DESIGN, SYNTHESIS AND *IN VITRO* ANTI-CANCER ACTIVITY OF NOVEL SACCHARINE DERIVATIVE AGAINST NEUROBLASTOMA (SY5Y-CELL LINE) TARGETING ANAPLASTIC LYMPHOM KINASE (ALK) RECEPTOR

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Abstract

This research focuses on the design, synthesis, characterization, and biological evaluation of novel Saccharin-7-Sulphonamide derivatives as potential anticancer agents targeting the Anaplastic Lymphoma Kinase (ALK) receptor. The compounds were tested against human neuroblastoma SY5Y cell lines using the MTT assay. Computational tools, including ChemSketch, Swiss ADME, Molinspiration, OSIRIS Property Explorer, and PyRx, were employed for molecular modeling and drug-likeness predictions, confirming compliance with Lipinski's rule. Molecular docking studies performed using AutoDock 4® TOOLS 1.5.6 revealed strong binding affinities of the synthesized derivatives with ALK.

The synthesized compounds were characterized by determining their percentage yield, solubility, melting point, and molecular formula. Purity was confirmed using TLC (Rf value determination), and further structural elucidation was conducted through IR, NMR, and mass spectrometry. Among the five synthesized derivatives (SA1–SA5), SA2 demonstrated the highest anticancer activity, exhibiting an IC50 value of 5.253 ± 1.761 µM at concentrations of 20, 40, and 50 µM. The remaining compounds (SA1, SA3, SA4, SA5) displayed moderate activity with IC50 values ranging from 1 to 40 µg/ml. Toxicity predictions using OSIRIS Property Explorer indicated favourable drug-likeness profiles for all derivatives.

The study concludes that SA2 is a promising lead compound for further development as an anticancer agent targeting the ALK receptor in neuroblastoma. These findings support the potential of saccharin-based sulphonamide derivatives as effective anticancer candidates, warranting further in-depth biological evaluation.

Keywords: Saccharin-7-Sulphonamide derivatives, Anaplastic Lymphoma Kinase (ALK), Neuroblastoma SY5Y, Molecular docking.

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]

Neuroblastoma is a cancer that develops from immature nerve cells found in several areas of the body. Most commonly it arises in and around the adrenal glands, which have similar origins to nerve cells and sit atop the kidneys. However, neuroblastoma can also develop in other areas of the abdomen and in the chest, neck and near the spine, where groups of nerve cells exist. Signs and symptoms of neuroblastoma vary depending on which part of the body is affected. Neuroblastoma in the abdomen - the most common form may cause signs and symptoms such as abdominal pain, a mass under the skin that isn't tender when touched, changes in bowel habits, such as diarrhoea or constipation. Neuroblastoma in the chest may cause signs and symptoms such as wheezing, chest pain, changes to the eyes, including drooping eyelids and unequal pupil size.

Anaplastic Lymphoma Kinase (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 rotein. Therefore, it catalyzes a tyrosine residue phosphorylation reaction on its substrate proteins. The phosphorylation and ephosphorylation of proteins are critical reactions catalyzedby different enzymes (kinases and phosphatases), which play critical roles in various cellular functions. ALK is usually expressed during the development of the nervous system.[2]

Saccharin is an 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 ha about 200–700 times the sweetening power of granulated sugar. Saccharin, in addition to being used as a table-top sweetener, is commonly used in soft drinks, baked foods, jams, canned fruit, candy, dessert toppings, and chewing gum. Saccharin became the first commercially available artificial sweetener. It is still made by the oxidation of *o*-toluene sulfonamide, as well as from phthalic anhydride.[3]

Unlike glucose and sucrose, saccharin cannot be metabolized by the body.[4] 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.[5] 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.[6]

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.[7] For these reasons, many people still believe that the use of saccharin should be limited in infants, children, and pregnant women. Due to the increasing demand for the use of non-nutritive artificial sweeteners as a tool to control the level of blood glucose and body weight, and due to uncertain and little information on the safety of using saccharin as a sweetener, the present study was set to analyze the effect of long-term consumption of saccharin on biochemical parameters when increasingly large doses are administered.[8]

MATERIALS AND METHODS

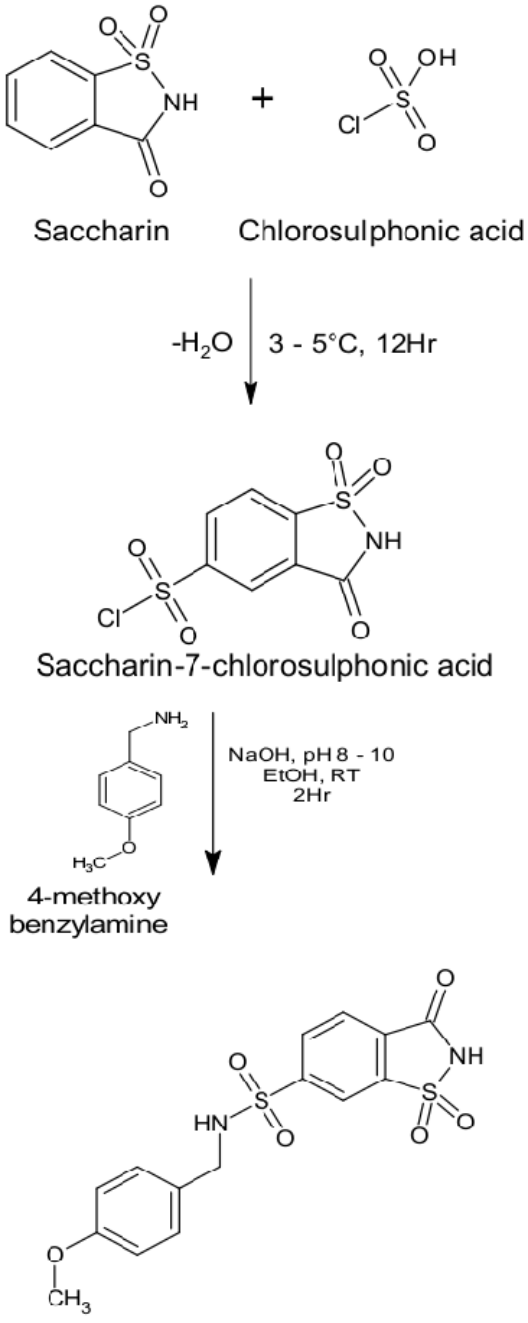
Step-1

An excess (4:1) of Chlorosulphonicacid was slowly added to the Saccharin. The mixture was mixed well and kept in the refrigerator over-night. Then, the mixture was poured over crushed ice and the precipitated Saccharin-7-chlorosulphonic acid was filtered and dried. [9]

Step-2

4-methoxybenzylamine were dissolved in ethanol and added slowly to the Saccharin-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. [10]

SYNTHESIS OF COMPOUNDS



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

R=4-methoxybenzyl,4-aminophenyl,4-methoxyphenyl,

4-nitrophenyl,4-chlorophenyl

MOLECULAR DESIGN

TARGET ENZYME:

H^+K^+ ATPase were 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) PBD ID – 5YLU.

MOLECULAR DOCKING BY AUTODOCK®:

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.

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

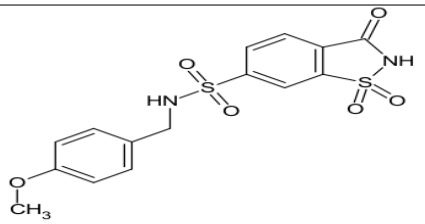
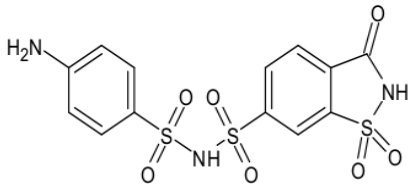
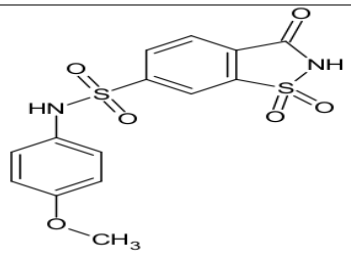
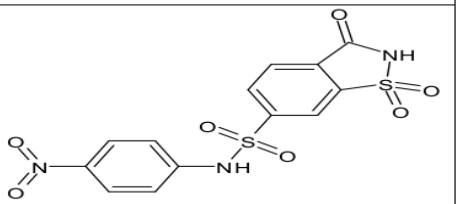
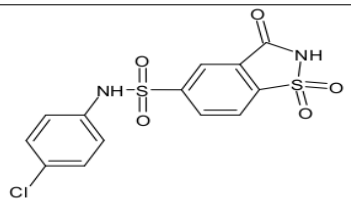
IN SILICO TOXICITY PREDICTION OSIRIS:

- 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®:

- ✓ 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 rediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

Table 1. NEWLY SYNTHESIZED 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-sulfonamide1,1-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-1*H*-1λ^6,2-benzothiazole-5-sulfonamide	

RESULT AND DISCUSSION

Table 2. PHYSICAL EVALUATION OF THE NEWLY SYNTHESIZED COMPOUNDS

Compound code	R	Molecular formula	Molecular weight	Melting point	Rf value	Percentage yield
SA1	4-methoxy	C15H14N2O6S2	384.41	186-187	0.65	78%
SA2	4-amino	C13H11N3O7S3	417.425	183-184	0.73	83%
SA3	4-methoxy	C14H12N2O6S2	368.38	184-185	0.58	92%
SA4	4-nitro	C13H9N3O7S2	383.36	204-205	0.78	95%
SA5	4-chloro	C13H9ClN2O5S2	372.80	195-196	0.76	94%

Table 3. TLC PROFILE

S.NO	SAMPLE CODE	MOBILE PHASE	Rf 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

INVITRO ANTICANCER ACTIVITY

Name of the study : Invitro Anticancer activity of test samples
Duration of study : 5 days
Cell culture : SY5Y - Cell line

Invitro 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 l of DMSO was added to each well after two further washes in PBS. A micro plate reader (LabtechLT-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.

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

Table 4. 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		

Table 5. 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		

Table 6. 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		

Table 7. 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		

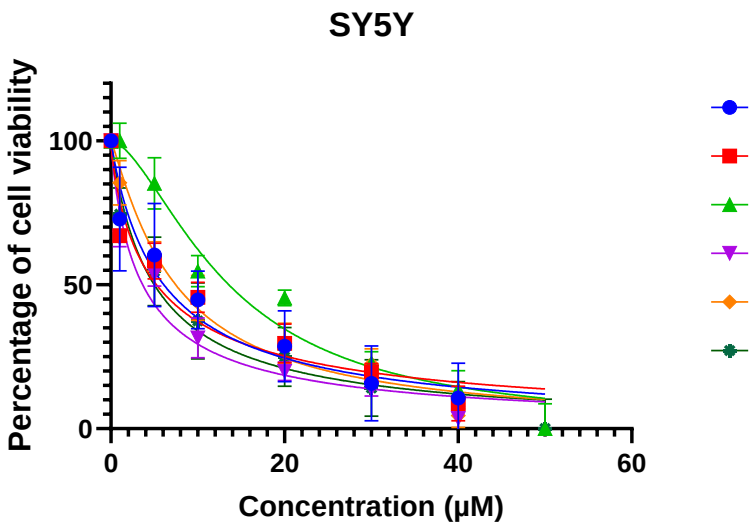
Table 8. GROUP 5

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		

Table 9. 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.1 SY5Y – CELL LINE



In-SilicoToxicityAssessment 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 10. 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

Table 11. 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

SUMMARY AND CONCLUSION

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 drug likness and acceptable pharmacokinetic parameter. Hence would be further studied for biological activities.The designed molecules were docked against the target chosen using AutoDock 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 [^{1}H 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.Among the synthesized Saccharin derivatives **SA2** showed a good activity with IC50value 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.

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