed the test tube from the ice water bath and placed the RBF in the bath. Added the contents of the test tube to the RBF and stir gently for 10 minutes. Filtered the solid into a test tube using a glass funnel and a small cotton plug. In another 25 mL RBF with a magnetic stir bar, dissolved 0.74 g of 2-naphthol(5.1 mmol) / 0.74 g of 1- naphthol (5.1 mmol) /0.56g of resorcinol (5.1 mmol) in 10 mL of 2.5 M aq. NaOH and placed in an ice-water bath. Added the contents of the test tube slowly while stirring and continued stirring for 10 minutes while in the ice-water bath. Slowly added 1.5 mL of conc. HCl. Added 1 g of NaCl and heated the RBF until dissolved. Cooled the reaction to room temperature then placed in an ice-water bath for 15 minutes. Finally filtered the solid using vacuum filtration with a Buchner funnel and washed the solid with approximately 5 ml of water.

2.13 Synthesis of NAA, NAB,NAR

In a test tube, added 1.5 mL of water and 1.5 mL of conc. HCl and placed the test tube in an ice water bath. In a 25 mL RBF, add 0.7 g of 4-nitroaniline (5 mmol), 0.38 g (5.5 mmol) of sodium nitrite (NaNO2), 1.5 mL of water is added . Stirred the contents rapidly using a stirrer. Removed the test tube from the ice water bath and placed the RBF in the bath. Added the contents of the test tube to the RBF and stir gently for 10 minutes. Filtered the solid into a test tube using a glass funnel and a small cotton plug. In another 25 mL RBF with a magnetic stir bar, dissolved 0.74 g of 2-naphthol (5.1 mmol) /0.74 g of 1- naphthol (5.1 mmol) /0.56g of resorcinol (5.1 mmol) in 10 mL of 2.5 M aq. NaOH and placed in an ice- water bath.

Added the contents of the test tube slowly while stirring and continued stirring for 10 minutes while in the ice-water bath. Slowly added 1.5 mL of conc. HCl. Added 1 g of NaCl and heated the RBF until dissolved. Cooled the reaction to room temperature then placed in an ice-water bath for 15 minutes. Finally filtered the solid using vacuum filtration with a Buchner funnel and washed the solid with approximately 5 mL of water.

,
Synthesis is based on the following equation,



2.2 CHARACTERIZATION

Ultraviolet-Visible spectroscopy

Ultraviolet-visible spectroscopy (UV-Visible) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full adjacent visible spectral regions. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. Absorption measures transition from the ground state to the excited state. UV-Visible spectrum was recorded using Evolution 1800 Spectrophotometer in the range of 200-500nm

2.3 Computational details

Gaussian 09 software package was used for DFT calculation and calculations were performed at B3LYP/6-31G (d,p) level. The ground state structures were optimized and frequency calculations were performed to ensure that the optimized structures are minima in the potential energy surface. HOMO and LUMO for all the molecules are identified. Gauss View 5 cewere done using HPv185e workstation computer equipped with Intel 7 core processor and 24 GB RAM and Microsoft Windows as the operating system

2.4 Molecular docking

Docking of α- Glucosidase with acarbose and six Azo dyes were performed using Auto dock Vina software.

Structure of α- Glucosidase were downloaded from RCSB protein data bank. Acarbose is downloaded from pubchem. Thus the binding energies of acarbose and six azo dyes with the interaction between $\boldsymbol{\alpha}$ glucosidase

CHAPTER 3

RESULTS AND DISCUSSION

The synthesis of an Azo dye requires two organic compounds- a diazonium salt and a coupling component. The general synthesis of Azo dyes is shown below: The diazonium salt reacts as an electrophile with an electron-rich coupling component, like a β-naphthol and naphthalene derivative through an electrophilic aromatic substitution mechanism. The hydroxyl group (such as β-naphthol) direct the aryl diazonium ion to the para site unless that position is occupied, in which case the ion attaches ortho.

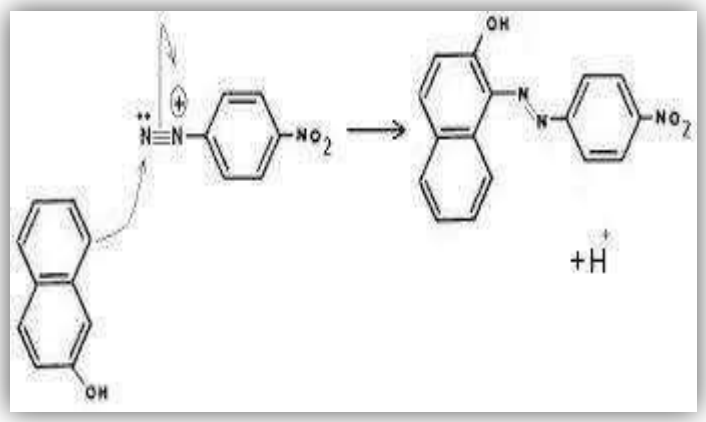


Fig. synthesis of NAB

Prepared compounds as Azo dyes were characterized by UV-Visible studies.

3.1UV- VIS SPECTRAL STUDY

The UV-Vis spectrum obtained was carried out in the range of 200 to 500 nm. For Azo dyes, we generally obtain a sharp peak over the range 350-400 nm. In the UV spectrum of the as synthesized Azo dye, an absorption peak was observed over the same range 350-400 nm to the absorption of azo group. This peak confirms the formation of Azo dye.

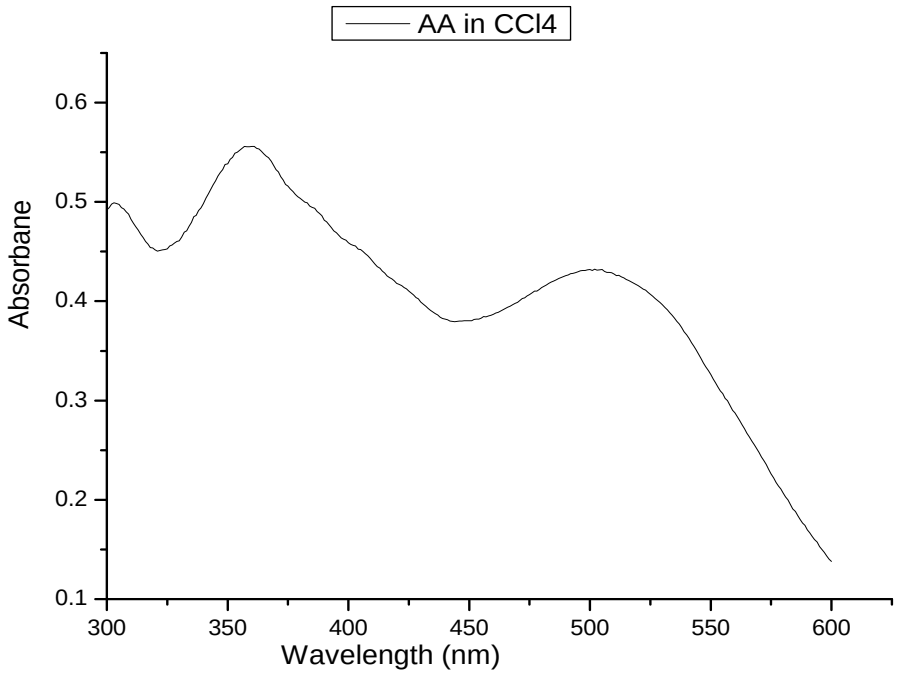


Figure. 3.1a UV absorption spectrum of compound AA

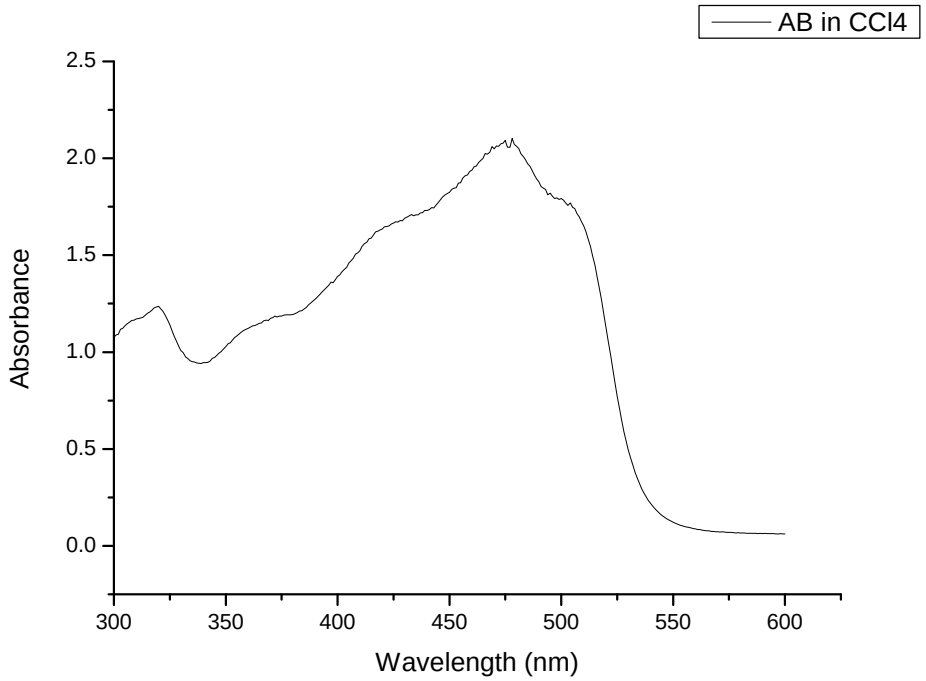


Figure.3.1b UV absorption spectrum of compound 2 AB

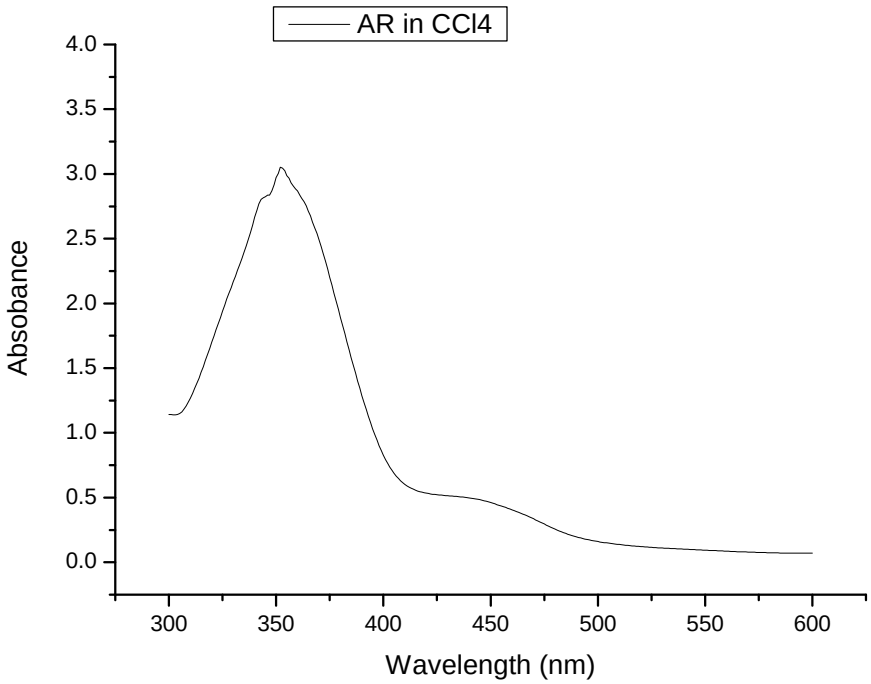
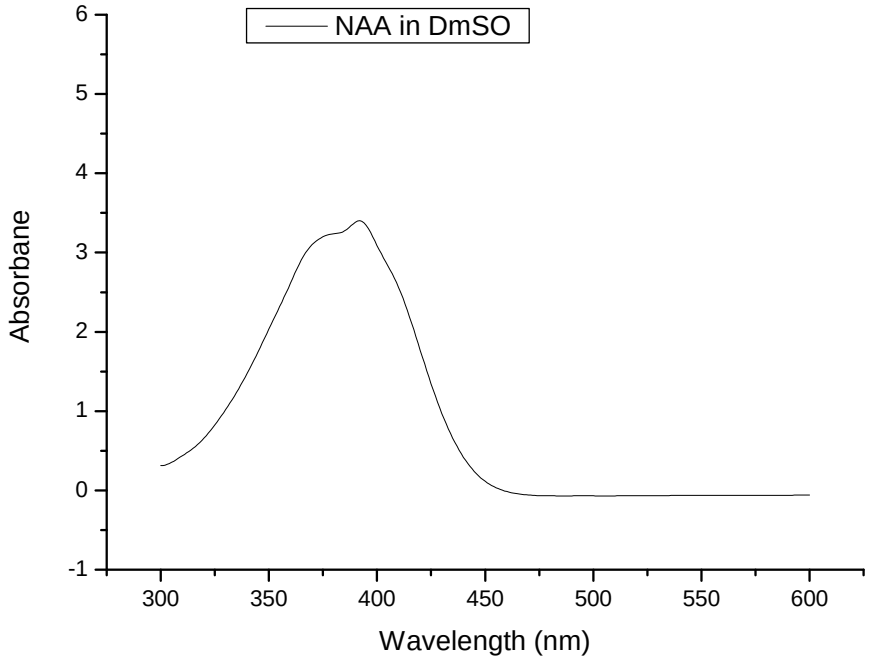


Figure. 3.1c UV absorption spectrum of compound 3 AR



G

Figure. 3.1d UV absorption spectrum of compound 4 NAA

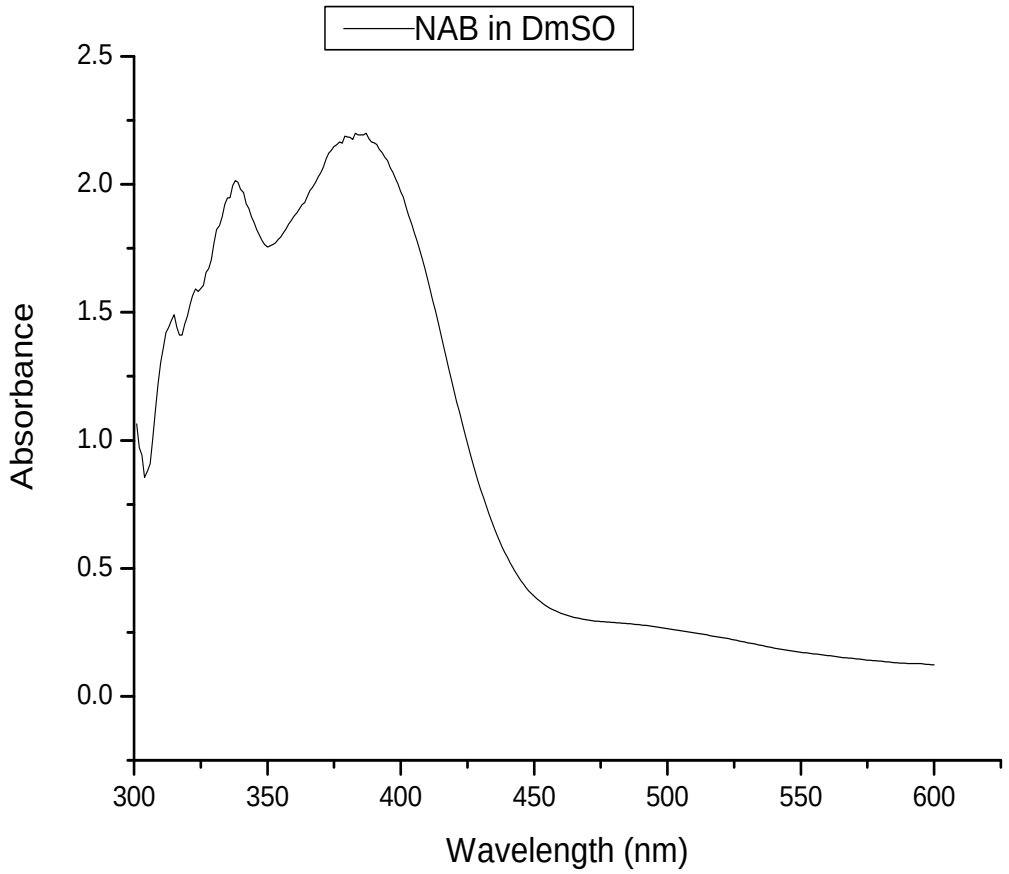


Figure.3.1e UV absorption spectrum of compound 5 NAB.

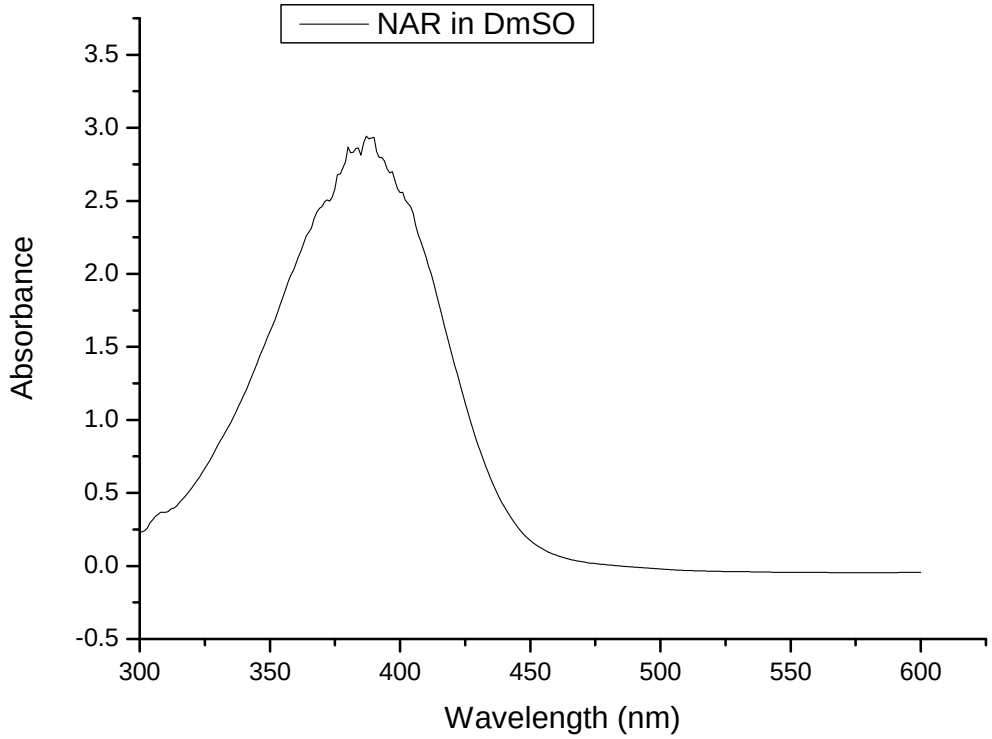


Figure.3.1f UV absorption spectrumof compound 6

NAR

UV Absorption maxima values of synthesized dyes

Azo Dye	λmax (nm)
AA	361
AB	353
AR	350
NAA	320
NAB	385
NAR	324

3.2 Geometry Optimization

The optimized molecular geometry represents an isolated molecule under ideal conditions with a stationary point at the potential energy surface. The convergence was confirmed by observing no imaginary vibrational frequencies. Several conformational isomeric ***cisoid*** and ***transoid*** structures of molecules were optimized at B3LYP/6-31G (d, p) level. The lowest energy structure of the molecule is given in below**. Figure, s**hows optimized geometry of synthesized dyes.

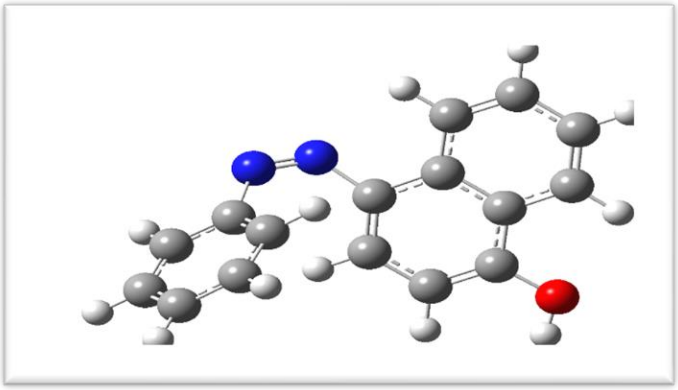


Figure 3.2a DFT Optimized structure of AA
Energy -801.62 Ha

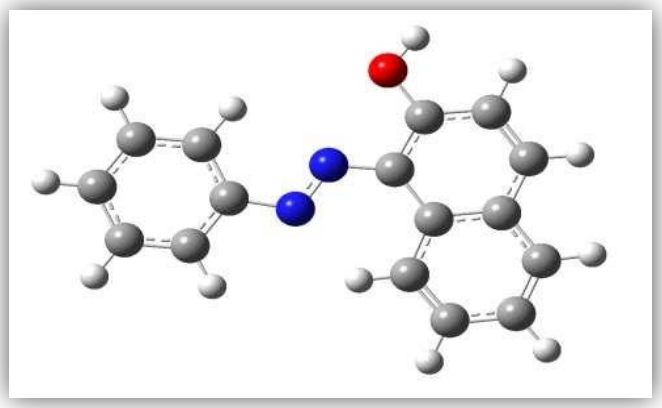


Figure.3.2b DFT Optimized structure of AB
Energy= -801.63 Ha

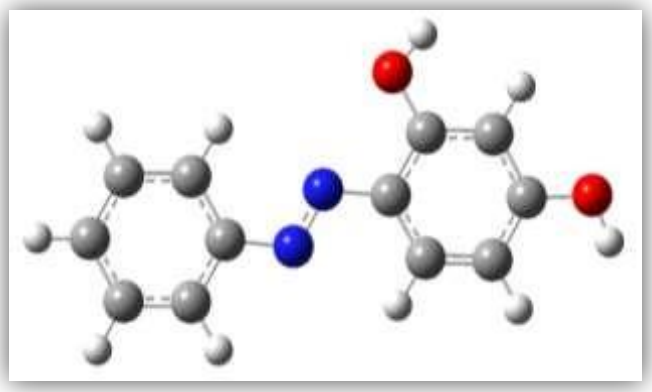


Figure.3.2c DFT Optimized structure of AR
Energy = -723.21Ha

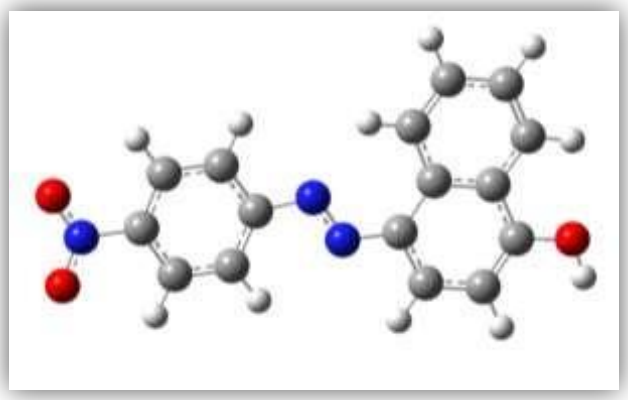


Figure 3.2d DFT Optimized structure of NAA

Energy = -1006.14 Ha

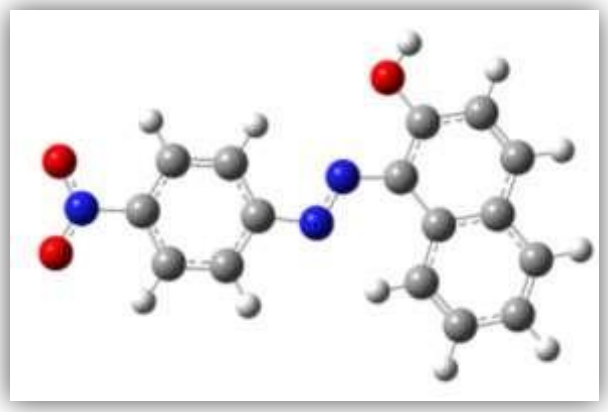


Figure.3.3e DFT Optimized structure of NAB
Energy = -1006.13 Ha

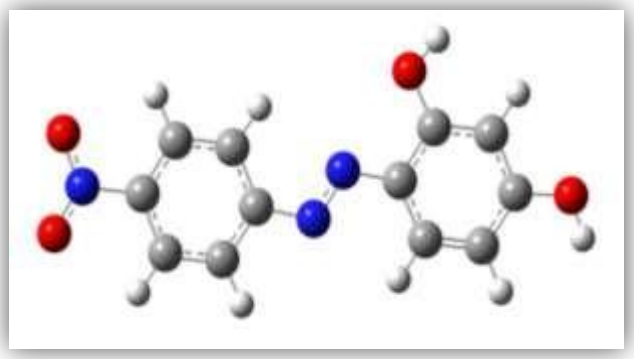
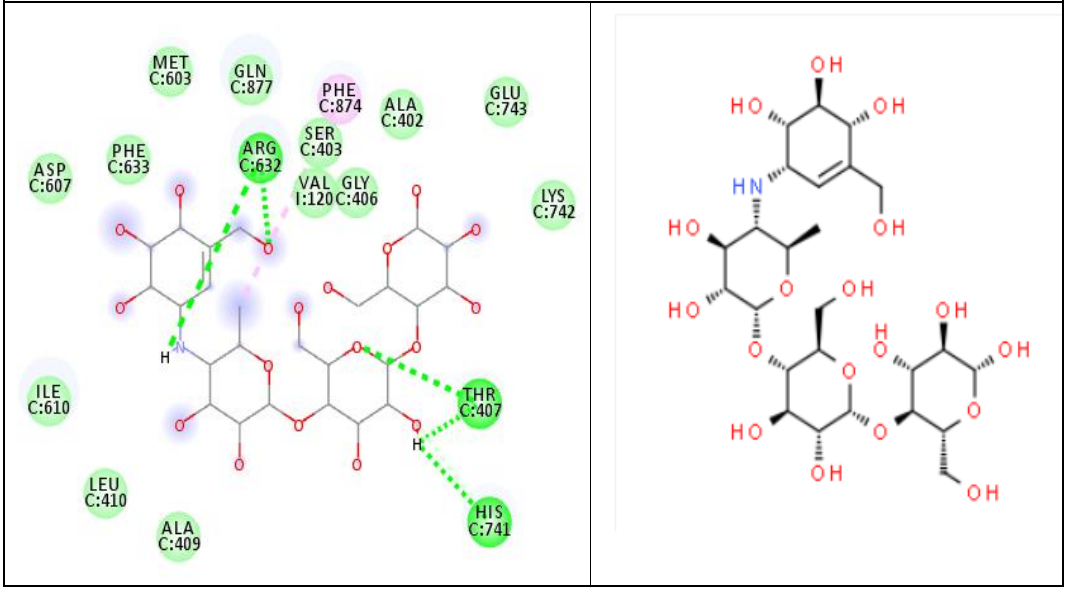


Figure. 3.2f DFT Optimized structure of NAR
Energy -927.71 Ha

3.3 Molecular docking studies

Molecular docking of α- Glucosidase with acarbose were done and results are shown in figure.

Fig 3.3a The active site of αglucosidase to acarbose Binding energy -7.7 kcal/mol



Docking studies of all compounds against alpha glucosidase are shown below and their binding energy was calculated

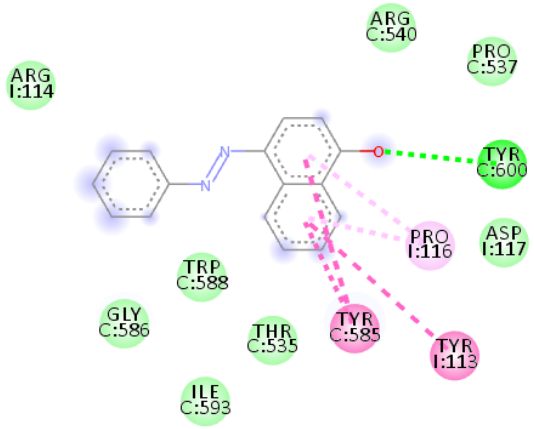
Fig 3.3b Docking of compound 1 AA with α glucosidase	
	Binding Energy = -7.2kcal/mol

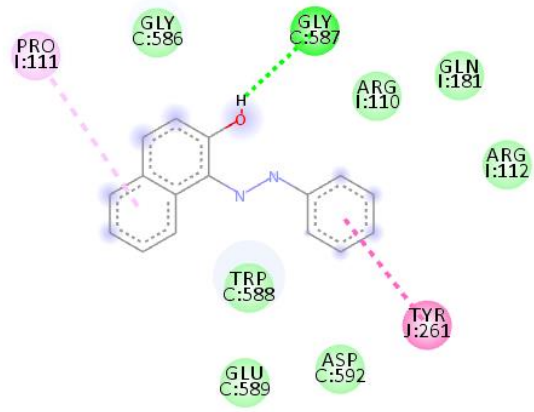
Fig 3.3c Docking of compound 2 AB with α glucosidase	
	Binding energy= -6.8 kcal/mol

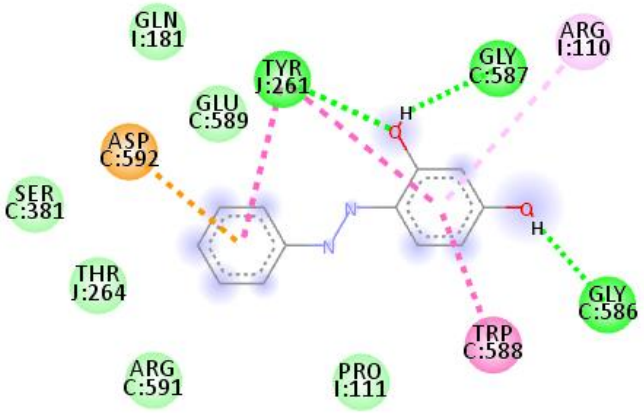
Fig 3.3d Docking of compound 3 AR with ***α*** glucosidase	
	Binding energy= -7.4kcal/mol

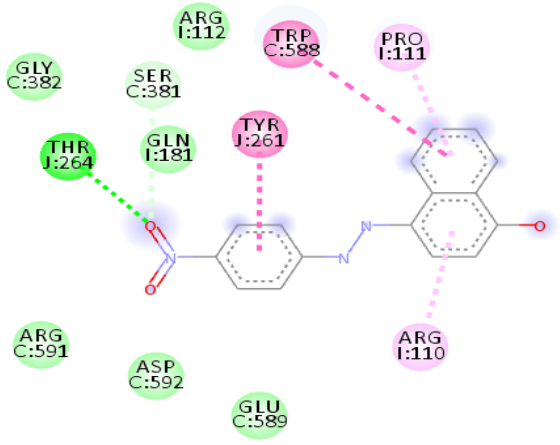
Fig 3.3e Docking of compound 4 NAA with ***α*** glucosidase	
	Binding energy = -7. 5 kcal/mol

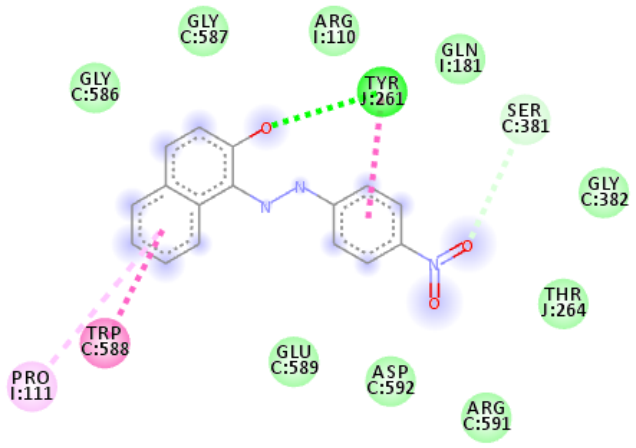
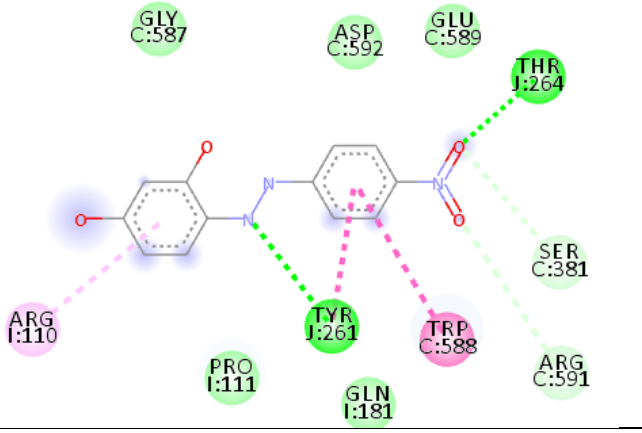
Fig 3.3f Docking of compound 5 NAB with αglucosidase	
	Binding energy = --7.8 kcal/mol

Fig3.3g Docking of compound 6 NAR with α glucosidase	
	Binding energy = -7.1kcal/mol

Azo dye	Binding energy Kcal/mol
AA	-7.2
AB	-6.8
AR	-7.4
NAA	-7.5
NAB	-7.8
NAR	-7.1

Chapter 4

CONCLUSIONS

The six azo dyes prepared by traditional approach i.e coupling of aryl diazonium salt of aniline and para nitroaniline and with electron rich aromatic compounds such as resorcinol and naphthol derivatives and characterized using uv spectrometer. The UV values of synthesized six azo dyes comes with in the range 350-400 nm .The combination of aromatic substituted amines with coupling agents could yield variety of azo compounds having versatile pharmacological properties including antioxidant, antidiabetic etc. In this project as a pharmaceutical application we studied the antidiabetic properties of six azo dyes using molecular docking with α glucosidase. These azo dyes can act as alpha glucosidase inhibitor by binding to the active site α glucosidase enzyme. The synthesized azo dyes were found to be most effective compared to the reference drug acarbose.The six azo dyes shows similar binding energy values with that of drug acarbose. Among the compound NAB (E)-1-(nitrophenyl) diazenyl naphthalene-2- ol was found to be more effective with higher binding energy than the other azo dyes.

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11 Diaryl azo derivatives as anti-diabetic and antimicrobial agents: synthesis, in vitro, kinetic and docking studies Tehreem Tahira , Mirza Imran Shahzada , Rukhsana Tabassumb , Muhammad Rafiqc , Muhammad Ashfaqb , Mubashir Hassand , Katarzyna Kotwica-Mojzyche and Mariusz Mojzychf https://doi.org/10.1080/14756366.2021.1929949