

Design, Synthesis, and In Vitro Evaluation of Novel Saccharin Derivatives for Anticancer Activity Against Neuroblastoma (SH-SY5Y Cell Line) Through Targeting Anaplastic Lymphoma Kinase (ALK).

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ABSTRACT

The study was designed, synthesised, characterized, and evaluated by using MTT Assay as a potential anticancer agent, new Saccharin-7-Sulphonamide derivatives will be tested on human Neuroblastoma SY5Y cancer cell lines. These are potential anticancer agents that target the Anaplastic Lymphoma Kinase receptor. As part of the computational design process, a variety of software tools were used to generate the structural models for the synthesized compounds, including Chemscketch, Swiss ADME, Molinspiration, OSIRIS property explorer, and PyRx. According to Lipinski rule predictions made using Swiss ADME, the properties of the Lipinski rule are not violated. As part of the assessment of the effectiveness of the synthesized compounds as anticancer agents, molecular docking studies were conducted with ALK, which demonstrated promising binding affinities between the synthesized compounds and ALK molecules. There was a determination and characterization of the percentage yield, molecular formula, solubility, melting point, appearance, and molecular formula of the compounds that were investigated. In order to confirm the purity, TLC was used and Rf values were calculated to determine the purity. It was further characterized by IR, NMR, and Mass spectroscopy in order to determine the nature of the compound. It has been demonstrated that all synthesized compounds exhibit anti-cancer activities, and at concentrations of 20, 40, and 50g/mol, SA2 exhibits significant efficacy against Neuroblastoma SY5Y cancer cell line. This study showed that CompoundSA2 exhibited the most potent activity with an IC50 value of $5.253 \pm 1.761 \mu\text{mol}$, whereas SA 1, 3, 4, and 5 exhibited moderate activities with IC50 values ranging from 1 to 40 $\mu\text{g/ml}$. This study suggests that synthesized Saacharin with Sulphanilamide derivatives, especially SA2, may prove to be promising candidates for further development as anticancer agents that target Neuroblastoma ALK receptors and are promising candidates for further development.

Aim: To develop the novel and potent anti-cancer agents against neuroblastoma [SY5Y-cell line]

Objective: To design molecules, synthesize, characterize and determine anti-cancer activity, acute toxicity and cytotoxicity.

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INTRODUCTION

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn't. These cells may form tumors,

which are lumps of tissue. Tumors can be cancerous or not cancerous (benign). Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors. Many cancers form solid tumors, but cancers of the blood, such as leukemias, generally do not.[1]

The four main stages of cancer are:[2]

Stage 1 — Cancer is normally localized in a small area

Stage 2 — The size of the cancer increases

Stage 3 — The size of cancer becomes larger and starts spreading to some parts of the body including lymph nodes

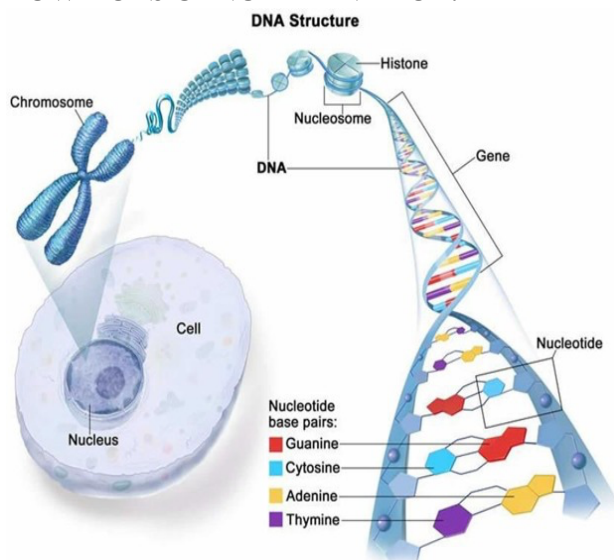
Stage 4 — Cancer has grown and has spread to most parts of the body

Types of cancer [3]

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. There are

- Carcinoma
- Leukemia
- Lymphoma
- Multiple Myeloma
- Brain and Spinal Cord Tumors
- Melanoma

HOW DOES CANCER DEVELOP?



Diagnosis [4]

Diagnosing cancer at its earliest stages often provides the best chance for a cure. For a few cancers, studies show that screening tests can save lives by diagnosing cancer early. Review the various guidelines with your doctor and together you can determine what's best for you based on your own risk factors for cancer.

- Cancer screening
- Lab tests used to diagnose cancer. Some common types of lab tests used to help diagnose cancer are listed below.

PET scan, MRI, CT scan, Complete blood count (CBC), Blood chemistry test.

Treatment [5]

Many cancer treatments are available. Your treatment options will depend on several factors, such as the type and stage of your cancer, your general health, and your preferences.

Targeted drug therapy, Immunotherapy, Bone marrow transplant, Radiation therapy, Chemotherapy, Surgery, Hormone therapy

BIOLOGICAL TARGET

Anaplastic Lymphoma Kinase (ALK) Receptor Tyrosine Kinase

Anaplastic lymphoma kinase (ALK) was first found as a tyrosine kinase in anaplastic large-cell lymphoma (ALCL) cell lines. In these cell lines, ALK fusion proteins (NPM-ALK) resulting from chromosomal translocation were found. The human ALK gene is located at chromosome region 2p23.2–p23.1. This gene, which contains 26 exons, encodes the full-length ALK protein with 1620 amino acids. [6]

ALK is an enzyme with tyrosine kinase activity, which catalyzes the transference of a gamma-phosphate group from adenosine triphosphate (ATP) to a tyrosine residue on a substrate protein. Therefore, it catalyzes a tyrosine residue phosphorylation reaction on its substrate proteins. The phosphorylation and dephosphorylation of proteins are critical reactions catalyzed by different enzymes (kinases and phosphatases), which play critical roles in various cellular functions. [7]

ALK is usually expressed during the development of the nervous system. During mouse development, ALK expression was found in the central and peripheral nervous system, such as spinal cord motoneurons, sympathetic ganglia, and dorsal root ganglia. A recent study showed that ALK was expressed by sympathetic neuroblasts during some stages of mouse embryonic development. After the birth of the mouse, the ALK expression level in the nervous system decreased.

Additionally, during the development of chicks, ALK expression was found in the developing central and peripheral nervous system, including spinal cord motoneurons, sympathetic ganglia, and dorsal root ganglia.

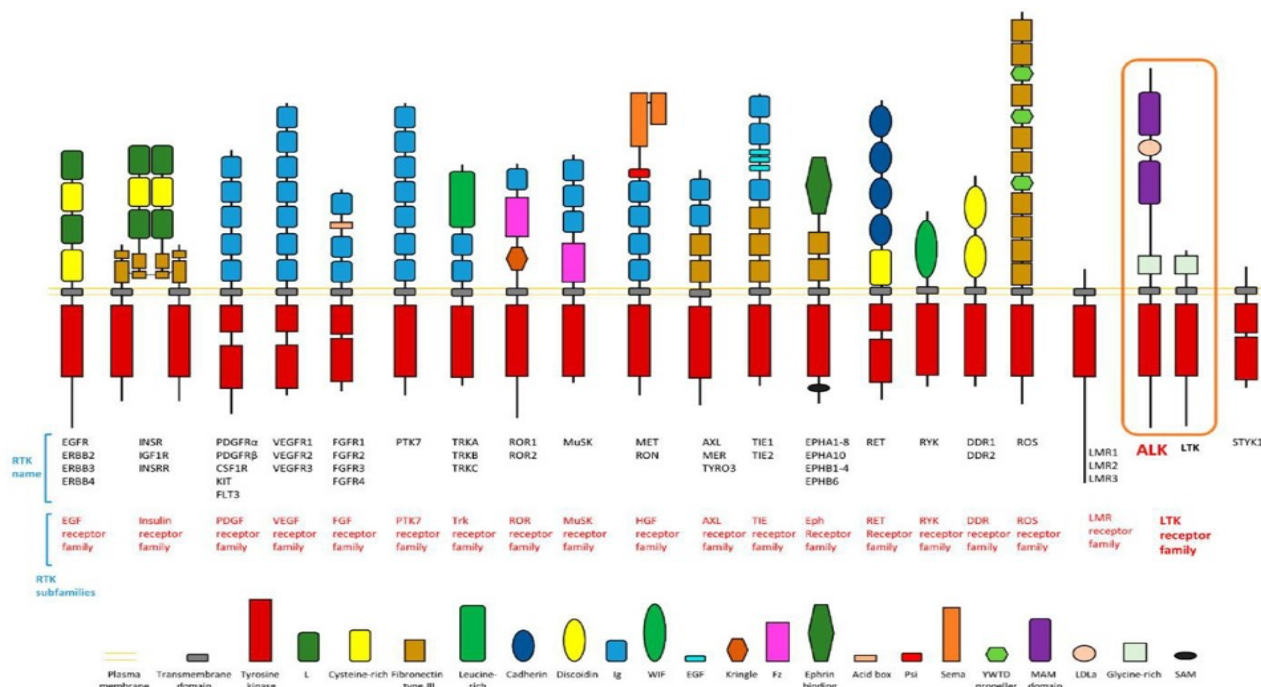
In adult mammals, a relatively low level of ALK expression exists in certain regions of a few organs, such as the hippocampus within the brain. Studies have shown that ALK is expressed in several regions of the hippocampus in the mouse brain,

including the dentate gyrus, cornu ammonis (CA) 1 region, and CA3 region.[8]

Although it is highly possible that the biological functions of mammalian ALK are related to the development and function of the nervous system, the direct biological roles of ALK are still not completely clarified. The study of ALK gene knockout mice indicates that ALK can affect the mouse brain functions. Some behaviors closely related to brain functions were observed to differ

between ALK gene knockout mice and wild-type mice. For instance, several studies showed that ALK knockout mice displayed elevated ethanol consumption compared to wild-type mice.[9]

This mini-review presents information on different aspects of ALK. Because several features of ALK biology are summarized and described in this review, a summarized illustration of these ALK features is presented.

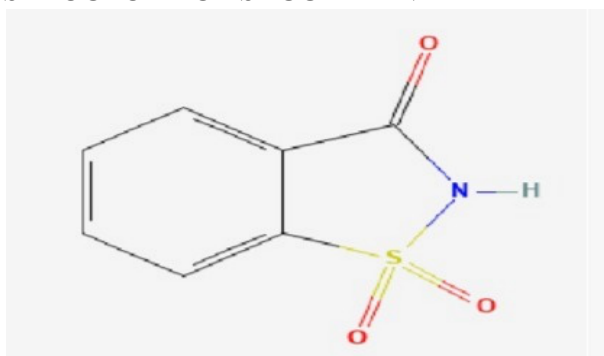


Domain structure of receptor tyrosine kinase families with anaplastic lymphoma kinase (ALK)

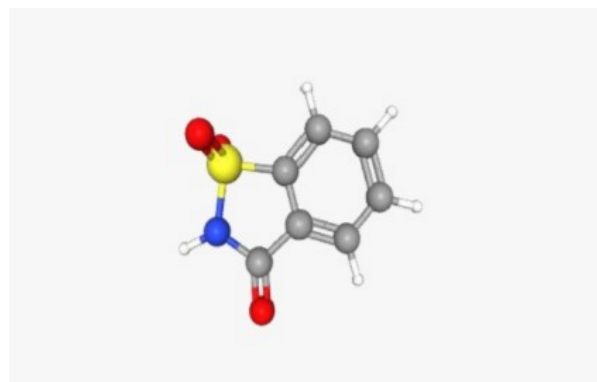
BASIC NUCLEUS: SACCHARIN

Saccharin, organic compound employed as a non-nutritive sweetening agent. It occurs as insoluble saccharin or in the form of various salts, primarily sodium and calcium. Saccharin has about 200–700 times the sweetening power of granulated sugar and has a slightly bitter and metallic after taste.

STRUCTURE OF SACCHARIN



3D STRUCTURE OF SACCHARIN



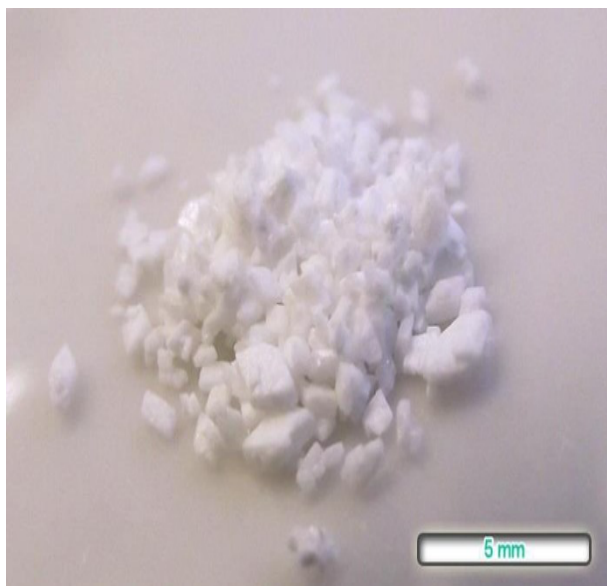
Saccharin was discovered by the chemists Ira Remsen and Constantin Fahlberg in 1879, while they were investigating the oxidation of o-toluenesulfonamide. Fahlberg noticed an unaccountable sweet taste to his food and found that this sweetness was present on his hands and arms,

despite his having washed thoroughly after leaving the laboratory.

Checking over his laboratory apparatus by taste tests, Fahlberg was led to the discovery of the source of this sweetness saccharin. Saccharin became the first commercially available artificial sweetener. It is still made by the oxidation of o-toluenesulfonamide, as well as from phthalic anhydride.[11]

Properties [12]

Chemical formula	C7H5NO3S
Molar mass	183.18 g·mol ⁻¹
Appearance	White crystalline solid
Density	0.828 g/cm ³
Melting Point	228.8 to 229.7 °C (443.8 to 445.5 °F; 501.9 to 502.8 K)
Solubility in water	1 g per 290 mL
Acidity (pKa)	1.6
Polar surface area	63.24 Å ²
Refractivity	42.39 m ³ . Mol ⁻¹
Polarizability	16.02 Å ³
Hydrogen Acceptor Count	3
Hydrogen Donor Count	1



That requirement was dropped in 2000 following new research that concluded humans reacted differently than rats and were not at risk of cancer at typical intake levels. The sweetener has continued to be widely used in the United States and is now the third-most popular artificial sweetener behind sucralose and aspartame.[13]

In the European Union, saccharin is also known by the E number (additive code) E954

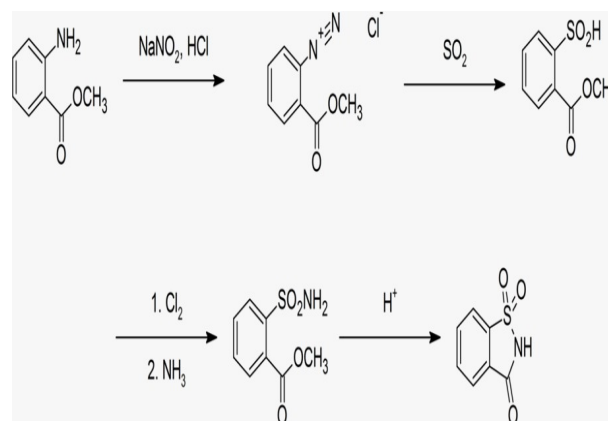
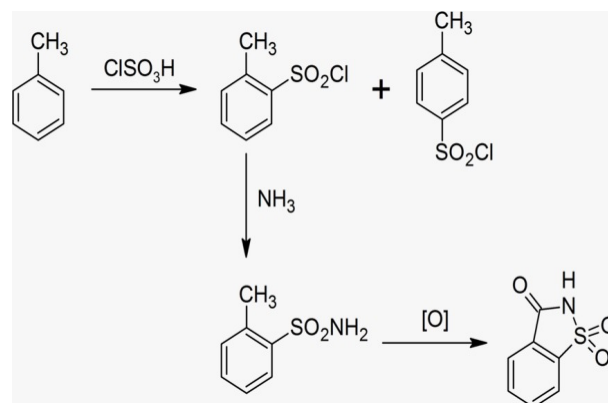
The current status of saccharin is that it is allowed in most countries, and countries such as Canada have lifted their previous ban of it as a food additive. The claims that it is associated with bladder cancer were shown to be unfounded in experiments on primates. (It is, however, prohibited to mail saccharin tablets or packets to France.) [14]

Saccharin was formerly on California's list of chemicals known to the state to cause cancer for the purposes of Proposition 65, but it was delisted in 2001.[15]

Preparation of Saccharin

Saccharin can be produced in various ways. The original route by Remsen and Fahlberg starts with toluene; another route begins with o-chlorotoluene. Sulfonation of toluene by chlorosulfonic acid gives the ortho and para substituted sulfonyl chlorides.

The ortho isomer is separated and converted to the sulfonamide with ammonia. Oxidation of the methyl substituent gives the carboxylic acid, which cyclizes to give saccharin free acid.



Pharmacokinetic Characteristics of Saccharin

Unlike glucose and sucrose, saccharin cannot be metabolized by the body.[16] Saccharin has been reported as a stable compound under a wide range

of conditions and no detectable metabolism of saccharin was shown either in animals or humans.[17]

Current research hypothesizes that because saccharin is highly polar, it is slowly and incompletely absorbed from the gut, but rapidly eliminated in the urine, leading to a reduction in its concentration within the plasma. The decrease in plasma concentrations after oral dosing is governed by the absorption rate, which is a phenomenon described as a flip-flop situation typical of molecules which are absorbed slowly but eliminated rapidly.[18]

Since saccharin is not metabolized and does not produce food energy, it has become an important sweetener, especially for diabetics. The affect of saccharin on insulin release is controversial. Ionescu et al. (1988) reported that saccharin triggered the release of insulin in genetically modified obese rats due to its taste, whereas Whitehouse et al. (2008) showed that saccharin did not affect blood insulin levels in animals that were treated with the sweetener from the time of conception to death.[19]

Toxicological studies [20]

In 1977, research showed bladder tumors in male rats with the ingestion of saccharin. The FDA proposed a ban on saccharin based on the Delaney Clause of the Federal Food, Drug, and Cosmetic Act enacted in 1958.

This clause prohibits the addition to the human food supply of any chemical that had caused cancer in humans or animals. Congress intervened after public opposition to the ban.

Possible danger of saccharin [21]

Saccharin is the possibility of allergic reactions. The reaction would be in response to it belonging to a class of compounds known as sulfonamides, which can cause allergic reactions in individuals who cannot tolerate sulfa drugs. Reactions can include headaches, breathing difficulties, skin eruptions, and diarrhea.

It's also believed that the saccharin found in some infant formulas and can cause irritability and muscle dysfunction.

For these reasons, many people still believe that the use of saccharin should be limited in infants,

children, and pregnant women. Without research to support these claims, the FDA has not imposed any limitations.

Saccharin and Oxidative Stress

Saccharin may induce oxidative stress on the liver cells through lowering catalase activity and the total antioxidant concentration (TAC) in plasma. It was demonstrated that saccharin harmfully affects both hepatic and renal tissues and alters biochemical markers, not only at high doses, but also at low doses in rats.[22]

Different methodologies have been applied to assess oxidative stress in animal models and humans. DNA bases are highly susceptible to reactive oxygen species (ROS) oxidation. The predominant detectable DNA oxidation product in vivo is 8-hydroxy-2'-deoxyguanosine (8- OHdG). 8-OHdG was established as sensitive marker of DNA damage, becoming a very

important biomarker for processes such as carcinogenesis, aging, and degenerative diseases.[23]

During the process of lipid peroxidation, a number of compounds are formed including alkanes, malondialdehyde, and isoprostanes, which are used as markers in lipid peroxidation assay. [24]

The discovery of isoprostanes has important implications for medicine as described by Morrow and Roberts. Measurement of F2-isoprostane is the most reliable approach to assess oxidative stress, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease. [25]

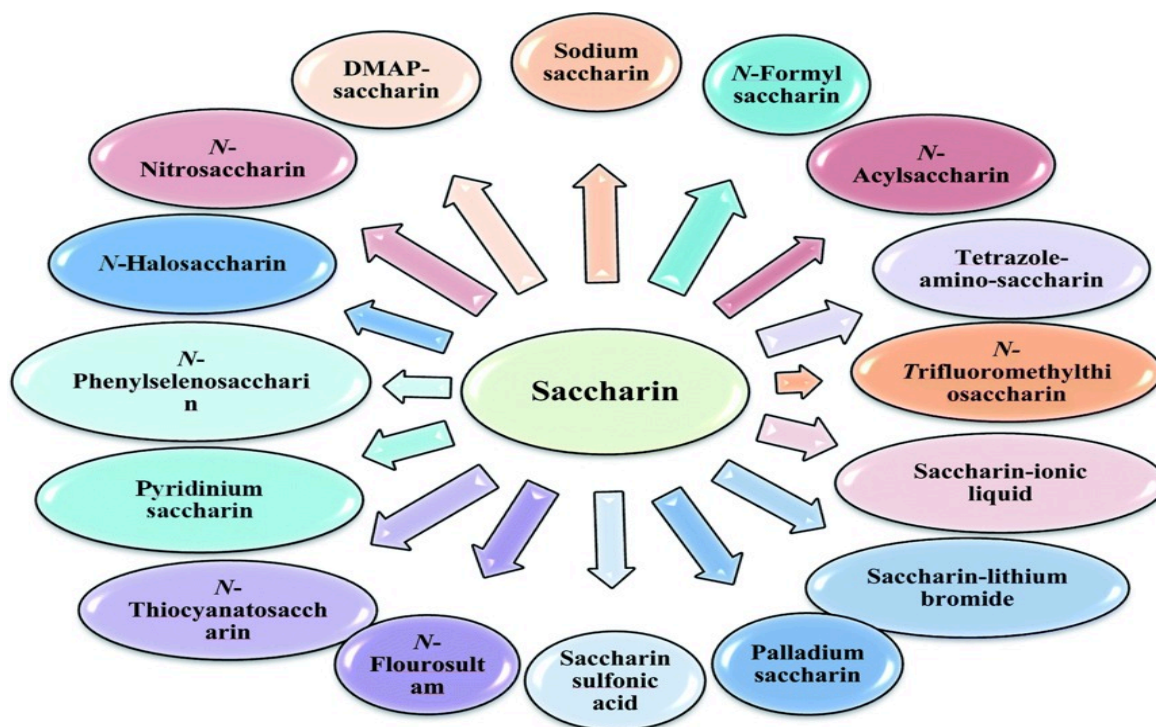
Pharmacological activity

On the basis of various literature surveys saccharine derivatives shows various pharmacological activity.

- Hyperglycemia
- Hypertension
- carbonic anhydrase inhibitors

Saccharin as catalyst

The catalytic applicability of saccharin and its derivatives on various organic transformations. The studies of saccharin and its derivatives as a catalyst for synthesis of a diverse range of a broad area of molecules.

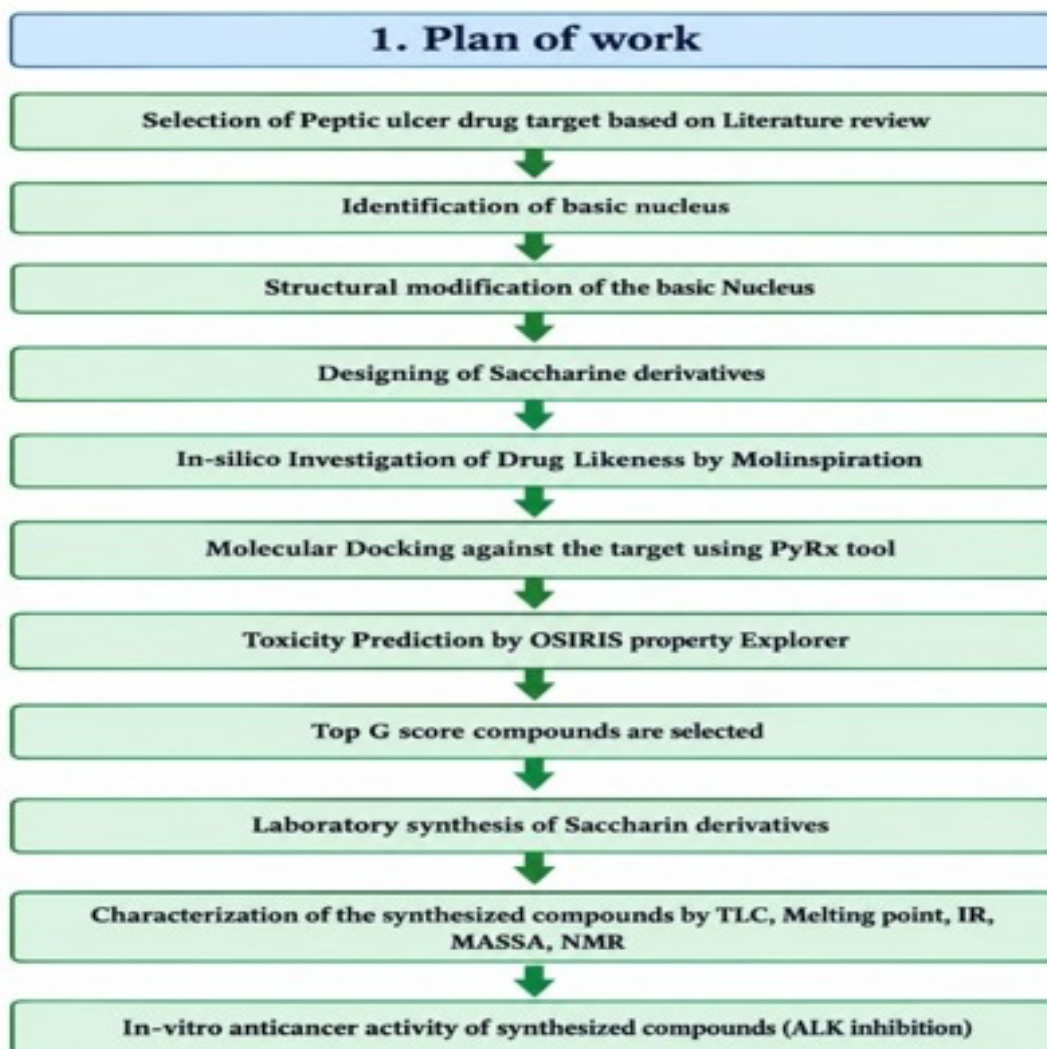


AIM: To develop the novel and potent anti-cancer agents against neuroblastoma [SY5Y-cell line]

OBJECTIVE: To design molecules, synthesize, characterize and determine anti-cancer activity, acute toxicity and cytotoxicity.

The plan of work includes:

- ❖ Insilico Drug likeness prediction.
- ❖ Design of Anaplastic Lymphoma Kinase inhibitors by docking studies using Autodock.
- ❖ Insilico Toxicity Assessment.
- ❖ Laboratory synthesis of those compounds with top Docking Scores.
- ❖ Characterization of the synthesized compounds by
 - Melting point
 - Infrared Spectroscopy
 - LC-Mass Spectroscopy
 - H1 Nuclear Magnetic Resonance Spectroscopy
- ❖ In-vitro anti -cancer activity of synthesized compound by MTT assay.



MOLECULAR DESIGN AND DOCKING COMPUTER AIDED DRUG DESIGN:

Drug discovery is the process by which drugs are discovered and/or designed. Rational Drug Design (RDD) is the inventive process of finding new molecules based on the knowledge of the biological target. Computer Aided Drug Design (CADD) computational tools and software's are used to simulate drug receptor interactions. [65]

Computer-aided drug design (CADD) techniques are used for the rapid assessment of chemical libraries in order to guide and speed up the early-stage development of new active compounds. CADD entails a vast number of computational methodologies like virtual screening, virtual library design, lead optimization and de novo design. The use of these methods depends on the nature of the target and the available information on the system.

Computer aided drug design (CADD) has offered valuable tools in the identification of compounds, minimizing the risk of later rejection of lead compounds. Even though high throughput screening (HTS) usually offers several hit compounds, success rates are often very low and many of the identified compounds are later rejected due to their physicochemical properties.

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme, which results in agonism or antagonism. Identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using docking programs. This method is known as virtual screening.

DOCKING: [66]

Docking program is used to fit the ligand molecule into the target structure in a variety of positions, conformations and orientations. Docking mode is known as pose. Each pose is scored based on its complementarities to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetic pose.

SCORING FUNCTIONS:

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

ΔG_0 – empirically derived that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.

ΔG_{hb} – contribution from hydrogen bonding

ΔG_{ionic} – contribution from ionic interactions

ΔG_{lip} – contribution from lipophilic interactions where is surface area of lipophilic contact between the ligand and receptor

ΔG_{rot} – entropy penalty due to freezing a rotatable bond in the ligand bond upon binding

$\Delta G_{bind} = -RT \ln K_d$

$$K_d = \frac{[Ligand][Receptor]}{[Complex]}$$

$\Delta G_{bind} = \Delta G_{desolvation} + \Delta G_{motion} + \Delta G_{configuration} + \Delta G_{interaction}$ Where: $\Delta G_{desolvation}$ is the enthalpic penalty for removing the ligand from solvent

ΔG_{motion} is the entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor $\Delta G_{configuration}$ is the conformational strain energy required to put the ligand in its "active conformation" $\Delta G_{interaction}$ is the enthalpic gain for "resolvating" the ligand with its receptor.

TYPES: [67]

Direct and indirect designs are the two strategies usually employed in drug design process. There are two major types of drug design.

- Ligand-based drug design (LBDD)
- Structure-based drug design

LIGAND-BASED:

Ligand-based drug design (or indirect drug design) relies on knowledge of ligand molecules that bind to the biological target of interest. These molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

STRUCTURE-BASED:

Structure-based drug design (or direct drug design) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

TARGET ENZYME:

H⁺K⁺ATPase was selected as the target enzyme for the study. The crystal structure of the enzyme was downloaded from the Protein Data Bank (An information Portal to Biological Macromolecular Structure) PDB ID – 5YLU.

MOLECULAR DOCKING BY AUTODOCK®:[68]

Autodock® tool 4.2.5.1 is an automated procedure for predicting the interaction of ligands with bio macromolecular targets. Progress in biomolecular x-ray continues to provide important protein and nucleic acid structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to the understanding of fundamental aspects of biology. The precise interaction of such agents or candidate molecules with their targets is important in the drug discovery process.

In any docking scheme, two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level. The ideal procedure would find the global minimum in the interaction energy between the substrate and the target protein and exploring all available degrees of freedom (DOF) for the system.

AutoDock® tool combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom. The quality of any docking result depends on the starting structure of both the protein and the potential ligand. The

protein and ligand structure need to be prepared to achieve the best docking results.

STEPS INVOLVED IN DOCKING:

- Protein preparation
- Ligand preparation
- Receptor grid generation
- Ligand docking (screening)

DOCKING PROCEDURE:

- ✓ Molecular docking study with a ligand molecule.
- ✓ Molecular Docking steps using PyRx software

Protein preparation

- STEP-1: Open Pyrx > Read molecule > Processed protein.pdb,
- STEP-2: Processed protein.pdb > Right click > Molecular surface view,
- STEP-3: Processed protein.pdb > Right click > Make macromolecule (pdbqt).

Ligand preparation

- STEP-4: Open babel > Import ligand > Right click > Minimize energy > Vina wizard
- STEP-5: Open vina wizard > Start > Select both > Macromolecule & Ligand > Forward Grid box
- STEP-6: Set grid box > Maximize > Forward (Blind Docking) Docking
- STEP-7: Forward > Docking progress > Status > Running Report-Binding energy
- STEP-8: Docking status > Finished > Docking score > Binding energy > Saved in Excel.
- STEP-9: Right click > Docked compound module 1 > Saved in .pdb format > Further visualized in Discovery studio.

Lipinski's rule:

Lipinski's rule is used to predict if a molecule is likely to be orally bioavailable or to evaluate drug likeness. The rule was formulated by Christopher A. Lipinski in 1997. The rule states that for drug likeness the molecule should have the following properties. The rule states that most "drug-like" molecules have

- Not more than 5 Hydrogen bond donors
- Not more than 10 Hydrogen bond acceptors
- Molecular weight less than 500 Daltons
- Calculated log P value less than 5

- Less than 10 rotatable bonds Also the 500 molecular weight cut off has been questioned.

Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally.

IN SILICO TOXICITY PREDICTION OSIRIS: [69]

- In silico toxicity prediction is done using OSIRIS® property explorer.
- The tool can be used to predict mutagenicity, tumorigenicity, skin irritation and reproductive effects.
- The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn.
- Compounds with undesired effects are shown in red colour, whereas green colour indicates the drug conform behavior.

MOLINSPIRATION®: [70]

- ✓ The designed and docked molecules are screened in silico using MOLINSPIRATION® cheminformatics software to evaluate drug likeness.
- ✓ It is a software available online for calculation of important molecular properties log P, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

MOLECULAR DESIGN:

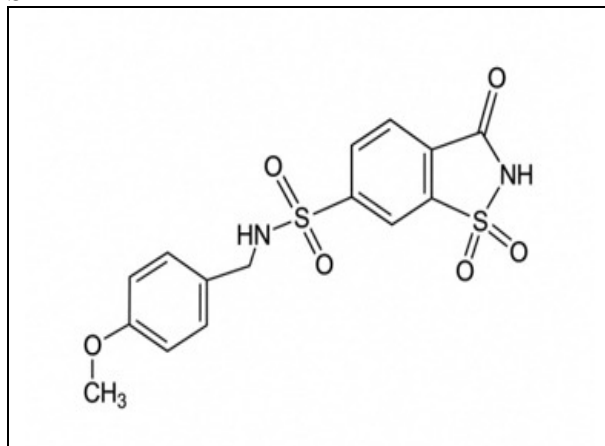
Molecular design is the process of finding new medicines based on the knowledge of biological target, it enabled the chemist to predict the structure and it also allows the medicinal chemist to evaluate the interaction between a compound and its target site before synthesizing a compound so as to increase the ability by reducing the side effects.

Various software's used:

- Chems sketch
- Mol inspiration
- Swiss ADME
- Osiris

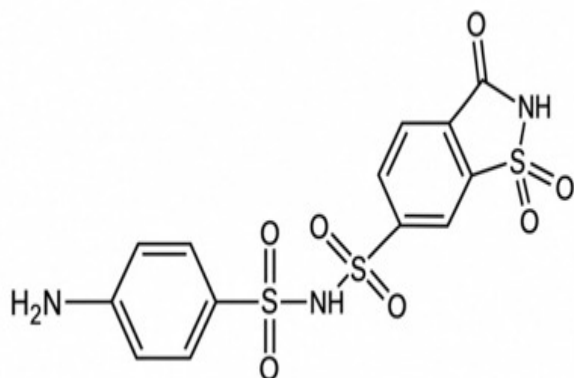
CHEMSKETCH

SA1



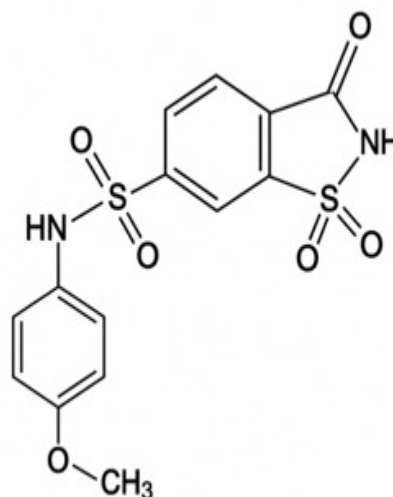
Molecular Formula	C ₁₅ H ₁₄ N ₂ O ₆ S ₂ Formula
Weight	382.405
Composition	C (47.11%) H (3.69%) N (7.33%) O (25.10%) S (16.77%)
Molar Refractivity	90.09 ± 0.4 cm ³
Molar Volume	252.9 ± 3.0 cm ³
Parachor	704.1 ± 6.0 cm ³
Index of Refraction	1.630 ± 0.02
Surface Tension	60.0 ± 3.0 dyne/cm
Density	1.511 ± 0.06 g/cm ³
Dielectric Constant	Not available
Polarizability	35.71 ± 0.5 10 ⁻²⁴ cm ³
RDBE	14
Monoisotopic Mass	382.029326 Da
Nominal Mass	382 Da
Average Mass	382.405 Da
M+	382.028778 Da
M-	382.029875 Da
[M+H] ⁺	383.036603 Da
[M+H] ⁻	383.0377 Da
[M-H] ⁺	381.020953 Da
[M-H] ⁻	381.02205 Da

SA2



Molecular Formula	C ₁₃ H ₁₁ N ₃ O ₇ S ₃ Formula
Weight	417.425
Composition	C (37.41%) H (2.66%) N (10.07%) O (26.83%) S (23.04%)
Molar Refractivity	91.53 ± 0.4 cm ³
Molar Volume	240.1 ± 3.0 cm ³
Parachor	713.1 ± 6.0 cm ³
Index of Refraction	1.687 ± 0.02
Surface Tension	77.7 ± 3.0 dyne/cm
Density	1.738 ± 0.06 g/cm ³
Dielectric Constant	Not available
Polarizability	36.28 ± 0.5 10 ⁻²⁴ cm ³
RDBE	16
Monoisotopic Mass	416.97591 Da
Nominal Mass	417 Da
Average Mass	417.425 Da
M+	416.975361 Da
M-	416.976458 Da
[M+H] ⁺	417.983186 Da
[M+H] ⁻	417.984283 Da
[M-H] ⁺	415.967536 Da
[M-H] ⁻	415.968633 Da

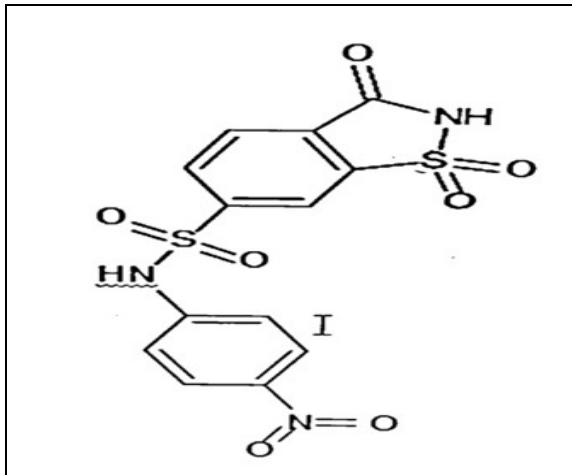
SA3



Molecular Formula	C ₁₄ H ₁₂ N ₂ O ₆ S ₂ Formula
Weight	368.378
Composition	C (45.65%) H (3.28%) N (7.60%) O (26.06%) S (17.41%)
Molar Refractivity	85.46 ± 0.4 cm ³
Molar Volume	230.7 ± 3.0 cm ³
Parachor	664.0 ± 6.0 cm ³
Index of Refraction	1.662 ± 0.02
Surface Tension	68.6 ± 3.0 dyne/cm
Density	1.596 ± 0.06 g/cm ³
Dielectric Constant	Not available
Polarizability	33.87 ± 0.5 10 ⁻²⁴ cm ³
RDBE	14
Monoisotopic Mass	368.013676 Da

Nominal Mass	368 Da
Average Mass	368.378 Da
M+	368.013128 Da
M-	368.014225 Da
[M+H] ⁺	369.020953 Da
[M+H] ⁻	369.02205 Da
[M-H] ⁺	367.005302 Da
[M-H] ⁻	367.0064 Da

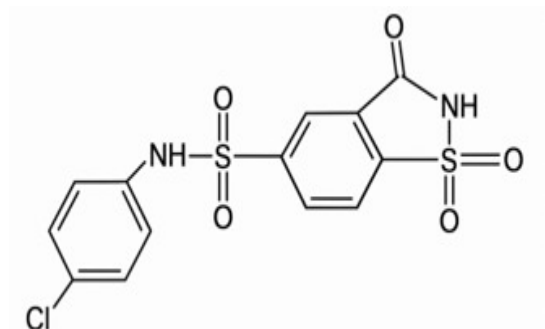
SA4



Molecular Formula	C ₁₃ H ₉ N ₃ O ₇ S ₂ Formula
Weight	383.349
Composition	C (40.73%) H (2.37%) N (10.96%) O (29.21%) S (16.73%)
Molar Refractivity	85.12 ± 0.4 cm ³
Molar Volume	218.5 ± 3.0 cm ³
Parachor	662.5 ± 6.0 cm ³
Index of Refraction	1.706 ± 0.02
Surface Tension	84.4 ± 3.0 dyne/cm
Density	1.753 ± 0.06 g/cm ³
Dielectric Constant	Not available
Polarizability	33.74 ± 0.5 10 ⁻²⁴ cm ³
RDBE	16
Monoisotopic Mass	382.98819 Da
Nominal Mass	383 Da
Average Mass	383.349 Da
M+	382.987641 Da

M-	382.988738 Da
[M+H] ⁺	383.995466 Da
[M+H] ⁻	383.996563 Da
[M-H] ⁺	381.979816 Da
[M-H] ⁻	381.980913 Da

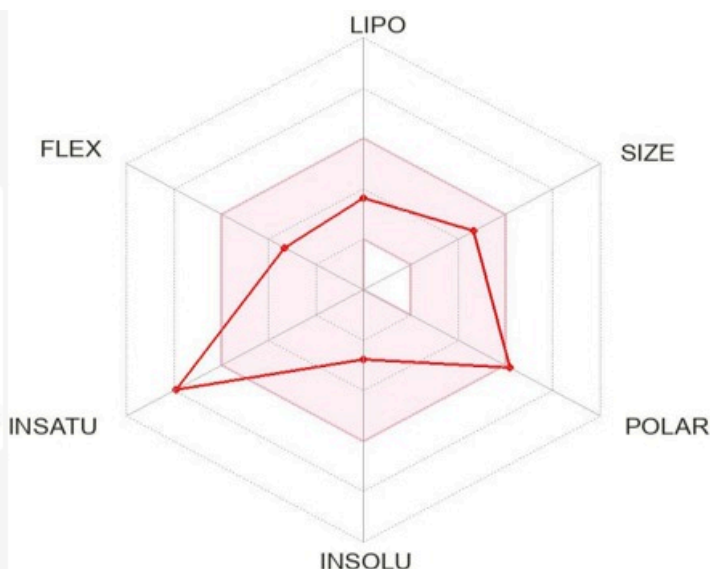
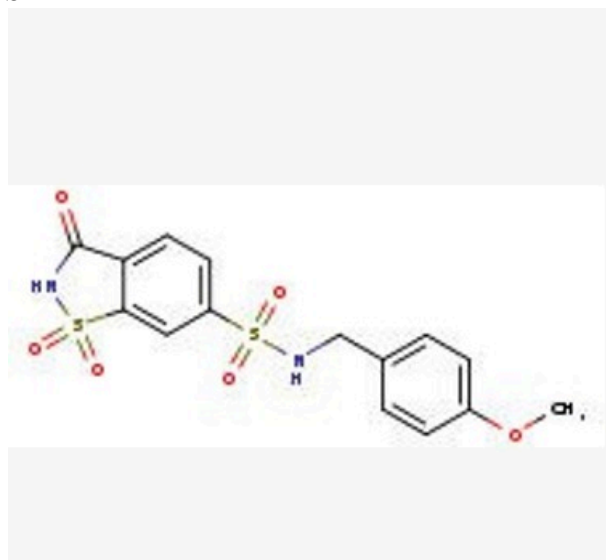
SA5



Molecular Formula	C ₁₃ H ₉ ClN ₂ O ₅ S ₂ Formula
Weight	372.794
Composition	C (41.88%) H (2.43%) Cl (9.51%) N (7.51%) O (21.46%) S (17.20%)
Molar Refractivity	83.92 ± 0.4 cm ³
Molar Volume	218.6 ± 3.0 cm ³
Parachor	642.6 ± 6.0 cm ³
Index of Refraction	1.693 ± 0.02
Surface Tension	74.5 ± 3.0 dyne/cm
Density	1.704 ± 0.06 g/cm ³
Dielectric Constant	Not available
Polarizability	33.27 ± 0.5 10 ⁻²⁴ cm ³
RDBE	14
Monoisotopic Mass	371.964139 Da
Nominal Mass	372 Da
Average Mass	372.794 Da
M+	371.96359 Da
M-	371.964688 Da
[M+H] ⁺	372.971416 Da
[M+H] ⁻	372.972513 Da
[M-H] ⁺	370.955765 Da
[M-H] ⁻	370.956863 Da

SWISS ADME

SA1

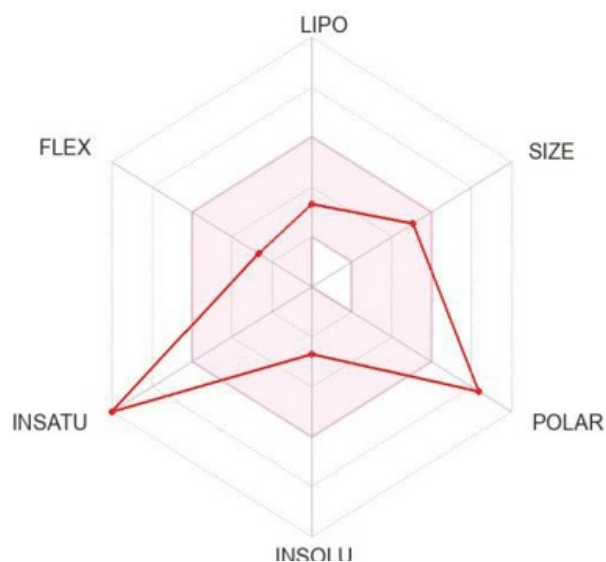
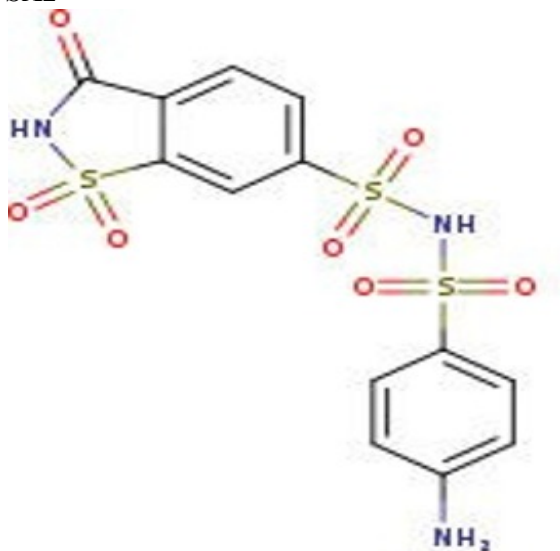


Physicochemical Properties	
Formula	C15H14N2O6S2
Molecular weight	382.41 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	12
Fraction Csp3	0.13
Num. rotatable bonds	5
Num. H-bond acceptors	7
Num. H-bond donors	2
Molar Refractivity	91.59
TPSA	135.40 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	1.19
Log $P_{o/w}$ (XLOGP3)	0.84
Log $P_{o/w}$ (WLOGP)	2.23
Log $P_{o/w}$ (MLOGP)	0.04
Log $P_{o/w}$ (SILICOS-IT)	0.50
Consensus Log $P_{o/w}$	0.96

Water Solubility	
Log S (ESOL)	-2.77
Solubility	6.56e-01 mg/ml; 1.72e-03 mol/l
Class	Soluble
Log S (Ali)	-3.27
Solubility	2.07e-01 mg/ml; 5.42e-04 mol/l

Class ?	Soluble
Log <i>S</i> (SILICOS-IT) ?	-5.50
Solubility	1.21e-03 mg/ml; 3.18e-06 mol/l
Class ?	Moderately soluble
Pharmacokinetics	
GI absorption ?	High
BBB permeant ?	No
P-gp substrate ?	No
CYP1A2 inhibitor ?	No
CYP2C19 inhibitor ?	No
CYP2C9 inhibitor ?	No
CYP2D6 inhibitor ?	No
CYP3A4 inhibitor ?	No
Log <i>K_p</i> (skin permeation) ?	-8.04 cm/s
Druglikeness	
Lipinski ?	Yes; 0 violation
Ghose ?	Yes
Veber ?	Yes
Egan ?	No; 1 violation: TPSA>131.6
Muegge ?	Yes
Bioavailability Score ?	0.55
Medicinal Chemistry	
PAINS ?	0 alert
Brenk ?	0 alert
Leadlikeness ?	No; 1 violation: MW>350
Synthetic accessibility ?	2.96

SA2

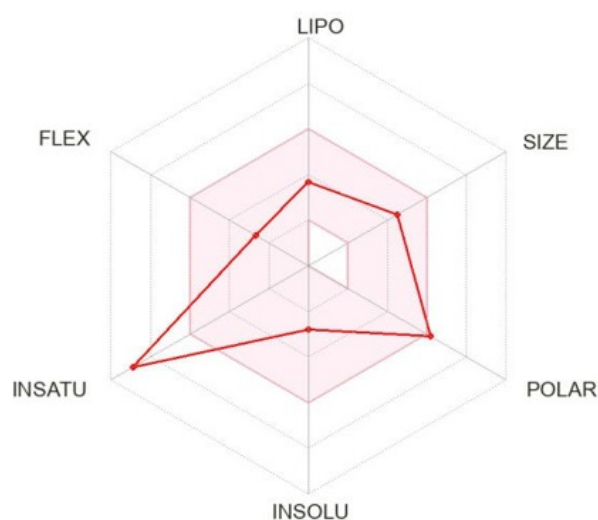
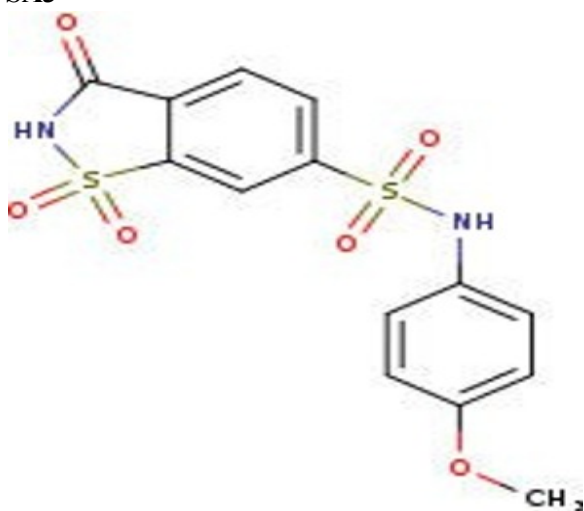


Physicochemical Properties	
Formula	C ₁₃ H ₁₁ N ₃ O ₇ S ₃
Molecular weight	417.44 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	12
Fraction Csp ³	0.00
Num. rotatable bonds	4
Num. H-bond acceptors	8
Num. H-bond donors	3
Molar Refractivity	92.83
TPSA 	194.71 Å ²
Lipophilicity	
Log <i>P</i> _{o/w} (iLOGP) 	0.35
Log <i>P</i> _{o/w} (XLOGP3) 	0.32
Log <i>P</i> _{o/w} (WLOGP) 	2.24
Log <i>P</i> _{o/w} (MLOGP) 	-1.09
Log <i>P</i> _{o/w} (SILICOS-IT) 	-1.74
Consensus Log <i>P</i> _{o/w} 	0.01

Water Solubility	
Log <i>S</i> (ESOL) 	-2.71
Solubility	8.19e-01 mg/ml; 1.96e-03 mol/l
Class 	Soluble
Log <i>S</i> (Ali) 	-3.97
Solubility	4.45e-02 mg/ml; 1.07e-04 mol/l
Class 	Soluble
Log <i>S</i> (SILICOS-IT) 	-4.50
Solubility	1.31e-02 mg/ml; 3.15e-05 mol/l
Class 	Moderately soluble
Pharmacokinetics	
GI absorption 	Low
BBB permeant 	No
P-gp substrate 	No
CYP1A2 inhibitor 	No
CYP2C19 inhibitor 	No
CYP2C9 inhibitor 	No
CYP2D6 inhibitor 	No
CYP3A4 inhibitor 	No
Log <i>K</i> _p (skin permeation) 	-8.62 cm/s
Druglikeness	
Lipinski 	Yes; 0 violation

Ghose ?	Yes
Veber ?	No; 1 violation: TPSA>140
Egan ?	No; 1 violation: TPSA>131.6
Muegge ?	No; 1 violation: TPSA>150
Bioavailability Score ?	0.55
Medicinal Chemistry	
PAINS ?	0 alert
Brenk ?	1 alert: aniline ?
Leadlikeness ?	No; 1 violation: MW>350
Synthetic accessibility ?	3.09

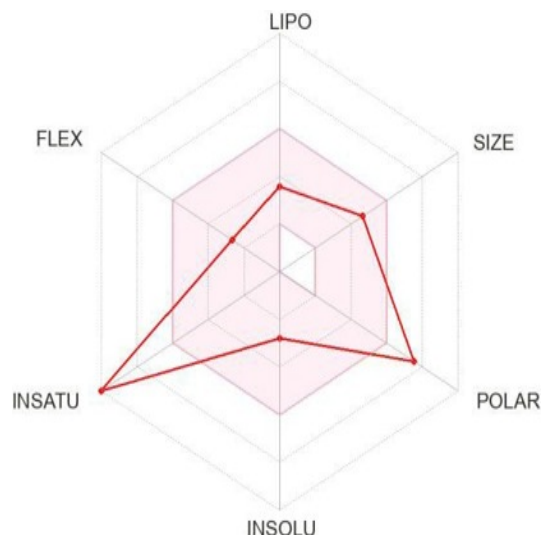
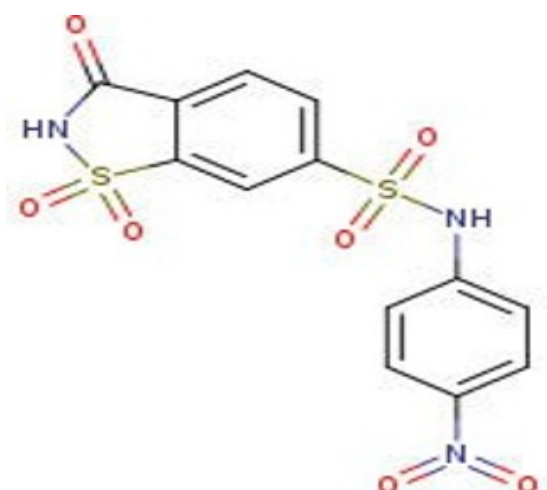
SA3



Physicochemical Properties	
Formula	C ₁₄ H ₁₂ N ₂ O ₆ S ₂
Molecular weight	368.38 g/mol
Num. heavy atoms	24
Num. arom. heavy atoms	12
Fraction Csp ³	0.07
Num. rotatable bonds	4
Num. H-bond acceptors	6
Num. H-bond donors	2
Molar Refractivity	88.32
TPSA ?	135.40 Å ²
Lipophilicity	
Log <i>P</i> _{o/w} (iLOGP) ?	0.88
Log <i>P</i> _{o/w} (XLOGP3) ?	0.90
Log <i>P</i> _{o/w} (WLOGP) ?	2.52

Log $P_{o/w}$ (MLOGP) ?	0.05
Log $P_{o/w}$ (SILICOS-IT) ?	0.12
Consensus Log $P_{o/w}$?	0.90
Water Solubility	
Log S (ESOL) ?	-2.80
Solubility	5.88e-01 mg/ml; 1.60e-03 mol/l
Class ?	Soluble
Log S (Ali) ?	-3.33
Solubility	1.73e-01 mg/ml; 4.69e-04 mol/l
Class ?	Soluble
Log S (SILICOS-IT) ?	-5.10
Solubility	2.90e-03 mg/ml; 7.87e-06 mol/l
Class ?	Moderately soluble
Pharmacokinetics	
GI absorption ?	High
BBB permeant ?	No
P-gp substrate ?	No
CYP1A2 inhibitor ?	No
CYP2C19 inhibitor ?	No
CYP2C9 inhibitor ?	No
CYP2D6 inhibitor ?	No
CYP3A4 inhibitor ?	No
Log K_p (skin permeation) ?	-7.91 cm/s
Druglikeness	
Lipinski ?	Yes; 0 violation
Ghose ?	Yes
Veber ?	Yes
Egan ?	No; 1 violation: TPSA>131.6
Muegge ?	Yes
Bioavailability Score ?	0.55
Medicinal Chemistry	
PAINS ?	0 alert
Brenk ?	0 alert
Leadlikeness ?	No; 1 violation: MW>350
Synthetic accessibility ?	2.86

SA4

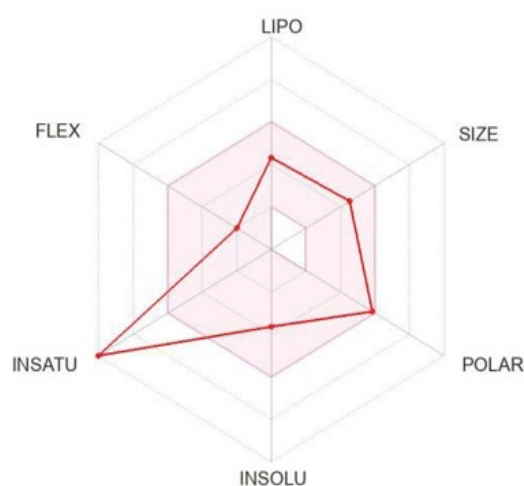
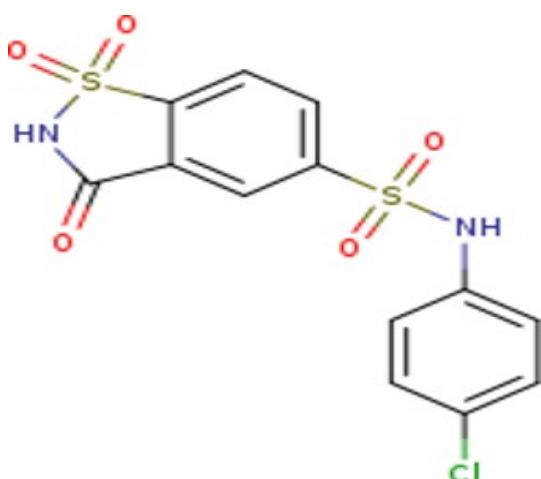


Physicochemical Properties	
Formula	C13H9N3O7S2
Molecular weight	383.36 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	12
Fraction Csp3	0.00
Num. rotatable bonds	4
Num. H-bond acceptors	7
Num. H-bond donors	2
Molar Refractivity	90.65
TPSA	171.99 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.26
Log $P_{o/w}$ (XLOGP3)	0.76
Log $P_{o/w}$ (WLOGP)	2.94
Log $P_{o/w}$ (MLOGP)	-0.62
Log $P_{o/w}$ (SILICOS-IT)	-1.65
Consensus Log $P_{o/w}$	0.34

Water Solubility	
Log S (ESOL)	-2.79
Solubility	6.26e-01 mg/ml; 1.63e-03 mol/l
Class	Soluble
Log S (Ali)	-3.95
Solubility	4.29e-02 mg/ml; 1.12e-04 mol/l
Class	Soluble
Log S (SILICOS-IT)	-4.82

Solubility	5.75e-03 mg/ml; 1.50e-05 mol/l
Class ?	Moderately soluble
Pharmacokinetics	
GI absorption ?	Low
BBB permeant ?	No
P-gp substrate ?	No
CYP1A2 inhibitor ?	No
CYP2C19 inhibitor ?	No
CYP2C9 inhibitor ?	No
CYP2D6 inhibitor ?	No
CYP3A4 inhibitor ?	No
Log K_p (skin permeation) ?	-8.10 cm/s
Druglikeness	
Lipinski ?	Yes; 0 violation
Ghose ?	Yes
Veber ?	No; 1 violation: TPSA>140
Egan ?	No; 1 violation: TPSA>131.6
Muegge ?	No; 1 violation: TPSA>150
Bioavailability Score ?	0.55
Medicinal Chemistry	
PAINS ?	0 alert
Brenk ?	1 alert: nitro_group ?
Leadlikeness ?	No; 1 violation: MW>350
Synthetic accessibility ?	2.95

SA5



Physicochemical Properties	
Formula	C13H9CLN2O5S2
Molecular weight	372.80 g/mol
Num. heavy atoms	23
Num. arom. heavy atoms	12
Fraction Csp3	0.00
Num. rotatable bonds	3
Num. H-bond acceptors	7
Num. H-bond donors	2
Molar Refractivity	96.84
TPSA 	126.17 Å ²
Lipophilicity	
Log <i>P</i> _{o/w} (iLOGP) 	0.83
Log <i>P</i> _{o/w} (XLOGP3) 	2.06
Log <i>P</i> _{o/w} (WLOGP) 	3.16
Log <i>P</i> _{o/w} (MLOGP) 	0.57
Log <i>P</i> _{o/w} (SILICOS-IT) 	0.73
Consensus Log <i>P</i> _{o/w} 	1.47

Water Solubility	
Log <i>S</i> (ESOL) 	-3.64
Solubility	8.59e-02 mg/ml; 2.31e-03 mol/l
Class 	Soluble
Log <i>S</i> (Ali) 	-4.34
Solubility	1.71e-02 mg/ml; 4.59e-05 mol/l
Class 	Moderately Soluble
Log <i>S</i> (SILICOS-IT) 	-5.59
Solubility	9.60e-04 mg/ml; 2.58e-06 mol/l
Class 	Moderately soluble
Pharmacokinetics	
GI absorption 	High
BBB permeant 	No
P-gp substrate 	No
CYP1A2 inhibitor 	No
CYP2C19 inhibitor 	No
CYP2C9 inhibitor 	No
CYP2D6 inhibitor 	No
CYP3A4 inhibitor 	No
Log <i>K</i> _p (skin permeation) 	-7.11 cm/s
Druglikeness	
Lipinski 	Yes; 0 violation

Ghose ?	Yes
Veber ?	Yes
Egan ?	Yes
Muegge ?	Yes
Bioavailability Score ?	0.55
Medicinal Chemistry	
PAINS ?	0 alert
Brenk ?	0 alert
Leadlikeness ?	No; 1 violation: MW>350
Synthetic accessibility ?	2.85

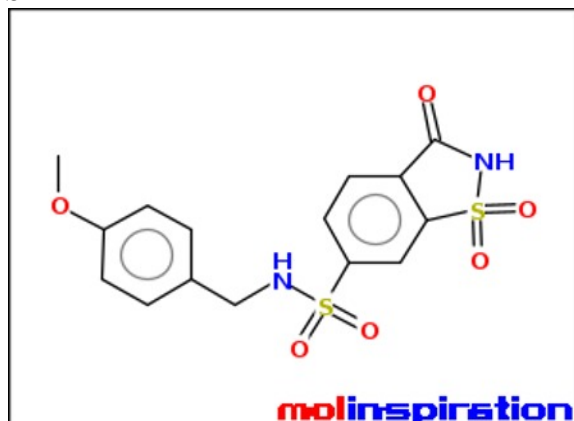
MOLINSPIRATION

For a molecule to be orally active, it must have drug likeness properties conforming to the Lipinski's Rule of five. The molecules were evaluated for their drug likeness behaviour using the online tool – Molinspiration® Cheminformatics.

Molinspiration software is used to calculate the following properties:

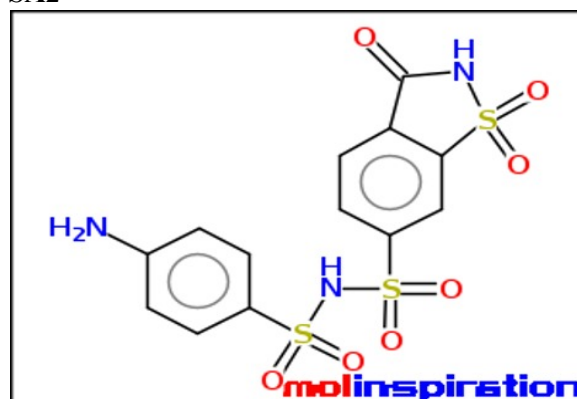
- Log P
- Molecular weight
- Number of H- bond donor
- Number of H- bond acceptor
- Number of rotatable bonds

SA1



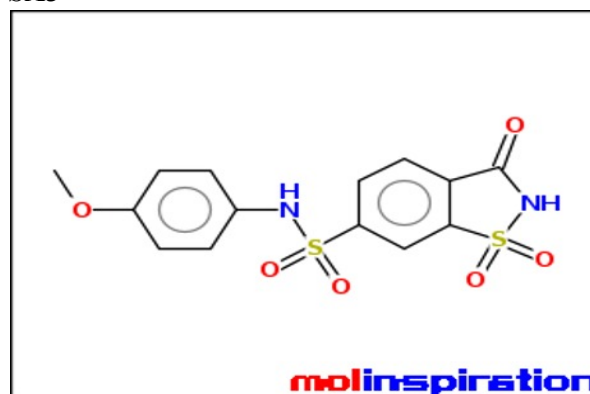
Molinspiration property engine		Molinspiration bioactivity score	
miLogP	0.73	GPCR ligand	-0.05
TPSA	118.64	Ion channel modulator	-0.27
natoms	25	Kinase inhibitor	-0.45
MW	382.42	Nuclear receptor ligand	-0.53
nON	8	Protease inhibitor	0.08
nOHNH	2	Enzyme inhibitor	0.00
nviolations	0		
nroth	5		
volume	293.61		

SA2



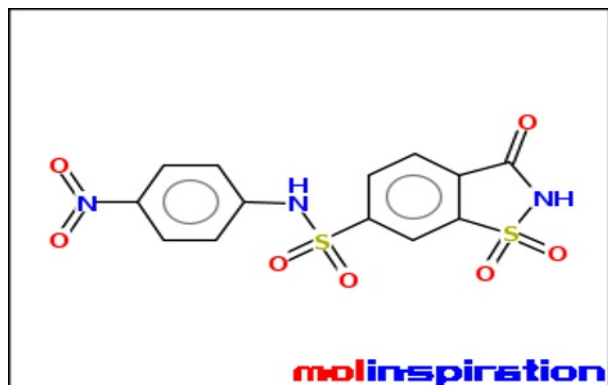
Molinspiration property engine		Molinspiration bioactivity score	
miLogP	-0.74	GPCR ligand	0.12
TPSA	169.54	Ion channel modulator	-0.13
natoms	26	Kinase inhibitor	-0.12
MW	417.45	Nuclear receptor ligand	-0.50
nON	10	Protease inhibitor	0.18
nOHNH	4	Enzyme inhibitor	0.09
nviolations	0		
nroth	4		
volume	293.98		

SA3



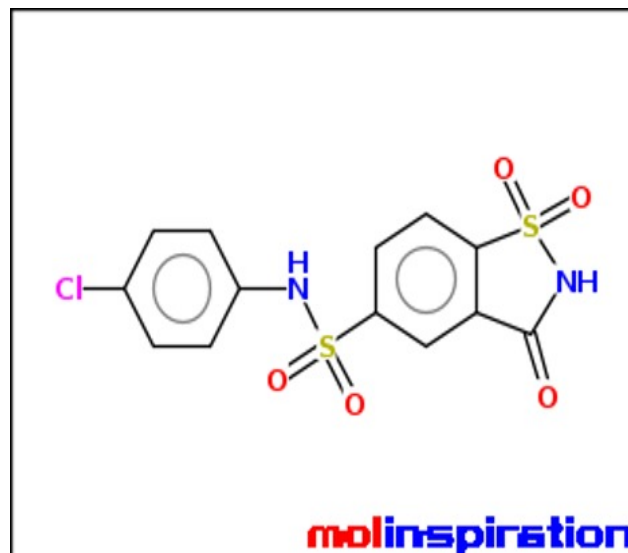
Molinspiration property engine		Molinspiration bioactivity score	
miLogP	1.03	GPCR ligand	-0.07
TPSA	118.64	Ion channel modulator	-0.30
natoms	24	Kinase inhibitor	-0.49
MW	368.39	Nuclear receptor ligand	-0.49
nON	8	Protease inhibitor	0.05
nOHNH	2	Enzyme inhibitor	-0.06
nviolations	0		
nrotb	4		
volume	276.81		

SA4



Molinspiration property engine		Molinspiration bioactivity score	
miLogP	0.93	GPCR ligand	-0.17
TPSA	155.23	Ion channel modulator	-0.26
natoms	25	Kinase inhibitor	-0.59
MW	383.36	Nuclear receptor ligand	-0.55
nON	10	Protease inhibitor	0.02
nOHNH	2	Enzyme inhibitor	0.11
nviolations	0		
nrotb	4		
volume	274.60		

SA5



Molinspiration property engine		Molinspiration bioactivity score	
miLogP	1.65	GPCR ligand	-0.05
TPSA	109.41	Ion channel modulator	-0.28
natoms	23	Kinase inhibitor	-0.44
MW	372.81	Nuclear receptor ligand	-0.44
nON	7	Protease inhibitor	0.03
nOHNH	2	Enzyme inhibitor	0.08
nviolations	0		
nrotb	3		
volume	264.80		

RESULTS OF CHEMSKETCH, SWISS ADME&MOL INSPIRATION

Table No.1 Lipinski's rule

Code	M.W	H- Bond acceptor	H- Bond donar	LogP	M.R (Cm ³ /mol)	No. of criteria
Rule	<500	<10	< 5	< 5	<150	Atleast-3
SA1	382.41	7	2	0.96	90.09	ALL
SA2	417.44	8	3	0.01	91.53	ALL
SA3	368.38	6	2	0.90	85.46	ALL
SA4	383.36	7	2	0.34	85.12	ALL
SA5	372.80	7	2	0.83	96.84	ALL

✓ The above molecules showed no violations and follows the Lipinski's rule of five

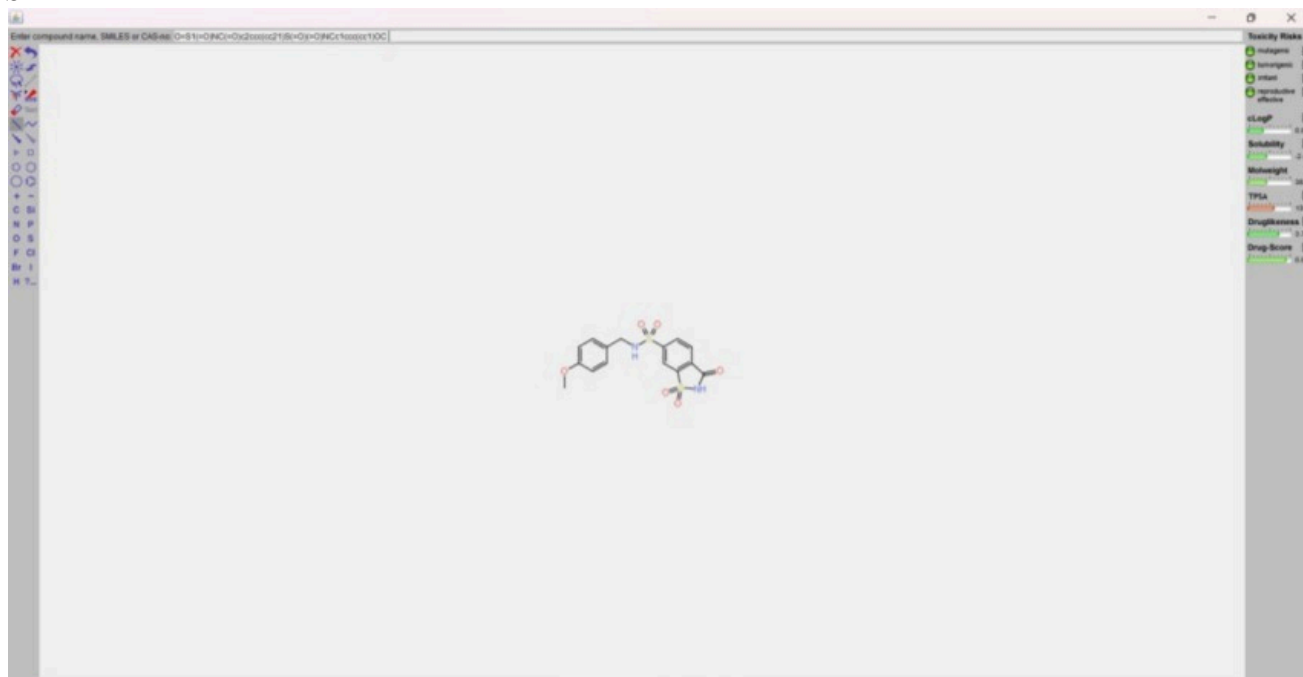
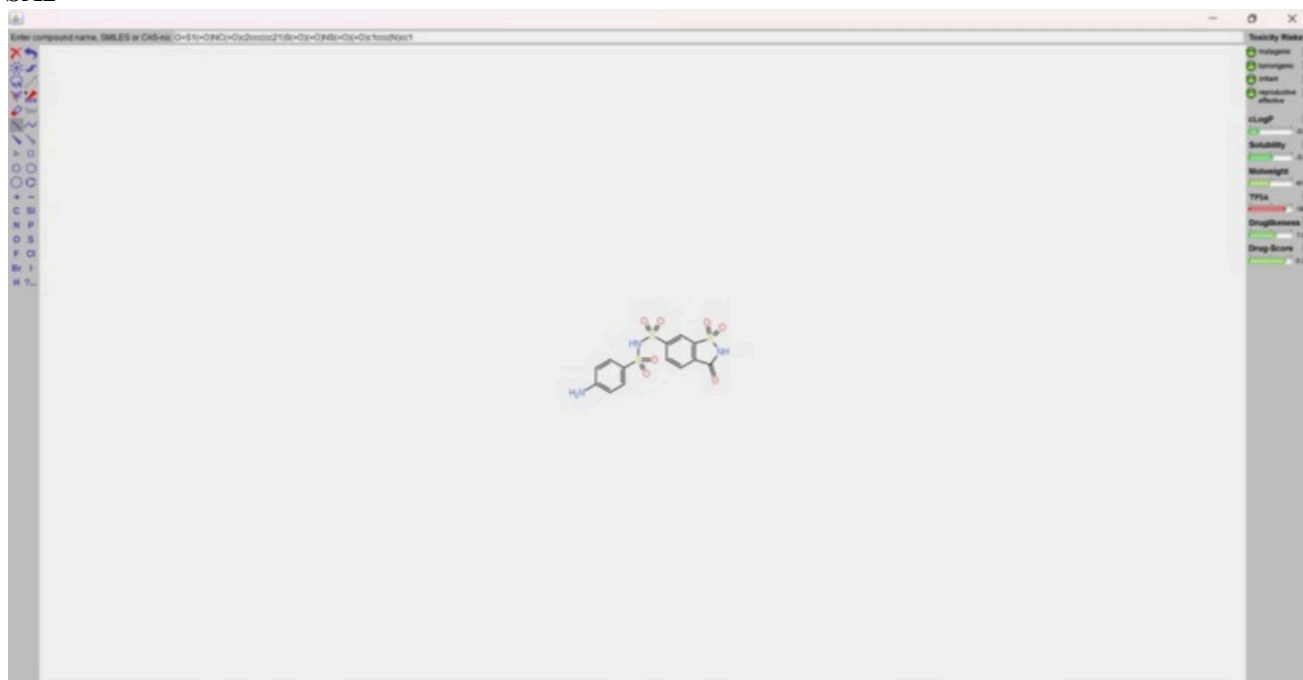
Table 2

Code	Solubility (Log S)	Glabsorption	BBB Permeant	Synthetic Accessibility
SA1	-2.77	High	No	2.96
SA2	-2.71	Low	No	3.09
SA3	-2.80	High	No	2.86
SA4	-2.79	Low	No	2.95
SA5	-3.64	High	No	2.85

OSIRISIS

On accessing the OSIRISIS Property explorer, which is JAVA applet, allows us to draw chemical structures. On drawing the chemical structures the toxicity risk parameters are calculates on-the-fly

whenever a structure is valid. Prediction results are valued color coded. Properties with high risks of undesired effects are shown in red color. Where as a green color indicates drug-conform behavior.

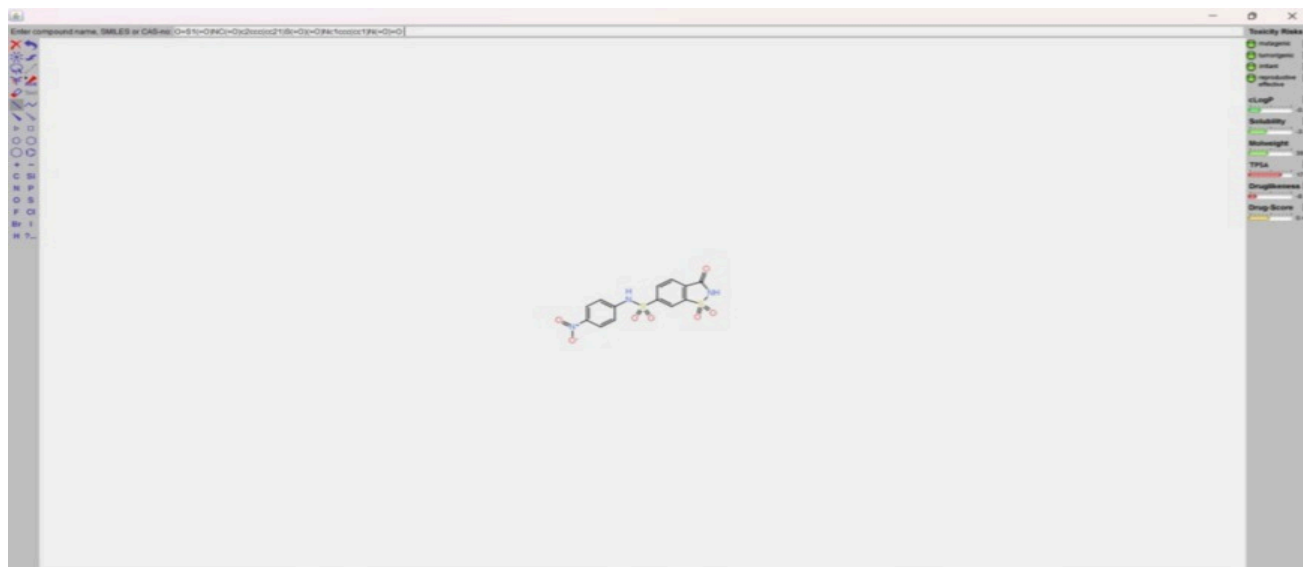
SA1**SA2**

Design, Synthesis, and In Vitro Evaluation of Novel Saccharin Derivatives for Anticancer Activity Against Neuroblastoma (SH-SY5Y Cell Line) Through Targeting Anaplastic Lymphoma Kinase (ALK).

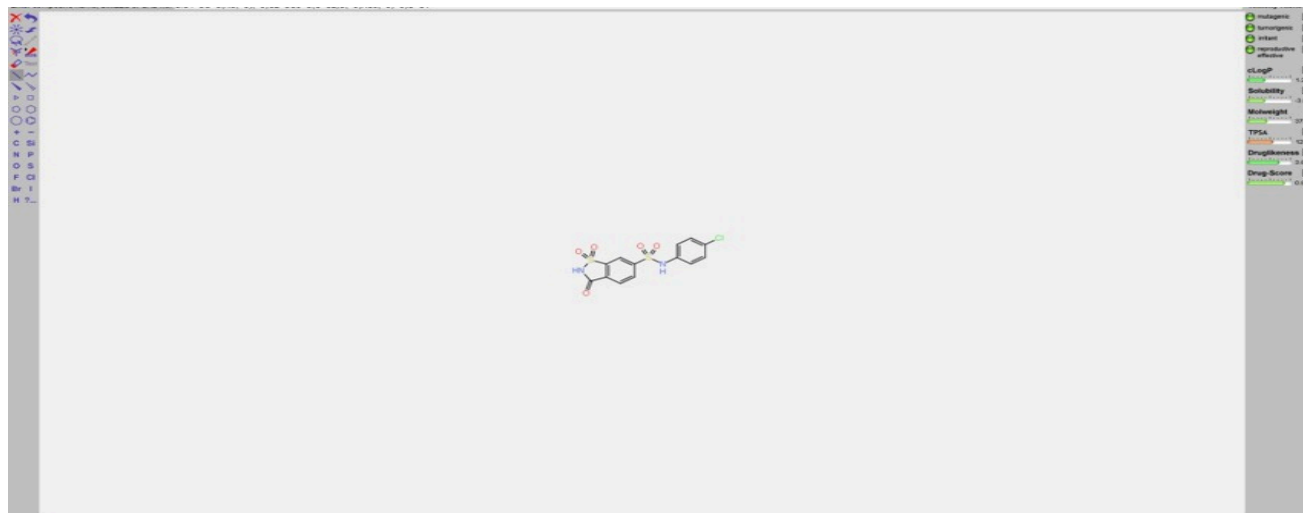
SA3



SA4



SA5



In-Silico Toxicity Assessment results

Toxicity prediction was done by OSIRIS® Property explorer, the online software of Thomas Sander Actelion Pharmaceuticals Ltd, Switzerland.

The chemical structure was drawn in OSIRIS® Property explorer to show the biological property of the compound. Properties with high risks of

undesired effects like mutagenicity, tumorigenicity, were indicated in red.

Green colour was used to indicate drug conform behaviour. The following are the results of toxicity prediction for Five selected molecules based on docking score.

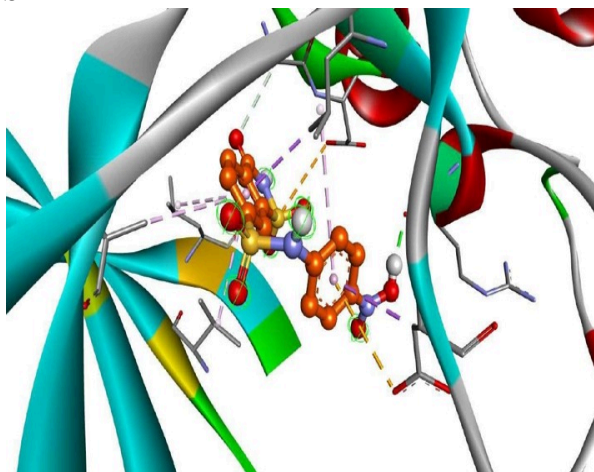
Table. No 3 OSIRIS PROPERTY RESULTS

Compounds	Mutagenicity	Tumorigenicity	Skin Irritancy	Reproductive Effect
SA1	G	G	G	G
SA2	G	G	G	G
SA3	G	G	G	G
SA4	G	G	G	G
SA5	G	G	G	G

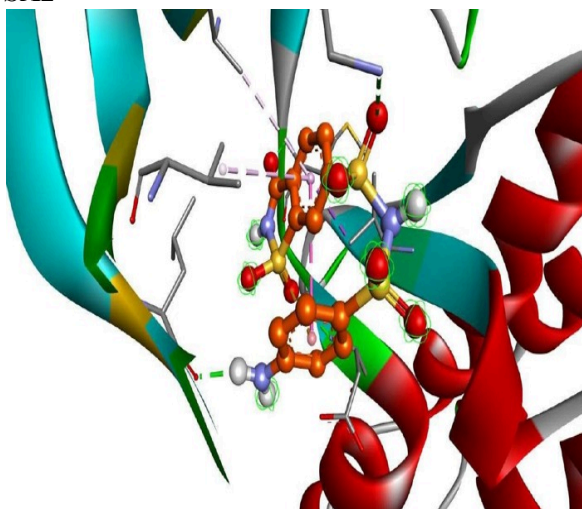
- ✓ The above molecules showed no toxicity risks (no red colour alert) were shown in OSIRIS property explorer.
- ✓ The nontoxic, drug like molecules were subjected to molecular docking studies.

MOLECULAR DOCKING BY 3D PICTURE

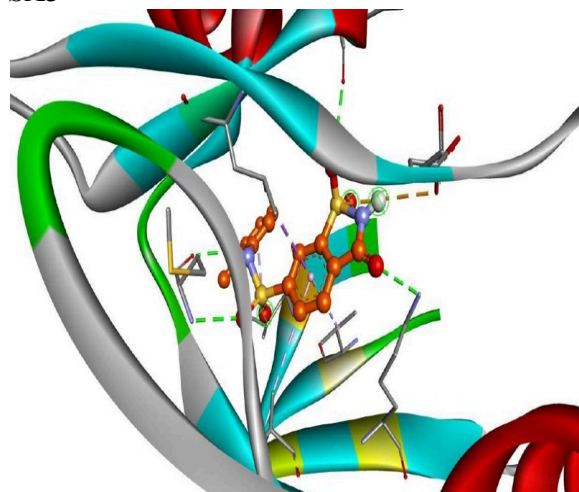
SA1



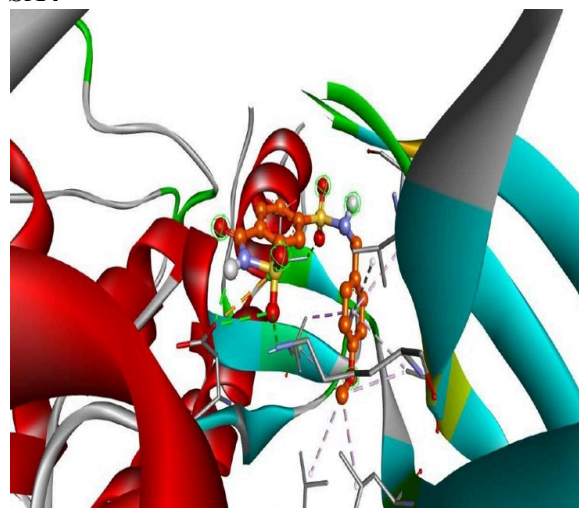
SA2



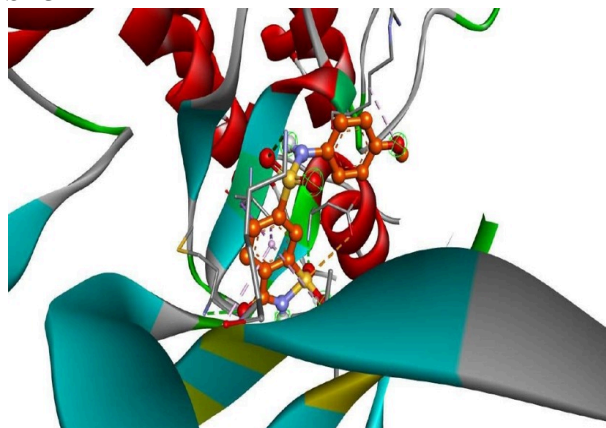
SA3



SA4



SA5



- Molecules which were found to be non-toxic in the in silico toxicity assessment tool were synthesized by suitable laboratory process and recrystallized using ethanol.
- Completion of the synthetic reaction was determined by comparing the Rf values of the reactants and products. Rf value of the products was found to be different from the Rf value of the reactants.

- Melting point of the products was determined by Digital Melting Point Apparatus. Sharp melting point denotes the purity of the compound.

RESULTS OF MOLECULAR DOCKING

- ✓ 50 scaffolds were sketched using chemsketch.
- ✓ The nontoxic molecules were docked against the targeted enzyme ALK to determine their effectiveness as anticancer agents.
- ✓ Compounds with good docking score and favourable interactions were selected and screened

Table No: 4 Molecular Docking Results

S.NO	COMPOUNDS	BINDINGENERGY (-Kcal/Mol)
01	SA1	-8.1
02	SA2	-8.2
03	SA3	-7.7
04	SA4	-8.1
05	SA5	-8.1

2. EXPERIMENTAL WORK

Table.no.1. List of Chemical Used

S.NO	CHEMICALS	GRADE	MANUFACTURER/SUPPLIER
1.	Saccharin	Laboratory reagent	Central Drug House
2.	Chlorosulphonic acid	Laboratory reagent	Nice Chemicals
3.	Ethanol	Laboratory reagent	Central Drug House
4.	4-methoxy benzalamine	Laboratory reagent	Central Drug House
5.	Sulphanilamide	Laboratory reagent	Central Drug House
6.	4-methoxyaniline	Laboratory reagent	Central Drug House
7.	4-nitroaniline	Laboratory reagent	Central Drug House
8.	4-chloroaniline	Laboratory reagent	Central Drug House
9.	Chloroform	Laboratory reagent	Central Drug House
10.	Methanol	Laboratory reagent	Central Drug House
11.	Ethanol	Laboratory reagent	Central Drug House

SYNTHETIC PROCEDURE

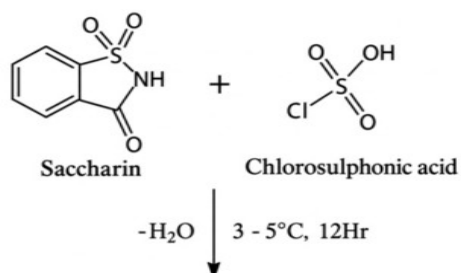
Step-1

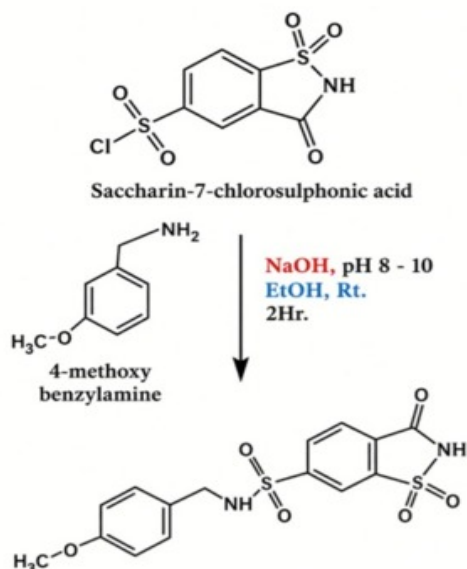
An excess (4:1) of Chlorosulphonic acid was slowly added to the Saccharine. The mixture was mixed well and kept in the refrigerator over-night. Then, the mixture was poured over crushed ice and the precipitated Saccharine-7 Chloro Sulphonic acid was filtered and dried.

Step-2

4-methoxy benzylamine were dissolved in ethanol and added slowly to the Saccharine-5- sulphonic acid with constant stirring for 2 hours. The pH was maintained at 8-10 using Sodium Hydroxide

solution. After 2 hours, the mixture was neutralized to pH 2 with Concentrated hydrochloric acid and poured over crushed ice. The precipitated product was filtered and dried.





N-(4-methoxybenzyl)-3-oxo-2,3-dihydrobenzo[d]isothiazole-5-sulfonamide 1,1-dioxide R=4-methoxybenzyl, 4-aminophenyl, 4-methoxyphenyl, 4-nitrophenyl, 4-chlorophenyl

Table.No.2. Physical Evaluation of the Synthesized Compounds

Compound code	R	Molecular formula	Molecular weight	Melting point	R _f value	Percentage yield
SA1	4- methoxy	C ₁₅ H ₁₄ N ₂ O ₆ S ₂	384.41	186-187	0.65	78%
SA2	4-amino	C ₁₃ H ₁₁ N ₃ O ₇ S ₃	417.425	183-184	0.73	83%
SA3	4- methoxy	C ₁₄ H ₁₂ N ₂ O ₆ S ₂	368.38	184-185	0.58	92%
SA4	4-nitro	C ₁₃ H ₉ N ₃ O ₇ S ₂	383.36	204-205	0.78	95%
SA5	4-chloro	C ₁₃ H ₉ ClN ₂ O ₅ S ₂	372.80	195-196	0.76	94%

TLC PROFILE

TLC was carried out on precoated plates (604 GF 254 MERCK), using a suitable solvent system.

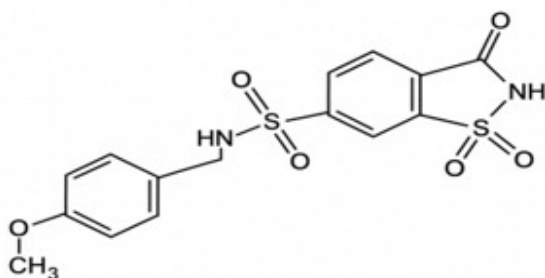
The selected 5 compounds were synthesized, recrystallized and purified. The purity was confirmed by a single spot obtained on the TLC Plate.

TableNo.3.

S.NO	SAMPLE CODE	MOBILE PHASE	R _f value
1	SA1	Methanol: Chloroform (9:1)	0.65
2	SA2	Methanol: Chloroform (9:1)	0.73
3	SA3	Methanol: Chloroform (9:1)	0.58
4	SA4	Methanol: Chloroform (9:1)	0.78
5	SA5	Methanol: Chloroform (9:1)	0.76

SPECTRAL DATA

1.INFRAREDSPECTRUM - SA1



IUPAC NAME: N-(4-methoxybenzyl)-3-oxo-2,3-dihydrobenzo[d]isothiazole-5-sulfonamide 1,1-dioxide

Fig.1 IR Spectrum of SA-1

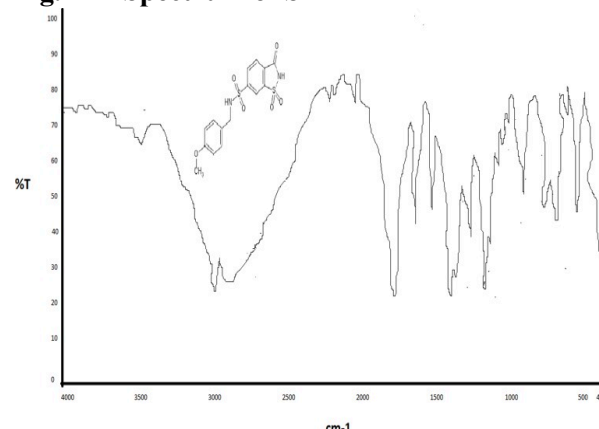


Table 8. Interpretation of IR Spectrum SA1

S. No	Wave Number (cm ⁻¹)	Functional Group
1	3100and2970	-NH (Secondary amino)
2	2670	-CH (Methylene)
3	1315and1170	S=O (Sulphonamide)
4	1725	-C=O (Keto)

Fig.2 ¹H NMR spectrum of SA1

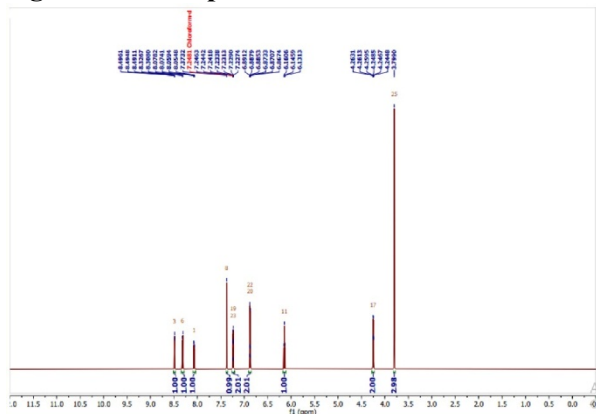


Table No 9: Interpretation of ¹H NMR Spectrum of Compound SA1

S.NO	Δ VALUE (PPM)	NATURE OF PROTON
A	3.615	-NH-
B	7.942 – 7.944	Ar-H
C	7.981 – 7.984	Ar-H
D	8.002 – 8.008	Ar-H
E	8.018 – 8.026	Ar-H

Fig.3 Mass spectrum of SA1

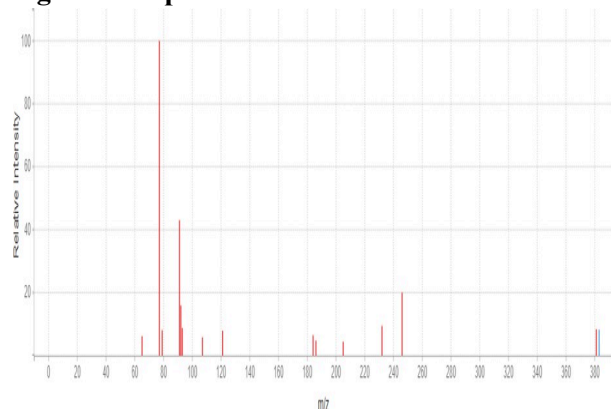
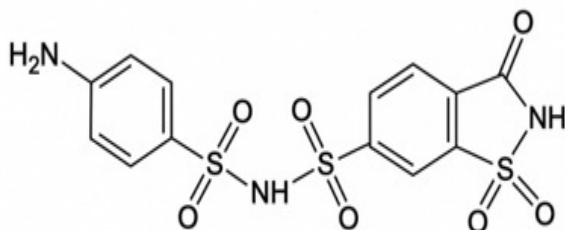


Table 10: Interpretation of Mass Spectrum of Compound SA1

Molecular Ion Peak m/z	383.03
Base Peak m/z	77.03

SA2



IUPAC NAME: N-[(4-aminophenyl) sulfonyl]-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide

Fig.4 IR Spectrum of SA2

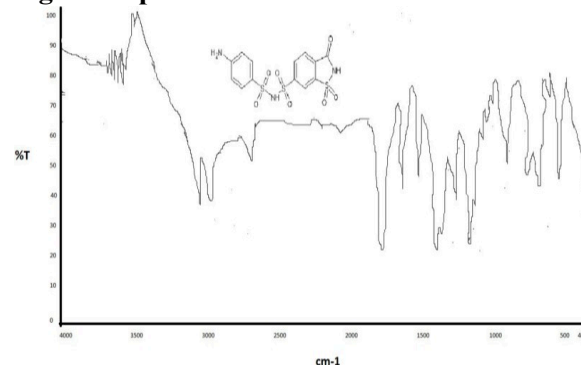


Table 11. Interpretation of IR Spectrum SA2

S.NO	WAVE NUMBER (cm ⁻¹)	FUNCTIONAL GROUP
1.	1718	-C=O (Keto)
2.	1593	-NH (Secondary amide)
3.	899	- C=C (Benzene ring)
4.	1336	-SO ₂ (Sulphonamide)
5.	3099	Benzene ring

Fig.5 ¹H NMR spectrum of SA2

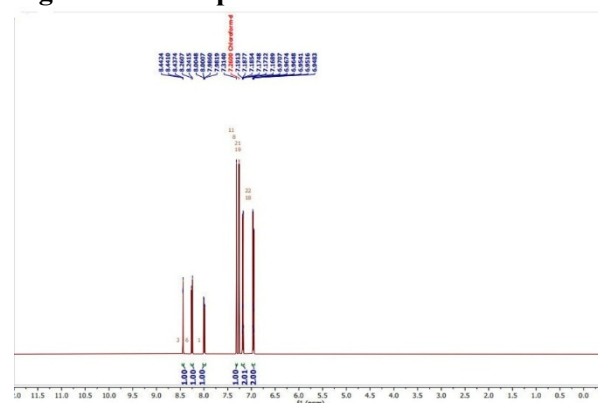


Table 12: Interpretation of ¹H NMR Spectrum of Compound SA2

S.NO	Δ VALUE (PPM)	NATURE OF PROTON
A	3.463	-NH-
B	7.946 – 7.948	Ar-H
C	7.964 – 7.967	Ar-H
D	7.984 – 7.988	Ar-H
E	8.006 – 8.012	Ar-H
F	8.022 – 8.025	Ar-H
G	8.030 – 8.031	Ar-H
H	8.165 – 8.184	Ar-H

Fig.6 Mass spectrum of SA2

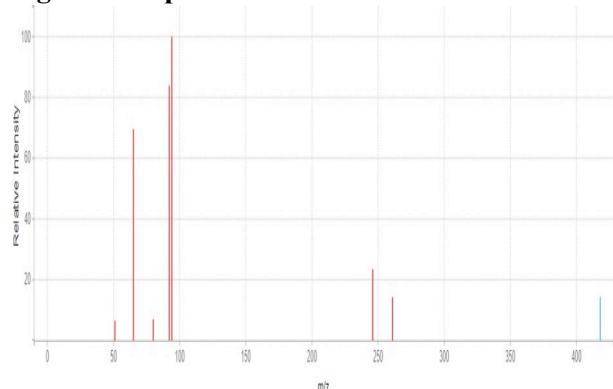
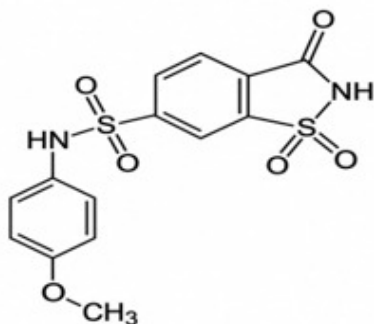


Table 13: Interpretation of Mass Spectrum of Compound SA2

Molecular Ion Peak m/z	417.98
Base Peak m/z	94.06

SA3



IUPAC NAME: N-(4-methoxyphenyl)-3-oxo-2,3-dihydrobenzo[d] isothiazole-5-sulfonamide 1,1-dioxide

Fig.7 IR Spectrum of SA3

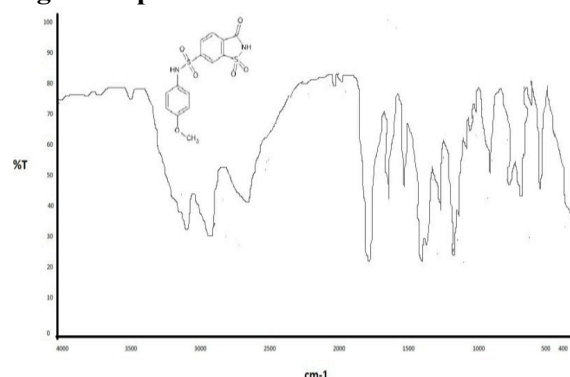


Table 14. Interpretation of IR Spectrum SA3

S. No	Wave Number (cm-1)	Functional Group
1	3100 and 2970	-NH (Secondary amino)
2	2670	-CH (Methylene)
3	1315 and 1170	S=O (Sulphonamide)
4	1725	-C=O (Keto)

Fig.8 1H NMR spectrum of SA3

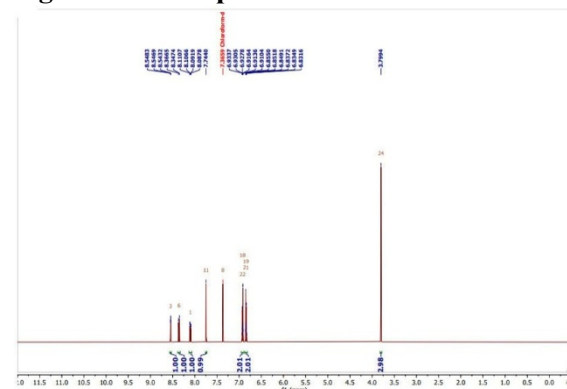


Table 15: Interpretation of 1HNMR Spectrum of Compound SA3

S.NO	Δ VALUE (PPM)	NATURE OF PROTON
A	3.783	-NH-
B	7.921 – 7.979	Ar-H
C	8.000 – 8.005	Ar-H
D	8.016 – 8.023	Ar-H
E	8.139 – 8.175	Ar-H

Fig.9 Mass spectrum of SA3

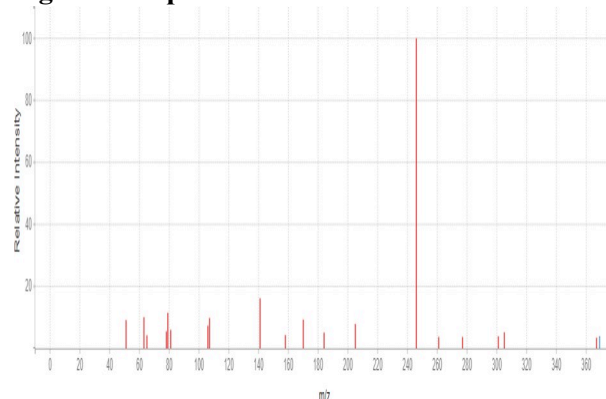
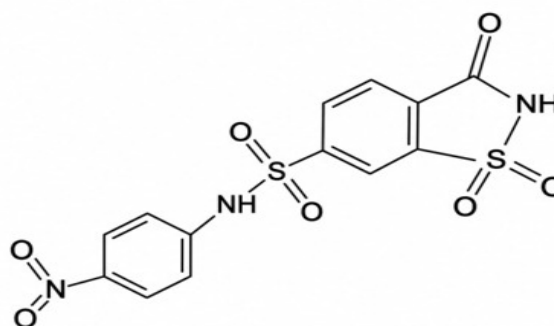


Table 16: Interpretation of Mass Spectrum of Compound SA3

Molecular Ion Peak m/z	369.02
Base Peak m/z	245.95

SA4



IUPAC NAME: N-(4-nitrophenyl)-1,1,3-trioxo-2,3-dihydro-1H-1λ6,2-benzothiazole-6-sulfonamide

Fig.10 IR Spectrum of SA4

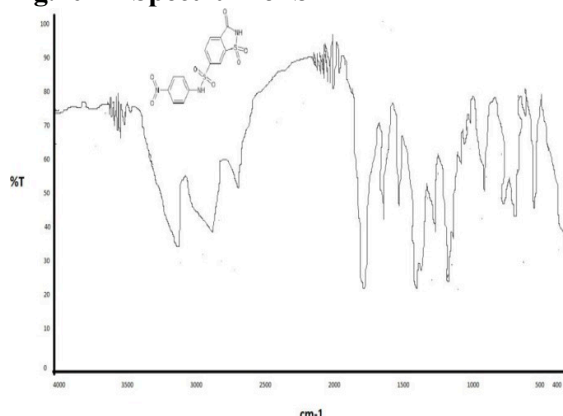


Table 17. Interpretation of IR Spectrum SA4

S. NO	WAVE NUMBER (cm ⁻¹)	FUNCTIONAL GROUP
1	1734	-C=O (Keto)
2	1636	-NH (Secondary amide)
3	705	- C=C (Benzene ring)
4	1335	-SO ₂ (Sulphonamide)
5	3415	Benzene ring
6	1545	NO ₂ (Nitro)

Fig.11 ¹H NMR spectrum of SA4

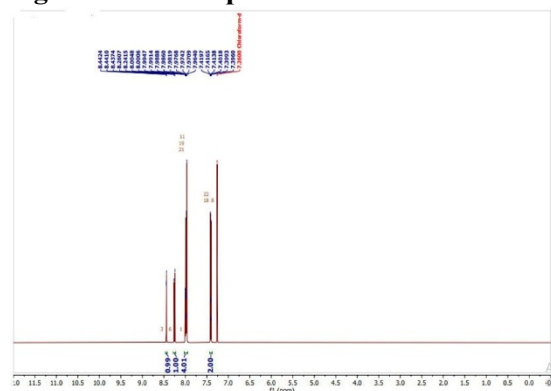


Table 18: Interpretation of ¹H NMR Spectrum of Compound SA4

SIGNALS	Δ -VALUE (PPM)	NATURE OF PROTON
A	4.00	Aromatic NH Proton
B	6.89	Benzene Proton
C	7.91	Benzene Proton
D	8.00	Secondary amide Proton
E	8.01	Benzene Proton
F	8.59	Benzene Proton
G	8.67	Benzene Proton

Fig.12 Mass spectrum of SA4

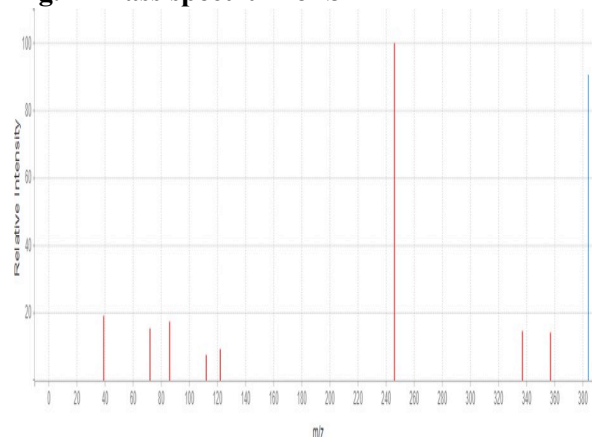
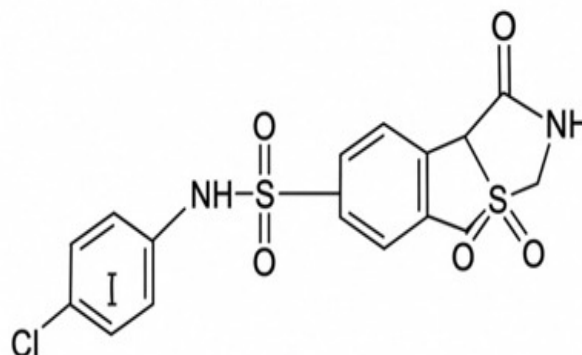


Table 19: Interpretation of Mass Spectrum of Compound SA4

Molecular Ion Peak m/z	383.99
Base Peak m/z	245.95

SA5



IUPAC NAME: N-(4-chlorophenyl)-1,1,3-trioxo-2,3-dihydro-1H-1λ6,2-benzothiazole-5-sulfonamide

Fig.13 IR Spectrum of SA5

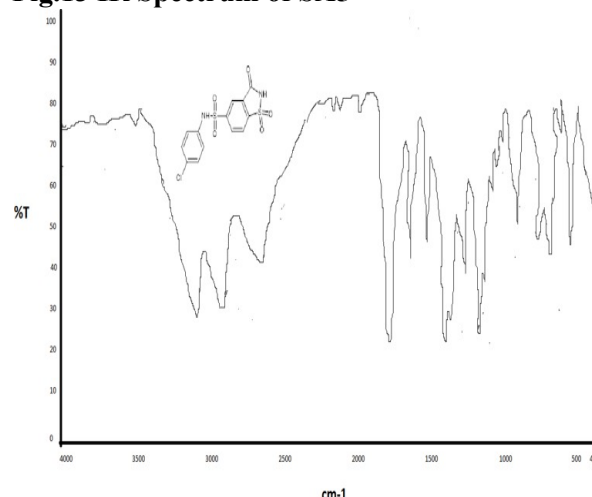


Table No. 20 Interpretation of IR Spectrum SA5

S. No	Wave Number (cm ⁻¹)	Functional Group
1	1475 and 1600	C=C (Aromatic)
2	1725	-C=O (Keto)
3	2970	-NH (Secondary amine)
4	1170 and 1370	S=O (Sulphon amide)
5	735	C-Cl (Chloro)
6	1725	-C=O (Keto)

Fig.14 ¹H NMR spectrum of SA5

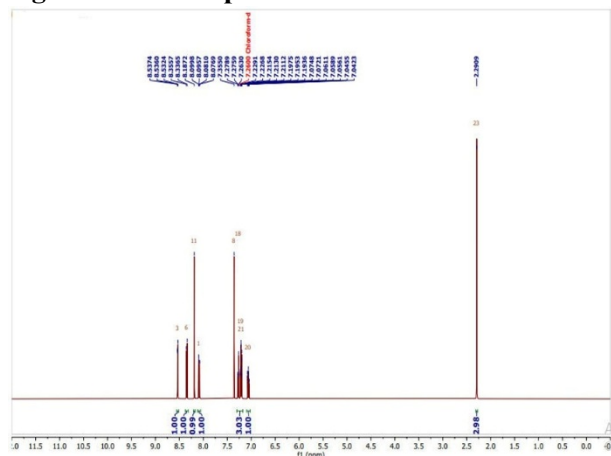


Table 21: Interpretation of ¹H NMR Spectrum of Compound SA5

SIGNALS	Δ - VALUE (PPM)	NATURE OF PROTON
A	2.00	Amine Proton
B	6.27	Aromatic NH Proton
C	6.61	Benzene Proton
D	7.61	Benzene Proton
E	7.91	Benzene Proton
F	8.00	Secondary Amide Proton
G	8.59	Benzene Proton
H	8.67	Benzene Proton

Fig.15 Mass spectrum of SA5

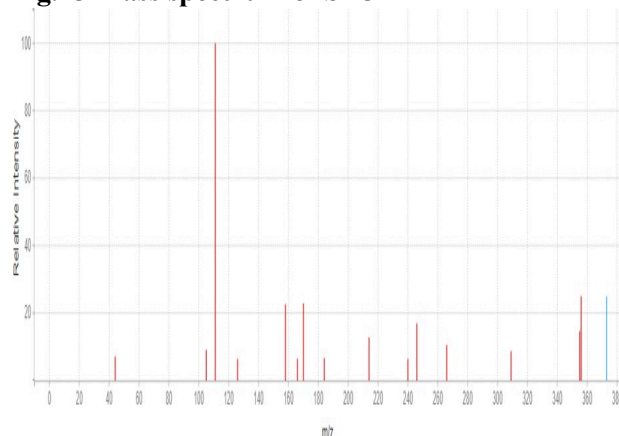


Table 22: Interpretation of Mass Spectrum of Compound SA5

Molecular Ion Peak m/z	372.97
Base Peak m/z	110.99

- All the 5 synthesized compounds had different Rf values from this respective reactant molecule and showed a single spot in the thin layer chromatogram.
- They exhibited a sharp melting point.
- In the IR spectrum, absorptions corresponding to the relevant functional groups were seen.
- From the NMR spectrum, numbers of protons present in the molecular structure were elucidated and the structure of the compound was confirmed.

Anticancer Activity

Name of the study: In vitro Anticancer activity of test samples

Duration of study: 5 days

Cell culture: SY5Y - Cell line

In vitro Anti-Cancer Activity of SA1 to SA5 on SY5Y-cell line

- Two washes of phosphate-buffered saline (PBS) were performed after the cells were exposed to the samples for 24 hours.
- Each well was then reacted with 10 ml of MTT reagent (5 mg/ml in PBS), including a blank (a non-reacted well containing only the medium).
- The plates were then incubated at 37°C for four hours. To dissolve the insoluble formazan products, 100 μl of DMSO was added to each well after two further washes in PBS. A micro plate reader (Labtech LT-4000MS, Labtech International Ltd., East Sussex, UK) was used to quantify the impact of unknown compounds on cancer cells (SY5Y Cell lines).
- The standard error of the mean was calculated by repeating each experiment three times (n = 3) for each treatment. Using this formula, growth inhibition in untreated (control) cells was compared to growth inhibition in treated cells.

$$\% \text{ of Growth inhibition} = \frac{\text{Control} - \text{Test absorbance}}{\text{Control}} \times 100$$

GROUP 1

Concentration (μM)	SA1		
X	A: Y1	A: Y2	A: Y3
0	100.0	100.0	100.0
1	88.7	87.30	65.60
5	78.2	79.70	56.75
10	65.3	63.70	52.10
20	53.4	54.40	38.60
30	48.3	40.70	29.70
40	43.6	37.63	26.50
50	38.7	28.60	17.50
ICF50(μM)	6.101±2.53		

GROUP 2

Concentration (μM)	SA2		
X	B:Y1	B:Y2	B:Y3
0	100.0	100.0	100.0
1	68.6	68.7	71.7
5	55.7	61.7	67.2
10	44.4	52.7	52.8
20	28.6	36.4	40.8
30	23.5	26.0	28.8
40	9.9	17.4	20.8
50	4.7	7.9	11.2
ICF50(μM)	5.253±1.761		

GROUP 3

Concentration (μM)	SA3		
X	C: Y1	C: Y2	C: Y3
0	100.0	100.0	100.0
1	104.5	98.5	97.3
5	97.3	87.6	87.5
10	72.7	67.7	74.3
20	67.6	64.2	65.3
30	53.7	48.6	51.7
40	49.8	41.4	45.5
50	42.7	31.8	36.5
ICF50(μM)	13.6±1.95		

GROUP 4

Concentration (μM)	SA4		
X	D: Y1	D: Y2	D: Y3
0	100.0	100.0	100.0
1	72.7	76.2	76.7
5	63.7	64.8	69.6
10	44.6	49.6	54.8
20	39.8	36.7	38.7
30	34.8	36.7	38.7
40	25.7	29.8	31.8
50	23.5	27.4	28.6
ICF50(μM)	3.654±1.027		

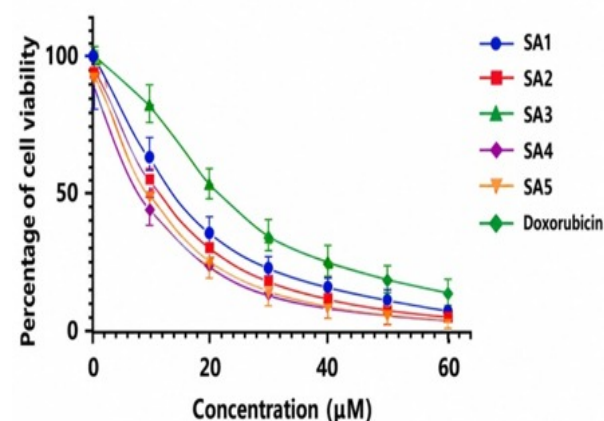
GROUP 5

Concentration (μM)	SA5		
X	E: Y1	E: Y2	E: Y3
0	100.0	100.0	100.0
1	96.5	87.9	88.5
5	77.4	76.3	71.5
10	71.2	65.7	61.5
20	61.3	53.7	53.9
30	55.6	49.5	50.4
40	42.8	41.5	37.8
50	36.6	37.7	39.4
ICF50(μM)	4.994±1.592		

GROUP 6

Concentration (μM)	Doxorubicin		
X	C: Y1	C: Y2	C: Y3
0	100.00	100.00	100.00
1	90.32	79.64	80.43
5	75.64	74.32	61.53
10	68.59	57.64	51.64
20	58.46	49.64	45.43
30	50.34	44.43	37.40
40	45.64	39.74	34.40
50	40.55	36.64	27.53
ICF50(μM)	4.994±1.592		

Fig.16 SY5Y – CELL LINE



RESULTS AND DISCUSSION

The molecular design of all synthesized compounds were done by using different software such as Chemschetch, swiss ADME, Molinspiration, OSIRIS property explorer and PyRx.

- The lipinski rule was predicted for all synthesized compound using SWISS ADME.
- It shows no violation in basic properties. The results were shown in Page.No.52

- In molecular docking, the molecules of the binding affinity with ALK shown in Page.No.60
- The solubility, GI Absorption, BBB Permeant and Synthetic Accessibility of the compounds were predicted and in Page.No.52
- The in-silico Toxicity Assessment results shown in Page.No.56
- The physical Evaluation of the compounds were predicted and shown in Page.No.64
- The purity of the compounds was found out by TLC and Rf value was calculated. The results are shown in Page.No.65
- The Characterisation of synthesized compounds were confirmed by IR spectra, NMR spectra and Mass spectra. The results are shown in Page.No.70 to 84
- All synthesized compounds were evaluated for anti-cancer activity.
- Anti-cancer activity of all synthesized compounds were evaluated and results were shown in group 1 to group 6
- At the concentration of 20 µg/ml, 40 µg/ml, 50 µg/ml, SA2 were found to be more effective against neuro blastoma cancer cell line SY5Y. Results were shown in Page.No.88
- Among the synthesized Saccharin derivatives SA2 showed a good activity with IC50 value of 50µg/ml. whereas the Compound SA1, SA3, SA4, SA5 showed moderate activity with IC50 value of 1,5,10,20,30,40, µg/ml.

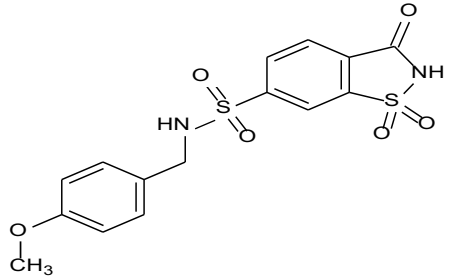
SUMMARY AND CONCLUSION

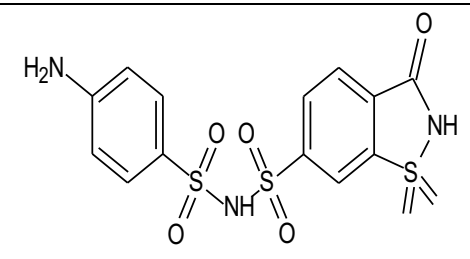
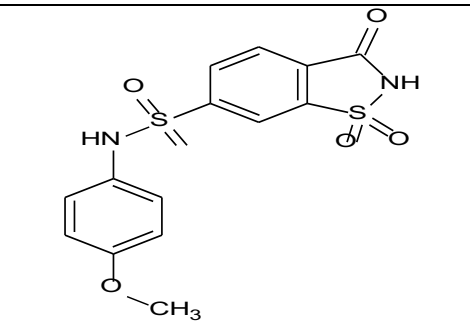
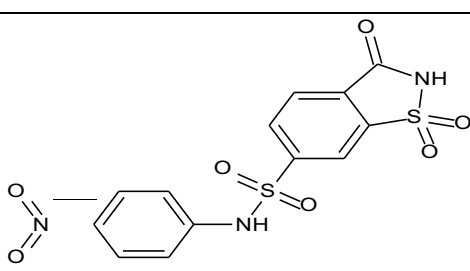
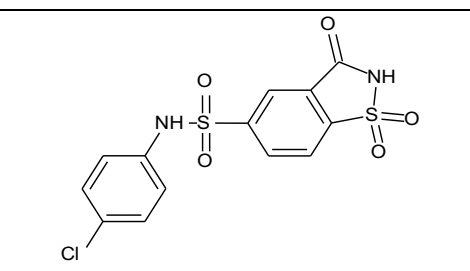
- Anaplastic Lymphoma Kinase is a vital enzyme present in the Neuroblastoma cells, located on the short arm of Chromosome 2 at position 23. It belongs to Tyrosine kinase

receptor member of the insulin receptor superfamily was chosen after the review of literature.

- A database of 60 molecules with high prospects of inhibiting the target ALK were carefully chosen by making changes to the known hit molecules, here the saccharin nucleus was chosen. Lead optimisation was done by observing in silico ADME studies and computation of drug like properties. All the ligands had showed druglikeness and acceptable pharmacokinetic parameter. Hence would be further studied for biological activities.
- The designed molecules were docked against the target chosen using Auto Dock 4® TOOLS 1.5.6 software.
- Five molecules (SA1, SA2, SA3, SA4, SA5) with good docking score [lower binding energy] with ALK and interactions were shortlisted for synthesis.
- The selected molecules were subjected to toxicity prediction assessment by OSIRIS® property explorer developed by Acetilon Pharmaceuticals limited which is available online. The results are colour coded as green colour which predicts the drug likeness and possibly better activity.
- The characterization of the synthesized compounds was done using TLC, Melting point Infra-red, Mass and Nuclear Magnetic Resonance [H1 NMR] spectroscopy methods.
- All the Synthesized compounds exhibited molecular ion peak (M⁺) of varying intensities.
- The final pure compounds were screened for Anti-Cancer activity by in vitro method called ALK Inhibitory assay.

5. LIST OF NEWLY SYNTHSIZED COMPOUNDS

Compound Code	Compound Name	Structure
SA1	N-(-4-methoxybenzyl)-3-oxo-2 dihydrobenzo[d]isothiazole-5-sulfonamide 1,1-dioxide	

SA2	N-[(4-aminophenyl)sulfonyl]-3-oxo-2,3-dihydro-1,2-benzothiazole-6-sulfonamide 1,1-dioxide	
SA3	N-(4-methoxyphenyl)-3-oxo-2,3-dihydrobenzo[d]isothiazole-5-sulfonamide 1,1-dioxide	
SA4	N-(4-nitrophenyl)-1,1,3-trioxo-2,3-dihydro-1H-1λ ⁶ ,2-benzothiazole-6-sulfonamide	
SA5	N-(4-chlorophenyl)-1,1,3-trioxo-2,3-dihydro-1H-1λ ⁶ ,2-benzothiazole-5-sulfonamide	

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