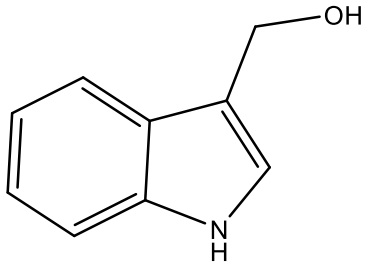
CHAPTER III

EXPERIMENTAL SECTION

III.1. NAME, STRUCTURE, PHYSICAL PROPERTIES AND APPLICATIONS OF THE BIOLOGICALLY ACTIVE MOLECULES, CYCLODEXTRINS, CALIXARENES AND SOLVENTS USED IN THE RESEARCH WORK

III.1.1. Biologically active molecules :

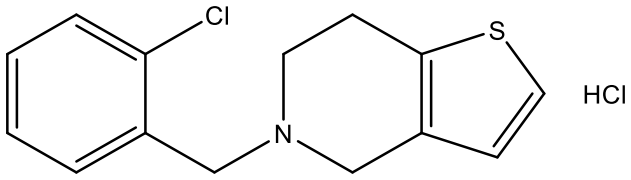
Indole-3-methanol : Indole-3-methanol (IM) is the product of the enzymatic hydrolysis of glucobrassicin in the human body.[1] IM has been examined concerning important role in cancer management. Recent studies showed that IM is expected to exhibit antioxidant activity. In human melanoma cells, IM result in proliferation arrest and apoptosis.[2]



CAS Number	700-06-1
Chemical formula	C_9H_9NO
Molar mass	147.17 g mol^{-1}
Appearance	Off white, Crystalline solid
Melting point	369-372 K
Solubility in water	10 mg mL^{-1}

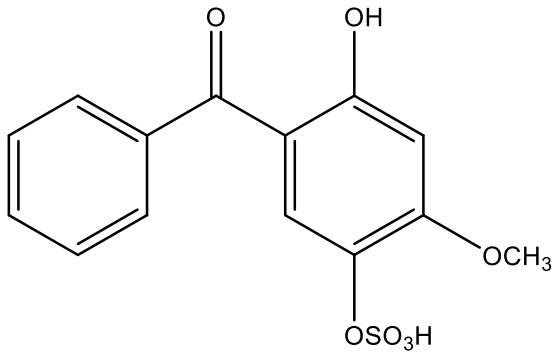
Ticlopidine hydrochloride : Ticlopidine hydrochloride (TCP) is a platelet antiaggregating agent whose use as a potent antithrombotic pharmaceutical ingredient.[3] Antiplatelet therapy prevents ischemic events in patients with high risk of arterial-

occlusive thrombosis and myocardial infarction.[4]



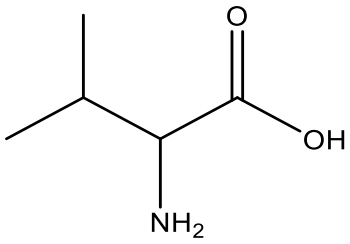
CAS Number	53885-35-1
Chemical formula	$C_{14}H_{15}Cl_2NS$
Molar mass	300.2 g mol^{-1}
Appearance	White crystalline solid
Melting point	478.15 K
Solubility in water	0.0219 mg mL^{-1}

Sulisobenzone : Sulisobenzone (SBZ) is a benzophenone based Food and Drug Administration (FDA) as well as European Union approved broad spectrum sunscreen agent. In Europe, the maximum amount of SBZ in commercial cosmetic products is fixed to 5%, while in the USA it is increased upto 10%, according to the U. S. Food and Drug Administration (FDA).[5] The sunscreen formulations helps to prevent from photo-aging as well as skin cancer. To increase the efficacy of the sunscreen, the formulation of a sunscreen requires the active chemical to penetrate less efficiently and to remain localized close to the skin surface for an extended period.[6]



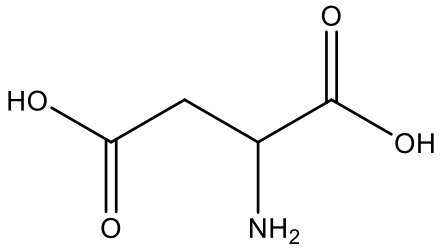
CAS Number	4065-45-6
Chemical formula	$C_{14}H_{12}O_6S$
Molar mass	308.31 g mol^{-1}
Appearance	Light tan powder
Melting point	418.15K
Solubility in water	250 mg mL^{-1}

L-Valine : L-Valine (Val) is the α-amino acid with chemical formula $C_5H_{11}NO_2$ that is commercially important for the need to supplement food or feed and to use in medical treatment and precursors for protein synthesis.[7] L-Valine has the ability to improve the lactation function of breeding animals and has been considered as one of the limiting amino acids in animal feed for poultry and pigs. The addition of L-Valine enhance moisturizing power in cosmetics and stimulate the synthesis of collagen. In the pharmaceutical industry, L-Valine is widely used as an active component of third-generation amino acids infusion.



CAS Number	72-18-4
Chemical formula	$C_5H_{11}NO_2$
Molar mass	117.151 g mol^{-1}
Appearance	White crystalline powder
Melting point	571.15 K
Solubility in water	83.4 g L^{-1}

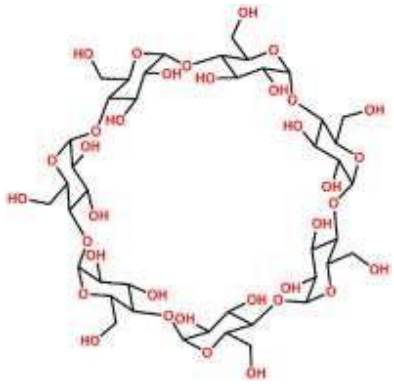
L-Aspartic acid : L-Aspartic acid (Asp), an endogenous amino acid are the primary active molecules in the human body to act as neurotransmitter. It also plays an important role in the neuroendocrine system, as well as in the development of the nervous system.[8]



CAS Number	56-84-8
Chemical formula	$C_4H_7NO_4$
Molar mass	133.103 g mol^{-1}
Appearance	White crystalline powder
Melting point	543.15 K
Solubility in water	4.5 g L^{-1}

III.1.2. Cyclodextrins and Calixarenes :

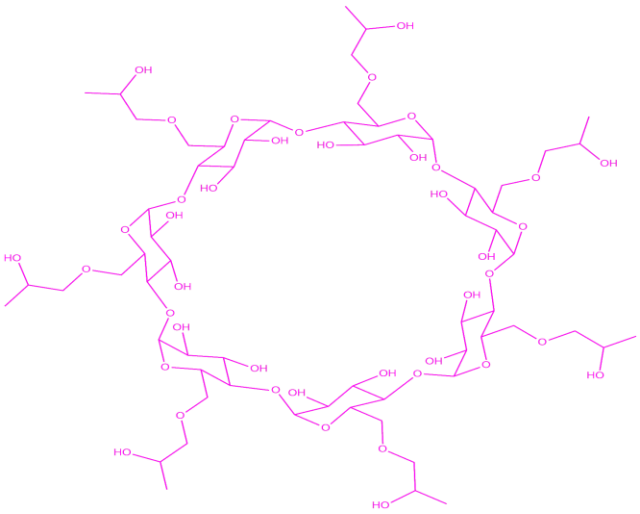
β-Cyclodextrin : β-Cyclodextrin (β-CD) is a most common cyclic oligosaccharide consisting of 7 glucopyranose units linked by α-1,4 glucosidic bonds. They can be used to form complexation with different 'guest' molecules and thus increasing solubility as well as bioavailability.[9]



CAS Number	7585-39-9
Chemical formula	$C_{42}H_{70}O_{35}$
Molar mass	1134.98 g mol^{-1}

Appearance	White powder
Solubility in water	18.5 g L^{-1}

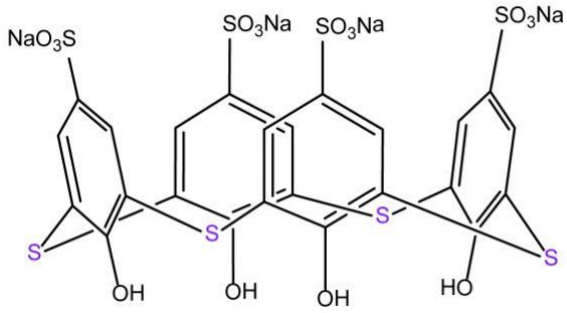
Hydroxypropyl-β-cyclodextrin : Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a chemically modified β-cyclodextrin derivative that possesses an increased safety profile than its naturally occurring parent compound.[10] HP-β-CD is used as an excipient for cardiac dysrhythmia, inflammation, and fungal disease medications.[11] Furthermore, HP-β-CD has been proposed as a vaccine adjuvant because it markedly enhances humoral immune responses to an influenza vaccine without any adverse effects.[12]



CAS Number	128446-35-5
Chemical formula	$C_{63}H_{112}O_{42}$
Molar mass	1541.54 g mol^{-1}
Appearance	White powder
Solubility in water	>500 g L^{-1}

***p*-Sulfonatothiacalix[4]arene :** *p*-Sulfonatothiacalix[4]arenes (TSC4X) are water soluble calixarene derivatives where phenolic units linked by sulphur groups at the 2- and 6-positions having ability to form inclusion complexes with several metal ions and organic systems.[13] Due to their canonical structures, they are widely used in ion sensing, molecular

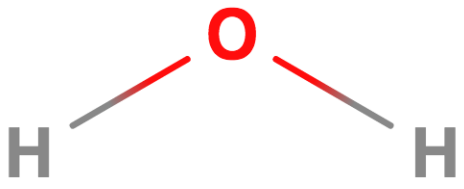
recognition, different pharmacological applications.[14] Depending on the guest size and preferred orientation, thiacalixarene can accommodate the guest molecule in their cavity through the driving forces such as hydrophobic and π-stacking interactions.



CAS Number	211561-04-5
Chemical formula	$C_{24}H_{12}Na_4O_{16}S_8$
Molar mass	904.78 g mol^{-1}
Appearance	White powder
Solubility in water	Highly soluble

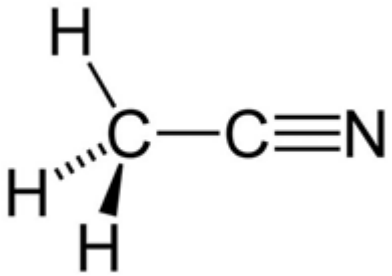
III.1.3. Solvents :

Water : Water is a polar inorganic chemical substance containing hydrogen and oxygen as an elements that is at room temperature a tasteless and odorless liquid, nearly colorless with a hint of blue. It is vital for maintaining health, growing food, generating energy and managing the environment. It is described as the "universal solvent" for its ability to dissolve different substances. This allows it to be the "solvent of life".



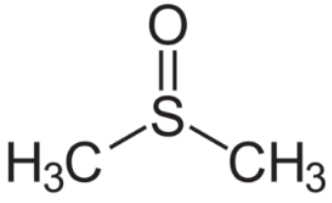
CAS Number	7732-18-5
Chemical formula	H_2O
Molar mass	18.015 g mol^{-1}
Appearance	Almost colorless, transparent, with a slight hint of blue liquid
Melting point	273.15 K
Boiling point	373.13 K
pK_a	13.995
Density	0.9998396 g/mL at 0 °C 0.9970474 g/mL at 25 °C
Refractive index	1.3330 (20°C)
Viscosity	0.890 cP
Dipole moment	1.8546 D
Specific heat capacity	75.375±0.05 J/mol K

Acetonitrile : Acetonitrile is a chemical compound and a derivative of acetic acid. This colourless, ether-like odor liquid is the simplest organic nitrile. It is used as a polar aprotic solvent in organic synthesis. In the laboratory, Acetonitrile is one of the most frequently utilized eluents in chromatographic separation technique due to its low viscosity, high chemical stability and strong eluting power. It dissolves a wide range of ionic and nonpolar compounds.



CAS Number	75-05-8
Chemical formula	C_2H_3N
Molar mass	41.05 g mol^{-1}
Appearance	Colorless liquid
Melting point	227 to 229 K
Boiling point	354.4 to 355.2 K
pK_a	25
Density	786 kg m^{-3}
Refractive index	1.344
Dipole moment	3.92 D
Specific heat capacity	91.69 J/K mol
UV-vis (λmax)	195 nm

Dimethyl sulfoxide : Dimethyl sulfoxide (DMSO) is a colorless organosulfur liquid. This colorless liquid is a significant polar aprotic solvent that dissolves both nonpolar and polar compounds and is miscible in water as well as a wide range of organic solvents. Many individuals experience a garlic-like taste in their mouth after coming into contact with DMSO. DMSO is widely used as an extractant in cell biology and biochemistry. It is also used to dissolve test compounds in *in vitro* drug discovery and drug design screening programs, including high-throughput screening programs.



CAS Number	67-68-5
Chemical formula	$(CH_3)_2SO$
Molar mass	78.13 g mol^{-1}
Appearance	Colourless liquid

Melting point	292 K
Boiling point	462 K
pK_a	35
Density	1.1004 g cm^{-3}
Refractive index	1.479
Viscosity	1.996 cP at 20^0C
Dipole moment	3.96 D

III.2. EXPERIMENTAL METHODS

III.2.1. Preparation of Solid Inclusion Complexes

The solid inclusion complexes were in general prepared by mixing host and guest in 1:1 molar ratio. A mixed solution of host and guest in 1:1 molar ratio was prepared in a definite volume of solvent. Then the mixed solution was stirred in a magnetic stirrer at around 48-55℃ for 30-48 hours till the emergence of a precipitate. The precipitate was filtered and then stored in a hot air oven till the solvent gets removed. The obtained solid inclusion complex was kept in a dessicator prior to analysis.

III.3. DESCRIPTION AND USE OF THE INSTRUMENTS INVOLVED IN THE RESEARCH WORK

III.3.1. Mass Measurement

Mass measurements were carried out on digital electronic analytical balance by Mettler Toledo, AG 285, Switzerland.

It can measure mass to a really very high precision and accuracy. The weighing pan of a high precision (0.0001g) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation.

III.3.2. Water Distiller

Water was distilled by an auto-connected distiller unit by Borosil Glass Works Limited, India.

A heating element in the boiling chamber heats the water until it boils. The steam rises from the boiling chamber. Volatile contaminants (gases) are discharged through a built-in vent. Minerals and salts are retained in the boiling chamber as hard deposits or scale. The steam enters a coiled tube (condenser), which is cooled by cool water. Water droplets form as condensation occurs. The distilled water is collected in a storage tank.

III.3.3. Magnetic Stirrer for Preparation of Solution and Solid Inclusion Complexes

The solutions of various biologically-active molecules and cyclodextrins have been prepared on magnetic stirrer. The solid inclusion complexes have also been prepared on the magnetic stirrer cum hot plate made by IKA.

III.3.4. Surface Tension Measurement

The surface tension experiments were completed by platinum ring detachment method employing a Tensiometer (K9, KRŰSS; Germany) at the experimental temperature.

The precision of the measurement was within the uncertainty level ±0.1 mNm^{-1}. Temperature of the system has been well-maintained by circulating auto-thermostated water (within ± 0.01 K) through a double-wall glass vessel holding the solution. Surface tension measurement technique works based on force develop at the surface.

III.3.5. UV-Visible Spectroscopic Measurement

Compounds that absorb Ultraviolet and/or Visible light have characteristic absorbance curves as a function of wavelength. Absorbance of different wavelengths of light occurs as the molecules move to higher energy states. UV-Visible spectra were recorded by Agilent 8453 UV-Visible spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The measurements were carried out at different temperature maintained by an automated digital thermostat.

The UV-Visible spectrophotometer comprises of two light sources, a deuterium lamp for ultraviolet light and a tungsten lamp for visible light. Initially the light source gets bounce back on a mirror, then, passes through a slit and hits a diffraction grating. The light beam strikes a second mirror before it gets split by a half mirror. One of the beams is allowed to undergo through a reference cuvette (which contains the solvent only), the other beam passes through the sample cuvette. The intensities of the light beams are then measured at the end and consequently the absorbance of the sample can be easily calculated.

III.3.6. Fluorescence Spectroscopic Measurement

The application of fluorescence spectroscopy is growing remarkably as a strong and effective tool to review the physical and chemical behavior of macromolecules. For X-ray

fluorescence analysis X-ray sources are needed, which emit constant characteristic radiation over an extended time. Until recently tubes with several kW power were needed to shorten measurement time and to reinforce precision. The main advantage of spectro-fluorometry over UV-Visible spectrophotometry is its high sensitivity of upto 10 to 100 times. Steady State fluorescence spectra were recorded by PTI QuantaMaster Fluorescence spectrophotometer.

III.3.7. FT-IR Spectroscopic Measurement

Infrared (IR) spectroscopy is a commonly used analytical technique for chemical sample containing different functional groups is irradiated over a range of wavelengths. It is worth mentioned that different bonds absorb energy that can be recorded as a series of spectroscopic peaks. This technique is traditionally used to determine the presence of functional groups that have well known spectroscopic signatures, and it can also be used to identify hydrogen bonds, formation of inclusion complex in supramolecular chemistry. The existence of strong shifting or deviation of peak position is often evidence for supramolecular complex formation between molecules with complementary hydrogen bonding motifs. FTIR spectra were recorded in a Perkin Elmer FT-IR spectrometer with a resolution of 2 cm^{-1} in the region of 4000-400 cm^{-1} at room temperature (25°C). This KBr optics based instrument records data in different modes (KBr pellets, non-aqueous solutions).

III.3.8. ^{1}H NMR Spectroscopic Measurement

The Nuclear Magnetic Resonance (NMR) provides us structural information at the molecular level of different biological, organic and inorganic substances in liquid environment. Configured for high resolution NMR experiments, it is invaluable to chemists, molecular biologists and material scientists. Most NMR signals appear from atoms that are related through bonds, such as hydrogen atoms on adjacent carbons, that correspond to atoms of the host and guest that interact. The strength of the shifts helps to understand the orientation of the guest within the host and other useful information. ^{1}H NMR were recorded at Bruker Avance 300 MHz NMR spectrometer in D_2O at 298.15 K. Signals are cited as δ values in ppm using residual protonated solvent signal as internal standard (HDO : δ 4.79 ppm). Data are presented as chemical shift.

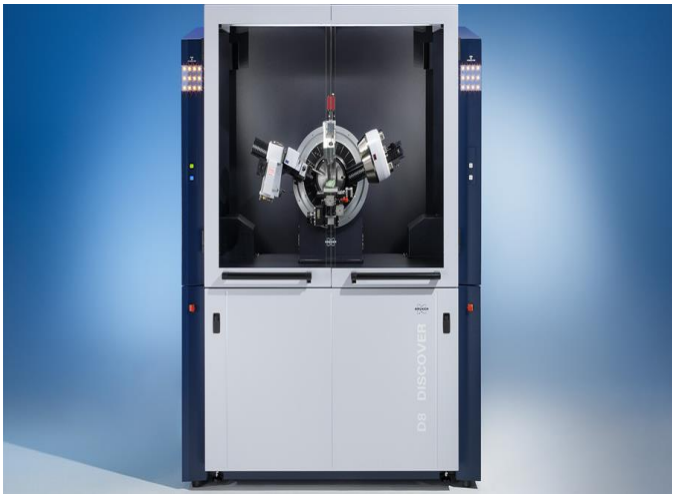
III.3.9. Mass Spectrometry Measurement

Mass spectrometry is a powerful tool for characterizing supramolecular complexes and assemblies. It has great importance in supramolecular chemistry as soft ionization methods can be used to detect weakly associated assemblies and their fragments. Classic

examples include the analysis of supramolecular capsules in which dimeric capsules containing guests can be identified as well as empty capsules and monomeric components following their molecular ion peaks. Different MS techniques are available such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI). However, electrospray ionization (ESI-MS) and high-resolution electrospray ionization (HRMS) being a soft ionization technique now-a-days are extensively used for the characterization of both the noncovalent interactions and the supramolecular structures of cucurbit[n]urils, calixarenes, crown ethers, catenanes, rotaxanes and cyclodextrins.

III.3.10. Powder X-Ray Diffraction (PXRD)

Powder X-ray diffraction (PXRD) is one such characterization technique that augment the advantage of simultaneously characterizing both the precursor and end product with a detailed qualitative presentation of their microstructural behaviors. PXRD may be a versatile non-destructive characterization tool that gives an in depth view of the chemical composition and crystallographic structure of the sample. PXRD provides only one-dimensional information where diffraction peaks with similar *d* values overlap and determine the crystallinity degree or amorphization of the examined samples.



III.3.11. Differential Scanning Calorimetry (DSC)

A technique during which the difference in energy inputs into a substance and a reference material is measured as a function of temperature whilst the substance and reference material are subjected to a controlled temperature program. Two distinct modes, power-compensation DSC and heat-flux DSC, can be distinguished, depending on the method of measurement used. In general, for the power compensation DSC curve, heat flow rate should be plotted on the ordinate with endothermic reactions upwards, and for the heat-flux DSC curve with endothermic reactions downwards. We can measure melting point and enthalpy of our host guest system through DSC. Differential Scanning Calorimetry (DSC) spectra were measured by Perkin Elmer Pyris 6 DSC calibrated using pyris manager software. The samples were heated in the temperature range 30–500°C in an inert nitrogen atmosphere at a heating rate of 10°C/min. The samples were taken in an aluminum container containing a certain amount of sample.

III.3.12. Scanning Electron Microscopy (SEM)

The surface morphology and size of the pure materials and the changes produced during the formation of inclusion complexes could be analyzed by SEM. Scanning electron microscopy (SEM) morphological images were obtained using JEOL JSM IT 100 scanning electron microscope (SEM) throughout this research work. The images were captured at required excitation voltages and magnifications for different samples.

III.3.13. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis is a technique during which the mass of a substance is measured as a function of temperature whereas the substance is subjected to a controlled temperature program. The recorded curve is called thermogravimetric or TG curve. Thermal

events like melting, crystallization and glass transition, don't cause a change within the mass of the sample, , but thermal changes accompanying mass change, such as decomposition, sublimation, reduction, desorption, absorption and vaporization, are often measured by TG.

III.3.14. Molecular Docking Study

Molecular modelling (MM) is not strictly an analytical tool that can be used directly. It is, however, a valuable way of visualizing supramolecular structures and predicting their spatial arrangement. Molecular docking (MD) is commonly used to predict the bound conformations of ligands and protein binding sites. Different softwares like AutoDock 4.2, MOE2015, PyRx, Gaussian have been employed for molecular modelling purpose. The MD methods are able to predict certain properties like Gibb's free energy, enthalpy, binding constant associated with the model that can usefully be compared to data gathered on the real system. This is useful when several different interpretations of an experiment arise as one model could also be shown to suit the data set and so be the foremost probable explanation. The main limitations of molecular modelling and computational techniques, in general, are the accuracy of the output data, and the size of simulation can also depend on system sizes and consequently on timescales.