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REVIEW ON COMPUTATIONAL INSIGHTS OF NOVEL ANTICANCER AGENT EMRICASAN BY TRACING RIPK3PATHWAY HASTENING NECROPTOSIS

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ABSTRACT

Cancer is a group of diseases involving uncontrolled growth of abnormal cells anywhere in a body. These abnormal cells are termed cancer cells, malignant cells, or tumor cells. Cancer is the second leading cause of death globally, accounting for an estimated 19.3 million cases and 10 million cancer deaths in 2020. Even though the cases of cancers are increasing day by day, except cytotoxic, targeted, and hormonal drugs no other way of treatment has been successfully developed so far. So it is need to develop a novel anticancer drugs. Thus, the objective of this review is to provide an insight over the computational study of novel anticancer agent Emricasan by tracing RIPK3 pathway hastening necroptosis. The review also contains brief idea about necroptosis process and pathological aspects of cancer. Drug emricasan is a caspase 8 inhibitor so, this review includes the QSAR study & molecular docking score of ligand - receptor complex, Emricasan and TNFR1.

KEYWORDS: Novel Anticancer Agent, Necroptosis, TNFR1, Caspase 8, Emricasan, Docking Score.

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells with the potential to invade or spread to other parts of the body. A tumor is an abnormal mass of cells. Tumors can either be benign (non-cancerous) or malignant (cancerous).

❖ **Benign tumors**

Benign tumors grow locally and do not spread. As a result, benign tumors are notconsidered cancer. They can still be dangerous, especially if they press against vital organslike the brain.

❖ **Malignant tumors**

Malignant tumors have the ability to spread and invade other tissues. This process, knownas metastasis, is a key feature of cancer. There are many different types of malignancy based on where a cancer tumor originates.

Cancer metastasis

Metastasis is the process whereby cancer cells break free from a malignant tumor and travelto and invade other tissues in the body. Cancer cells metastasize to other sites via the lymphatic system and the bloodstream. Cancer cells from the original—or primary—tumor can travel to other sites such as the lungs, bones, liver, brain, and other areas. These metastatic tumors are "secondary cancers" because they arise from the primary tumor.

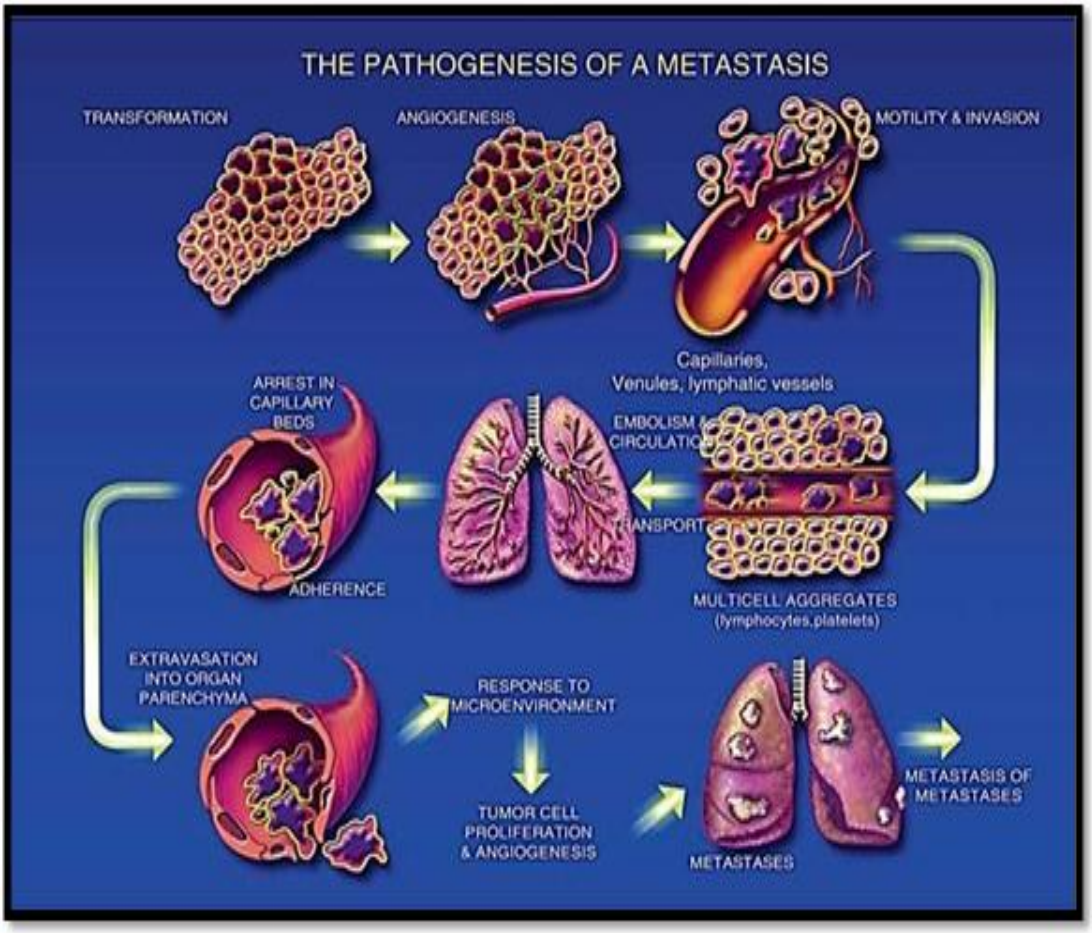
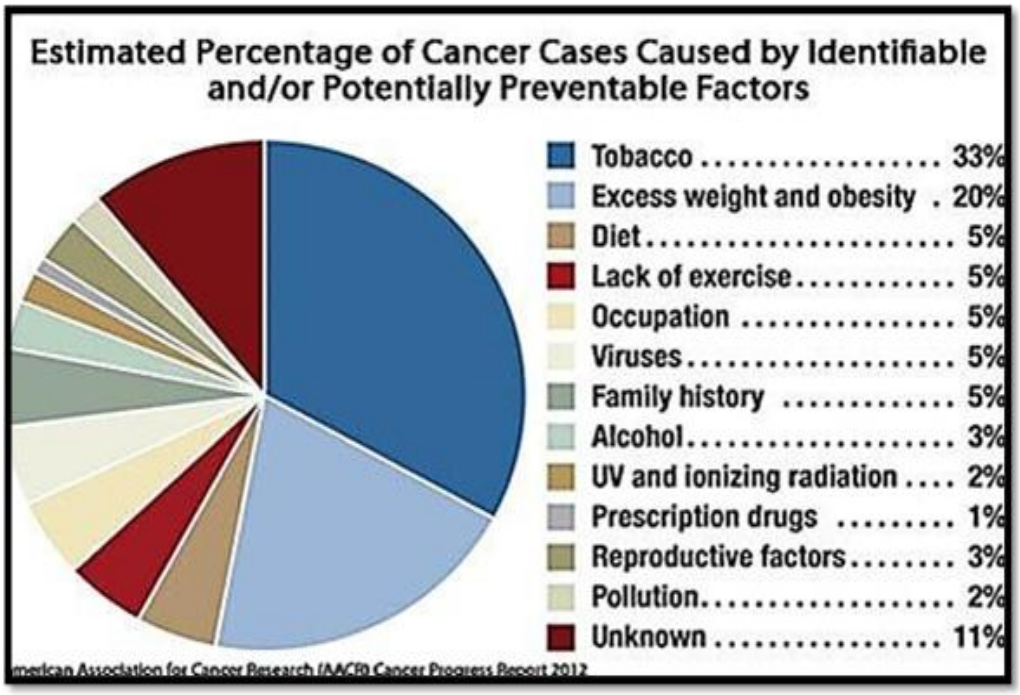


Fig. 1: Pathogenesis of metastasis.

What causes cancer?



Common Cancer Symptoms and Signs

Symptoms and Signs of cancer may include

- Fever
- Pain
- Fatigue
- Skin changes (redness, sores that won't heal, jaundice, darkening)
- Unintended weight loss or weight gain

Other more obvious signs of cancer may include

- Lumps or tumors (mass)
- Difficulty swallowing
- Changes or difficulties with bowel or bladder function
- Persistent cough or hoarseness
- Short of breath
- Chest pain

Types of cancer

Cancers are classified by the type of cell that the tumor cells resemble and is therefore presumed to be the origin of the tumor.

- **Carcinoma**

Cancer of epithelial cells eg.skin, mouth, throat, lung cancer.

- **Sarcoma**

Cancer of bone muscle or connective tissue.

- **Melanoma**

A malignant tumour associated with skin cancer.

- **Leukaemia**

Cancer of blood forming organs

- **Lymphoma**

Cancer of infection fighting organs.

- **Myeloma**

Cancer of bone marrow cell

Cell cycle of cancer cell

The cell cycle, the process by which cells progress and divide, lies at the heart of cancer. In normal cells, the cell cycle is controlled by a complex series of signalling pathways by which a cell grows, replicates its DNA and divides.

This process also includes mechanisms to ensure errors are corrected, and if not, the cells commit suicide (apoptosis). In cancer, as a result of genetic mutations, this regulatory process malfunctions, resulting in uncontrolled cell proliferation.

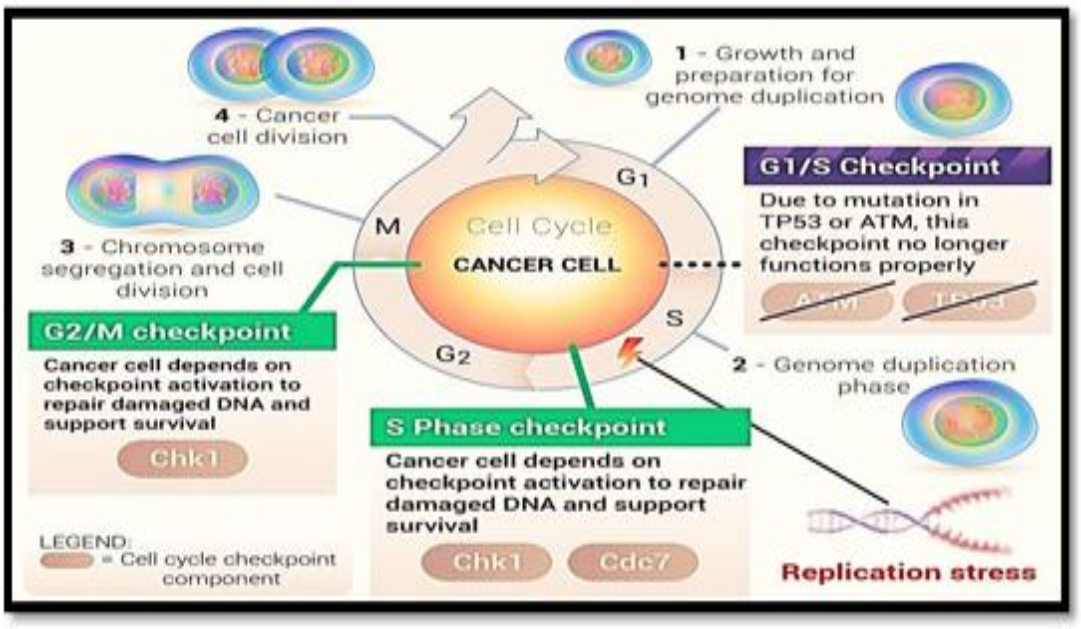


Fig. 2: Cell cycle of cancer cellPhases of cell cycle.

The eukaryotic cell cycle consists of four distict phases: G1 phase, S phase (synthesis), G2 phase (Collectively known as interphase) and M phase (Mitosis and cytokinesis).

Mitotic phase (Chromosome seperation)

- Prophase
- Prometaphase
- Metaphase
- Anaphase
- Telophase

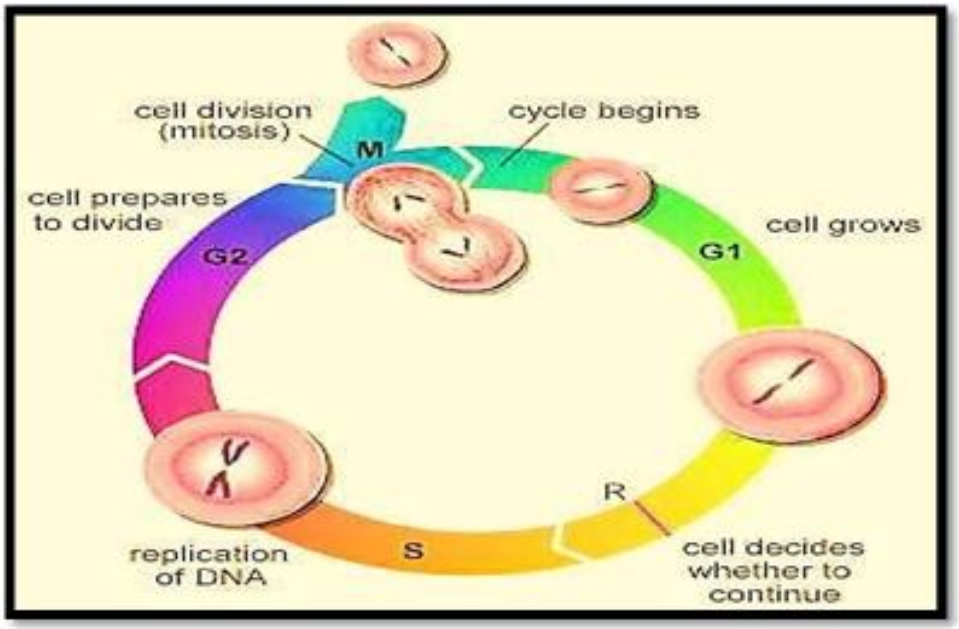


Fig. 3: Interphases of cancer cell growth.

G0 Phase

After being born through mitosis, some cells are not meant to divide themselves to produce daughter cells. Neurons, for example – animal nerve cells – do not divide. Their "parent cells"are stem cells, and the "daughter" neuron cells are programmed not to go through the cell cycle themselves because uncontrolled neuron growth and cell division could be very dangerous forthe organism. So instead of entering G1 phase after being "born," neurons enter a phase scientists call "G0 phase." This is a metabolic state meant only to maintain the daughter cell, not prepare for cell division. Neurons and other non-dividing cell types may spend their whole lives in G0 phase, performing their function for the overall organism without ever dividing orreproducing

G1 Phase

In G1 phase, the newly formed daughter cell grows. The "G" is most often said to stand for "gap," since these phases appear to an outside observer with a light microscope to be relatively inactive "gaps" in the cell's activity. However given what we know today, it might be more accurate to say the "G" stands for "growth" – for the "G" phases are flurries of protein and organelle production as well as literal increase in the size of the cell. During the first "growth" or "gap" phase, the cell produces many essential materials such as proteins and ribosomes. Cells that rely on specialized organelles such as chloroplasts and mitochondria make a lot more of those organelles during G1 as well. The cell's size may increase as it assimilates more material from its environment into its machinery for life.

S. Phase

During S phase, the cell replicates its DNA. The "S" stands for "synthesis" – referring to the synthesis of new chromosomes from raw materials. This is a very energy-intensive operation, since many nucleotides need to by synthesized. Many eukaryotic cells have dozens of chromosomes – huge masses of DNA – that must be copied.Production of other substances and organelles is slowed greatly during this time as the cell focuses on replicating its entire genome.When the S phase is completed, the cell will have two complete sets of its genetic material. This is crucial for cell division, as it ensures that both daughter cells can receive a copy of the "blueprint" they need to survive and reproduce.

G2 Phase

Just like the first "gap" phase of the cell cycle, the G2 phase is characterized by lots of protein production. During G2, many cells also check to make sure that both copies of their DNA are correct and intact. If a cell's DNA is found to be damaged, it may fail its "G2/M checkpoint" – so named because the this "checkpoint" happens at the end of the G2 phase, right between G2 and "M phase" or "Mitosis." This "G2/M checkpoint" is a very important safety measure for multicellular organisms like animals. Cancers, which can result in the death of the entire organism, can occur when cells with damaged DNA reproduce. By checking to see if a cells' DNA has been damaged immediately before replication, animals and some other organisms reduce the risk of cancer. Interestingly, some organisms can skip G2 altogether and go straightinto mitosis after DNA is synthesized during S phase. Most organisms, however, find it safer to use G2 and its associated checkpoint!If the G2/M checkpoint is passed, the cell cycle beginsagain. The cell divides through mitosis, and new daughter cells begin the cycle

that will take them through G1, S, and G2 phases to produce new daughter cells of their own.

Mitosis

During mitosis, the "parent" cell goes through a complex series of steps to ensure that each "daughter" cell will get the materials it needs to survive, including a copy of each chromosome. Once the materials are properly sorted, the "parent" cell divides down the middle, pinching its membrane in two. Each of the new "daughters" are now independently living cells. But they'resmall, and have only one copy of their genetic material. This means they can't divide to produce their own "daughters" right away. First, they must pass through "interphase" – the phase between divisions, which consists of three distinct phases.

Prophase: It is the first phase of mitosis, the process that separates the duplicated genetic material carried in the nucleus of a parent cell into two identical daughter cell. During prophase, the complex of DNA and proteins contained in the nucleus, known as chromatin condeses.

1) **Prometaphase:** It is the second phase of mitosis, the process that separates the duplicated genetic material carried in the nucleus of a parent cell into two identical daughter cells.during prometaphase, the physical barrier that encloses the nucleus, called the nuclear envelope, breaks down.
2) **Metaphase:** During metaphase, the cells chromosomes align metaphase, the cells chromosomes align themselves in the middle of the cell through a type of cellular ' tug of war'
3) **Anaphase:** It is stage of mitosis after the metaphase, when replicated chromosomes are split and the newly coppied chromosomes are moved to opposite poles of the cell. Chromosomes also reach there overall maximum condensation in late anaphase, to help chromosomes segreggation and the reformation of nucleus.
4) **Telophase:** The final phase of cell division, between anaphase and interphase, in which the chromatids or chromosomes moves to opposite ends of the cell and two nuclei are formed.[1-2]

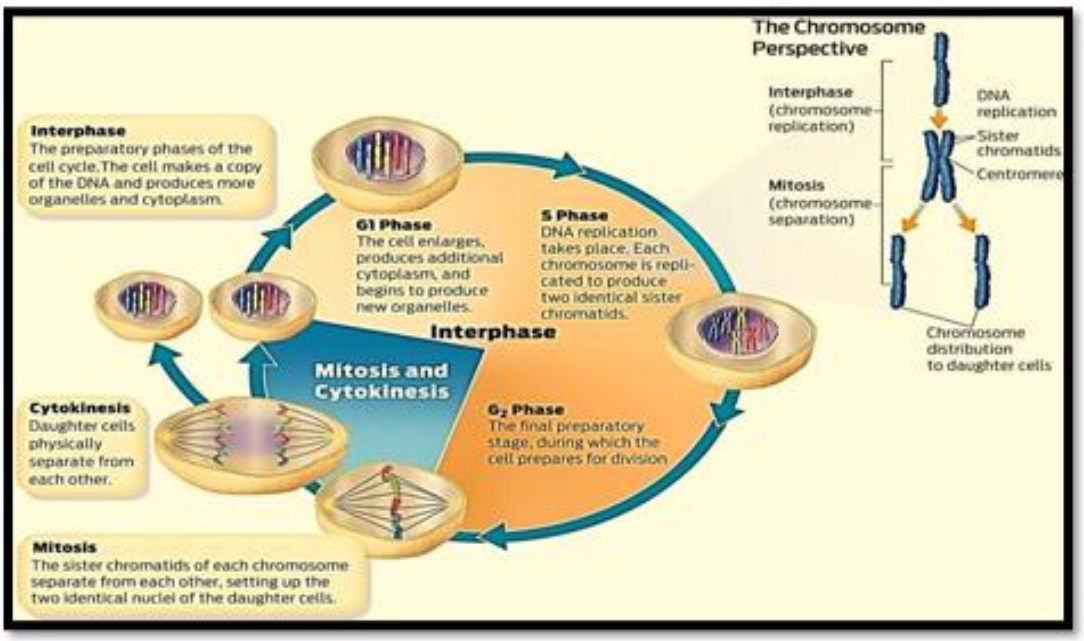


Fig. 4: Phases of cell cycle and interphase.

QSAR: (Quantitative Structure- Activity Relationship)

Its staying power may be attributed to the strength of its initial postulate that activity was a function of structure as Described by electronic attributes, hydrophobicity, and steric properties as well as the rapid and extensive development in methodologies and computational techniques that have ensued to delineate and refine the many variables and approaches that define the paradigm. The overall goals of QSAR retain their original essence and remain focused on the predictive ability of the approach and its receptiveness to mechanistic interpretation.

The formulation of thousands of equations using QSAR methodology attests to a validation of its concepts and its utility in the elucidation of the mechanism of action of drugs at the molecular level and a more complete understanding of physicochemical phenomena such as hydrophobicity. It is now possible not only to develop a model for a system but also to compare models from a biological database and to draw analogies with models from a physical organic database. This process is dubbed model mining and it provides a sophisticated approach to the study of chemical-biological interactions.

Parameters used in QSAR

1. Electronic parameters

Parameters are of critical importance in determining the types of intermolecular forces that underlie drug-receptor interactions. The three major types of parameters that were initially suggested and still hold way are electronic, hydrophobic, and steric in nature. Extensive studies

using electronic parameters reveal that electronic attributes of molecules are intimately related to their chemical reactivities and biological activities. A search of a computerized QSAR database reveals the following: the common Hammett constants (σ, σ+, σ-) account for 700018500 equations in the Physical organic chemistry (PHYS) database and nearly 1600/8000 in the Biology (BIO) database, whereas quantum chemical indices such as HOMO, LUMO, BDE, and polarizability appear in 100 equations in the BIO database. The introduction of substituent groups into the framework and the subsequent alteration of reaction rates helps delineate the Overall mechanism of reaction. Early work examining the electronic role of substituents on rate constants was first tackled by Burckhardt and firmly established by Hammett. Hammett employed, as a model reaction, the ionization in water of substituted benzoic acids and determined their equilibrium constants K. Thisled to an operational definitionof u, the substituent constant. It is a measure of the size of the electronic effect for a given substituent and represents a measure of electronic charge distribution in the benzene nucleus.

2. Hydrophobicity parameters

More than a hundred years ago, Meyer and Overton made their seminal discovery on the correlation between oil water partition coefficients and the narcotic potencies of small organic molecules. Ferguson extended this analysis by placing the relationship between depressant action and hydrophobicity in a thermodynamic context; the relative saturation of the depressant in the bio phase was a critical determinant of its narcotic potency. At this time, the success of the Hammett equation began to permeate structure-activity studies and hydrophobicity as a determinant was relegated to the background. In a landmark study, Hansch and his colleagues devised and used a multipara meter approach that included both electronic and hydrophobic terms, to establish a QSAR for a series of plant growth regulators. This study laid the basis for the development of the QSAR paradigand also firmly established the importance of lipophilicityin bio systems. Over the last 40 years, no other parameter used in QSAR has generated more interest, excitement, and controversy than hydrophobicity. Hydrophobic interactions are of critical importance in many areas of chemistry. These include enzyme-ligand interactions, the assembly of lipids in biomembranes, aggregation of surfactants, coagulation, and detergency. The integrity of biomembranes and the tertiary are determined by a polar-type interactions. Molecular recognition depends on hydrophobic interactions between ligands and receptors. Hydrophobicities of solutes can readily be determined by measuring partition coefficients designated as P. Partition coefficient deal with neutral species, whereas distribution

ratios incorporate concentrations of charged and polymeric species as well.

3. Steric parameters

The quantitation of steric effects is complex at best and challenging in all other situations, particularly at the molecular level. An added level of confusion comes into play when attempts are made to delineate size and shape. Nevertheless, steric are of overwhelming importance in ligand-receptor interactions as well as in transport phenomena in cellular systems. The first steric parameter to be quantified and used in QSAR studies was Taft's Es constant.

4. Development of receptor theory

The central theme of molecular pharmacology, and the underlying basis of SAR studies, has focused on the elucidation of the structure and function of drug receptors. It is an endeavour that proceeds with unparalleled vigour, fuelled by the developments in genomics. It is generally accepted that endogenous and exogenous chemicals interact with a binding site on a specific macromolecular receptor. This interaction, which is determined by intermolecular forces, may or may not elicit a pharmacological response depending on its eventual site of action.

The idea that drugs interacted with specific receptors began with Langley, who studied the mutually antagonistic action of the alkaloids, pilocorpine and atropine. He realized that both these chemicals interacted with some receptive substance in the nerve endings of the gland cells. Paul Ehrlich defined the receptor as the "binding group of the protoplasmic molecule to which a foreign newly introduced group binds". In 1905 Langley's studies on the effects of curare on muscular contraction led to the first delineation of critical characteristics of a receptor: recognition capacity for certain ligands and an amplification component that results in a pharmacological response Receptors are mostly integral proteins embedded in the phospholipid bilayer of cell membranes. Rigorous treatment with detergents is needed to dissociate the proteins from the membrane, which often results in loss of integrity and activity.

Pure proteins such as enzymes also act as drug receptors. Their relative ease of isolation and amplification have made enzymes desirable targets in structure based ligand design and QSAR studies. Nucleic acids comprise an important category of drug receptors. Nucleic acid receptors (aptamers), which interact with a diverse number of small organic molecules, have been isolated by in vitro selection techniques and studied.

5. Biological parameters

In QSAR analysis, it is imperative that the biological data be both accurate and precise to develop a meaningful model. It must be realized that any resulting QSAR model that is developed is only as valid statistically as the data that led to its development. The equilibrium constants and rate constants that are used extensively in physical organic chemistry and medicinal chemistry are related to free energy values AG. Thus for use in QSAR, standard biological equilibrium constants such as Ki or K, should be used in QSAR studies. Likewise only standard rate constants should be deemed appropriate for a QSAR analysis. Percentage activities (e.g., % inhibition of growth at certain concentrations) are not appropriate biological endpoints because of the nonlinear characteristic of dose-response relationships. These types of endpoints may be transformed to equieffective molar doses. Only equilibrium and rate constants pass muster in terms of the free-energy relationship's or influence on QSAR studies. Biological data are usually expressed on a logarithmic scale because of the linear relationship between response and log dose in the mid region of the log dose-response curve. Inverse logarithms for activity (log 1/C) are used so that higher values are obtained for more effective analogs.[2-3]

Literature review

Antitumor activity of emricasan by inhibition of caspase 8 & activation of Ripk1 and Ripk3 dependent regulated tnf-induced necroptosis.

Necroptosis

Necroptosis is a programmed form of necrosis or inflammatory cell death. Necrosis is associated with unprogrammed cell death resulting from cellular damage or infiltration by pathogens. Necroptosis has a viral defence mechanism, allowing the cell to undergo cellular suicide in a caspase –independent fashion in the presence of viral caspase inhibitors to restrict virus replication. Necroptosis has also been characterised as a component of inflammatory disease such as crohn's disease, pancreatitis and myocardial infection.

The signalling pathway responsible for carrying out necroptosis. TNFα leads to stimulation of its receptor TNFR1.TNFR1 binding protein TNFR-associated death protein TRADD and TNF receptor associated factor 2 TRAF2 signals to RIPK1 which recruits RIPK3 forming the necrosome also named ripoptosome. And ripoptosome phosphorylates the MLKL, allowing MLKL to insert into and permeabilize plasma membrane and organelles. Integration of MLKL, leads to the inflammatory phenotype and release of damage – associated molecular patterns

(DAMPs), which elicit immune responses. In necrotic cell death, cells undergoing necroptosis rupture and leak their contents into the intercellular space. And in necrosis, permeabilization of the cell membrane during necroptosis is tightly regulated.

The extrinsic stimulus through the TNF receptor by TNFα signals the recruitment of the TNF receptor- associated death domain (TRADD) which in turn recruits RIPK1. In the absence of active Caspase 8, RIPK1 AND RIPK3 auto and Transphosphorylate each other, leading to a formation of a microfilament like complex called the necrosome. Then necrosome activates to the pro- necroptotic protein MLKL through phosphorylation. MLKL activates the necrosis phenotype by inserting into the bilipid membranes of organelles and plasma membrane leading to expulsion of cellular contents into the extracellular space. The inflammatory rupturing of the cell releases Damage Associated Molecular Patterns (DAMPs) into the extracellular space. DAMP signals are known to recruit immune cells to the damaged /infected tissue.

There is substantial interplay between the apoptosis and necroptosis pathways. At multiple stages of their respective signalling cascades, the two pathways can regulate each other. The best characterized example of this co-regulation is the ability of caspase 8 to inhibit the formation of the necrosome by cleaving RIPK1. Conversely, caspase 8 inhibition of necroptosis can be bypassed the necroptotic machinery through the anti-apoptotic protein cFLIP which inactivates caspase 8 through formation of heterodimer.

The tumor necrosis factor receptor can signal for both apoptosis and necroptosis. The RIPK1 protein can also signal for both apoptosis and necroptosis depending on post translational modifications mediated by other signalling proteins. Furthermore, RIPK1 can be regulated by cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1, cIAP2) which polyubiquitinate RIPK1 leading to cell survival through downstream NF-kB signalling. cIAP1 and cIAP2 can also be regulated by the pro-apoptotic protein SMAC (second mitochondria-derived activator of caspases) which can cleave cIAP1 and cIAP2 driving the cell towards an apoptotic death.

In response to DNA damage, the RIPK1 and RIPK3 are phosphorylated and lead to deteriotion of the cell in the absence of caspase activation. The necrosome inhibits adenine nucleotide translocate in mitochondria to decrease cellular ATP levels. Uncoupling of the mitochondrial electron transport chain leads to additional mitochondrial damage and opening of the mitochondrial permeability transition pore, which releases mitochondrial proteins into the cytosol.

These different stimuli can enter the necroptotic cell death program by distinct entry sites. As an example, the prototype TNFR/TNFα necroptotic signalling pathway. Ligation of the plasma surface receptor TNFR1 by its natural ligand TNFα or by agonistic antibodies leads to oligomerization of receptors into aggregates and recruitment of various proteins including TNFR- associated death domain (TRADD), RIP1, cellular inhibitor of apoptosis (cIAP) proteins, TNF receptor associated factor (TRAF)2,and TRAF5 into a multimeric protein complex at the plasma membrane, the so called TNFR1 complex I. within this complex, RIP1 polyubiquinited via K63-linked ubiquitin chains by cIAP protein which in turn leads to the activation of the classical (canonical) signalling pathway of the transcription factor nuclear factor kappa B (NF-kB). Furthermore, TNFR is rapidly internalized upon ligand binding, which results alterations in the composition and post translational modification of receptor-associated proteins. RIP1 becomes deubiquitinated by the deubiquitinase cyclindiomatotis (CYLD), which in turn reduces its ability to interact with components canonical NFkB signalling and instead increases its affinity to proteins involve in cell death signalling.

When caspase activation is inhibited due to genetic or pharmacological inhibition, RIP1 forms instead a complex together with RIP3 to fuel into the necroptotic transduction pathway. These RIP1/RIP3-containing cytoplasmic necroptotic protein complex is called necrosome and constitutes a key molecular platform of necroptosis. This involves the reciprocal phosphorylation of RIP1 and RIP3 in an autocrine or paracrine manner, leading to activation of their kinase activity. Also, the mixed-lineage kinase domain-like protein (MLKL) has been identified as a substrate that is phosphorylated by RIP3 and shown to play an important role in the transduction of the necroptotic signal to cell death. Accordingly, the knockdown of MLKL was shown to result in inhibition of TNFα-mediated necroptosis. [4-6]

Signaling pathways to necroptosis

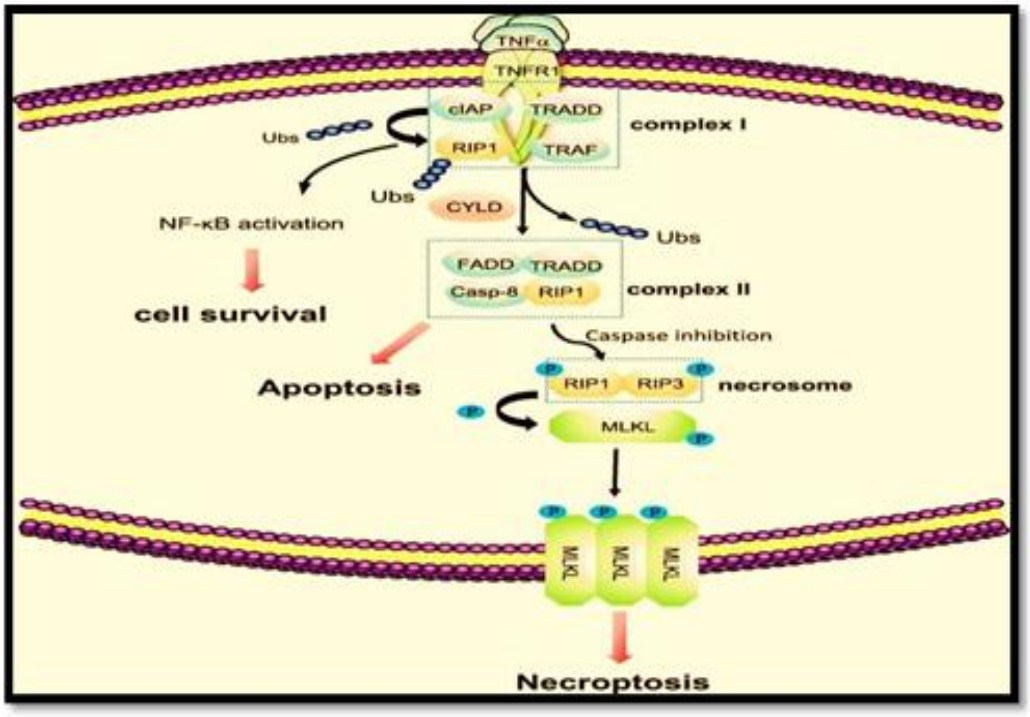


Fig. 5: Signalling pathways to necroptosis.

Activity of the drug emricasan

Drug emricasan

Emricasan is a potential drug invented in 1948 by Idun pharmaceuticals.

The drug was acquired by Pfizer in 2005. And then sold to canatus pharmaceuticals in 2010.

Emricasan acts as a pan -caspase inhibitor and has antiapoptotic and anti-inflammatory effects.

It is a caspase 8 inhibitor.

It was developed for the treatment of liver disease and has been granted fast track designation by the FDA for the treatment of non –alcoholic steatohepatitis cirrhosis.

Mechanism of emricasan

Emricasan involves selective irreversible inhibition of activated caspases, which are cysteine proteases that mediate apoptosis and inflammatory pathways and are implicated in a number of diseases, including liver diseases, inflammatory diseases and diabetes.

Emricasan is the first caspase inhibitor tested in human which has received orphan drug status by FDA. It is developed by Pfizer and made in such a way that it protects liver cells from

excessive apoptosis.[5]

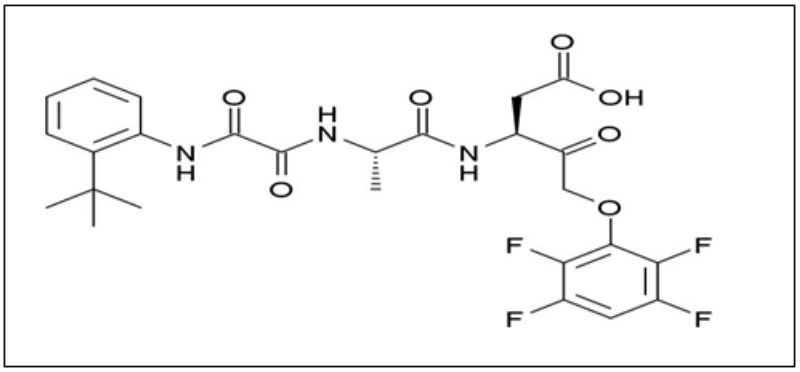
Biochemical /Physiological actions

Emricasan is an antiapoptotic pan- caspase inhibitor. It has been in clinical trials for the treatment of non –alcoholic steatohepatitis (NASH) with advanced fibrosis (scarring) and cirrhosis.

Emricasan has also been shown to protect infected astrocytes from ZIKV-induced cell death. It was developed for the treatment of liver disease, and has been granted fast track designation by the FDA for development after positive results in phase II clinical trials for non-alcoholic fatty liver disease.

Emricasan is the first caspase inhibitor developed for clinical use, and this novel mechanism of action has led to interest in research using emricasan for other potential applications such as an anticancer or antiviral drug.[5]

Emricasan structure



Molecular formula- $C_{26}H_{27}F_4N_3O_7$

Molecular weight- 569.5 g/mol

Selection of ligand (Emricasan)

- Emricasan is a caspase 8 inhibitor. So it inhibits the caspases activation and also prevents the further process of caspase dependent apoptosis.
- It prevents the apoptosis and then ultimately it helps in the activation of necroptosis pathway.
- The activation of the necroptosis pathway that is RIPK1 and RIPK3 can causes the necroptotic cell death.
- And this necroptotic pathway is used for the cancer cell death and for the treatment of

cancer.

Selection of receptors

- **TNFR1** receptor is used for molecular docking with emricasan.
- Docking of **caspase 8** and emricasan.

Need of work

Significance of tracing RIPK3 pathway

- The necroptosis signalling pathway responsible for carrying out necroptosis.
- And RIPK1 and RIPK3 are the important factors of this pathway, in which its activation causes the necroptosis.
- To use the mechanism of necroptotic cell death for the death of cancer cell.

Selection of drug

- Emricasan is a caspase 8 inhibitor.
- It inhibits the enzymes called caspases that are involved in inflammation and programmed cell death, or apoptosis.
- After the inhibition of caspases, RIPK1 stimulates to the RIPK3 and further Process of necroptosis are takes place.
- This necroptotic cell death pathway is used for cancer cell death and to select anticancer drug.

Why we are using computational docking?

- Computational docking is widely used for study of protein-ligand interactions and for drug discovery and development.
- Docking is then used to predict the bound confirmations and binding free energy of small molecules to the target.

AIMS AND OBJECTIVES

Aim

Computational insights of novel anticancer agents by tracing RIPK3 pathway hastening necroptosis.

Objectives

- To estimate ADME profile, Receptor occupancy, Binding energy, total docking score of

chemotherapeutic agent (emricasan).

- To study the role of RIPK1 and RIPK3 in TNF-induced necroptosis and action of emricasan as an anticancer agent through necroptotic pathway.
- To check the activity of emricasan by binding to the caspase 8 and TNFR1 Receptor.
- To find novel targets sites.
- To prove significant activity of emricasan.
- To estimate significant pharmacological activity of emricasan**.**

Plan of work

- To study QSAR parameters.
- Collection of necroptosis triggering agents.
- Collection of drugs, which causes necroptosis and is triggered by activation of RIPK1 and RIPK3.
- The collection of drugs which inhibits to the caspases activation, and prevents apoptosis.
- To find out the receptors, which are involve in activation of necroptotic pathway.
- Molecular docking study.
- Statistical analysis of data and interpretation of result.

MATERIALS AND METHODS

Instruments/Software's used

Table 1: Instruments and Software's.

Sr. no.	Instrument	Make
1	Molsoft software	Molinspiration
2	Molinspiration software	molinspiration
3	Hex 8.0.0 cuda software	Hex loria
4	Open babel software	Open babel
5	Accelrys discovery studio 4	Dassalt system

Target identification

The three dimensional structure, of caspase 8 & TNFR1 obtained from protein data bank (PDB). This structure was determined using X ray diffraction missing atom and loop were corrected by Prepare protein module under Accelrys. Discovery Studio 4(DS4); residue of were protonated of histamine receptor in PH 7.4 condition.

Ligand identification

Emricasan (with pub chem ID) was retrieved from NCBI pub chem compound database. The 2D and 3D structure of the ligand retrieved .The structure was downloaded in SDF format and

was then converted into PDB format using OPEN BABEL 2.2.199 and further used for docking studies.[9-10]

Docking FTO

The Graphical User Interface program "Auto-Dock Tools" "Hex 8.00 Cuda Software" was used to prepare, run, and analyse the docking simulation.

Procedure for assessment of ADME properties and bioactivity of selected novel anticancer agents-

1- Open website – www.mol.inspiration.com. Click on calculation of molecular property and prediction of bioactivity
2- Open website- https://pubchem.ncbi.hlm.nibo.gov.
3- Copy the name of constituent on compound search bar.
4- Copy the canonical smiles.
5- Paste the canonical smiles on search bar of mole inspiration website
6- Click on calculation to property to obtain ADME data.
7- Write mi log p no of rotations, no of violations.
8- Open website molsoft.com paste the canonical smiles on import bar. Click on ok button click on calculate the properties write the no of HBA, no of HBD, MOLPSA, MOL VOL
9- Click on predict bioactivity write down score of various receptor.[7-8]

Calculation of drug likeness score

Procedure

1. Open the mol soft .com.
2. Paste the canonical smile on import search bar then click OK. Click on calculate the Properties.
3. Take a screen shot by pressing WINDOWS & PRINT SCREEN SysRq.
4. Obtain a graph of drug likeness score. [8]

RESULT

In silico model[8-9-10]

Table 2: Assessment of novel anticancer agents for Lipinski rule of 5.

Anticancer agents	milog	TPSA	No of HBA	N violations	No of HBD	OHNH group	No of rotations	Volumes
Emricasan	2.11	150.90	10	1	4	10	11	474.31

Table 3: Bioactivity of the novel anticancer agent.

Anticancer Agents	GPCR ligand	ION channel modulator	Kinase Inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Emricasan	-0.05	-0.49	-0.44	-0.14	0.37	-0.05

Molecular properties and drug likeness[8]

Emricasan

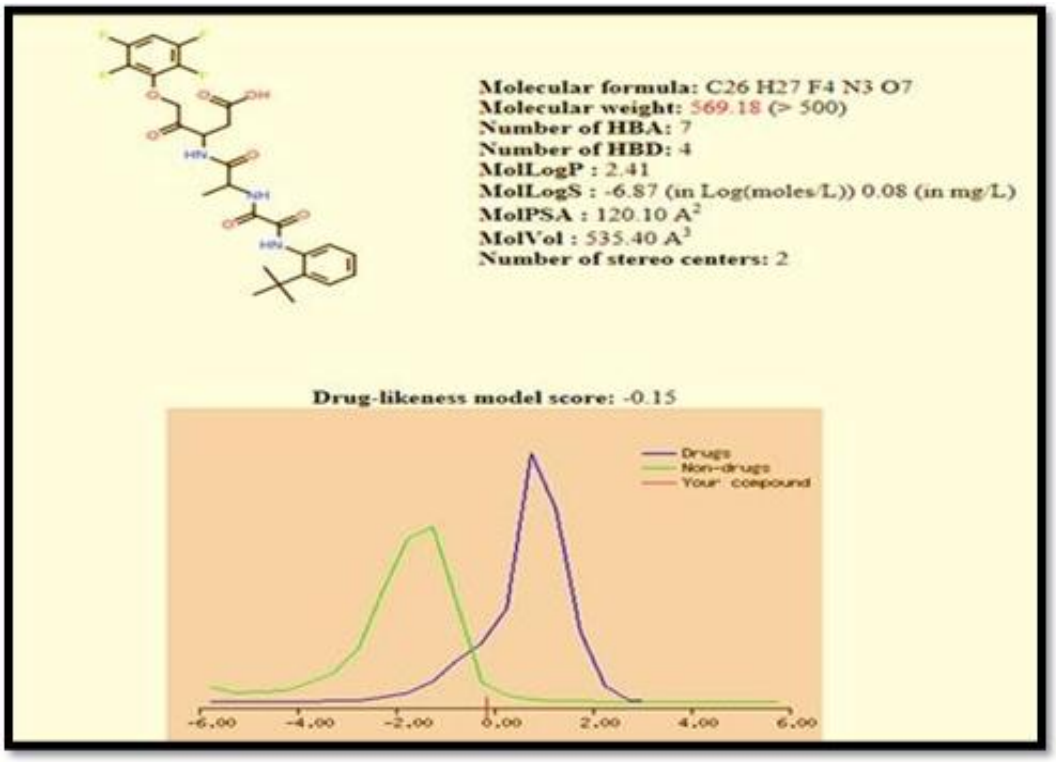


Fig. 6: Emricasan: drug likeness model score: -0.15 Docking Score of ligand-receptor complex.[9-10]

Table 4: Total docking score of the ligand-receptor complex.

Receptor-Ligand complex	Fractional Charge	Net charge	Receptor occupancy	E min	E max	E total/ E shape	RMS
Emricasan-TNFR1	-0.52	9	40.9%	-314.26	766.22	-314.26	-1
Emricasan-caspase 8	-1.46	-6	39%	-327.75	841.95	327.75	-1

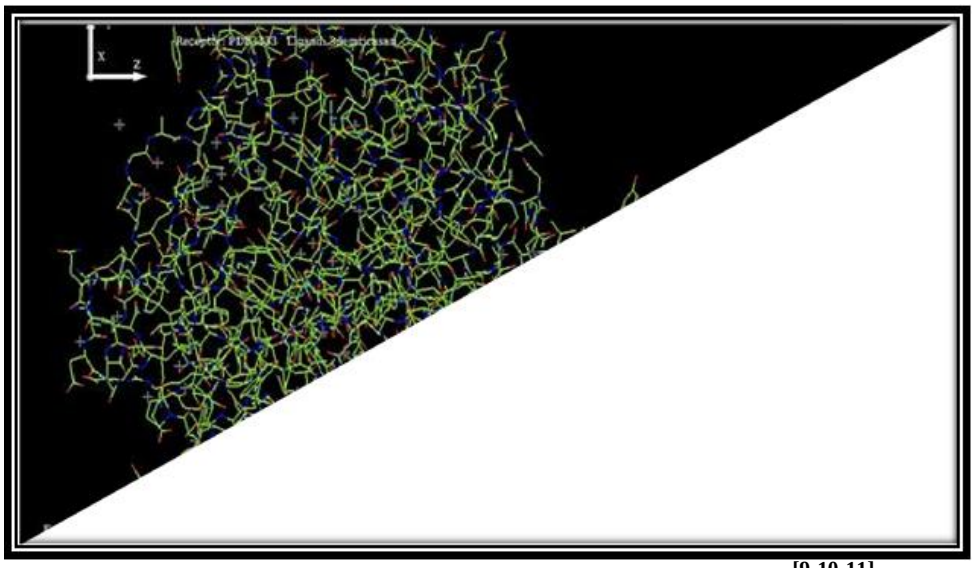


Fig. 7: Docked complex of Emricasan and TNFR1.[9-10-11]

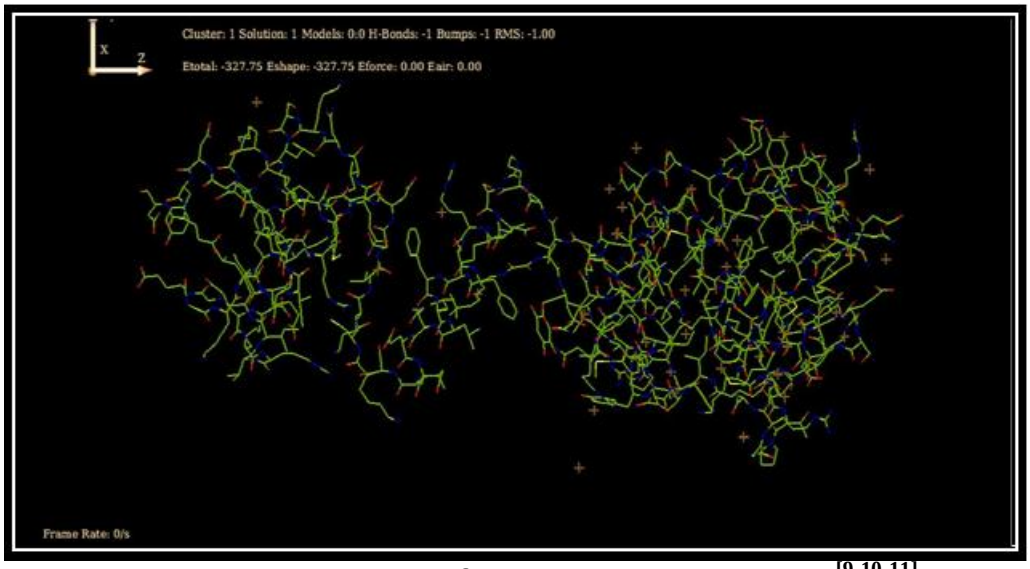


Fig. 8: Docked complex of Emricasan and Caspase 8.[9-10-11]

DISCUSSION

From the data (Table 2), it was observed that novel anticancer agent selected, Emricasan has high molecular weight (569.18). The novel anticancer agents that having molecular weight less than 500D which indicates that these anticancer agents follows one of the Lipinski rule for oral bioavailability. Molecular weight is also a significant parameter for determining the toxicity and absorption of the selected ligand, there is a limit of 500D for molecules to be selected as a drug candidate as more is the molecular weight more is the risk of side effects and toxicity. Emricasan has mi log P value (2.11). An Active anticancer agent's needs not only sufficient metabolic stability to maintain integrity in the intestine and liver but also should cross the Blood-Brain Barrier (BBB). At the molecular level, the BBB is not homogenous but consists

of a number of partially overlapping zones contained in a highly anisotropic lipid layer. The conformational mobility of the lipid chains is relatively low at or near the water (blood)/ lipid interface and interface at the center of the bilayer. In addition, the hydrophilic/lipophilic interface at the blood/membrane boundary consists of perturbed and bound water, charged polar lipid with the anticancer agents, similarly to that of receptor but with much looser steric requirements. High lipophilicity frequently leads to compounds with high rapid metabolic turn over and low solubility and poor absorption. As lipophilicity (log p) increases, there is an increased probability of binding to hydrophobic protein targets other than the desired one, and therefore, there is more potential for toxicity. The biological activity of an anticancer agents was almost entirely due to their Log P and their rate of metabolism was linearly related to log p. Furthermore, optimal activity is observed at log P = 2. The anticancer agents used to treat neurological disorders have log p value mostly between 2 to4. Subsequently, indicated that log p is predominantly a measure of anticancer agents volume or surface area, plus hydrogen bond acceptor potential. Thus, both hydrogen bonding potential and phytoconstituents volume contribute to permeability. Lipophilicity was the first of the descriptors to be identified as important for CNS penetration. Reasoned that highly lipophilic molecules will be partitioned into the lipid interior of membranes and will be retained there. The Polar Surface Area (PSA) and the molecular volume components were the most important descriptors, with psa\strongly predominating. Emricasan (150.90) were showing respective PSA values developed a dynamic PSA approach where by the set of available conformations were used and the contribution of each to the overall PSA was calculated using a Boltzmann distribution thereby taking into account conformational flexibility. On observation found that the anticancer agents can be targeted to the liver with a PSA more than 60–70 $Å^{2.}$ Their cutoff for PSA cutoff for CNS penetration is 90 $Å^2$. HBA and HBD of the corresponding molecules that were found to be less than the maximum level i.e.; HBA less than 10 and HBD less than 5. All the QSAR equations emphasize the importance of hydrogen bonding whether through polarity, PSA, hydrogen bond donor and acceptor counts, or simply counting heteroatoms capable of hydrogen bonding. [3-7]

Docking

Docking is done by Hex 8.0.0 for (TNFR1 and Caspase 8) receptor against anticancer drugs Emricasan. The selection of target protein was done from the literature. The selected protein was docked and the free energy of binding were obtained. The docking study showed that novel anticancer agent emricasan were showing energy score (841.95). Receptor occupancy of Emricasan and TNFR1 (40.9%).[9-10]

SUMMARY AND CONCLUSION

The novel anticancer agent Emricasan which were selected for study had shown drug likeness model score (-0.15). Docking energy of Emricasan & caspase 8 (841.95). And of Emricasan &TNFR1 (766.22). Receptor occupancy of emricasan and TNFR1 (40.9%). And receptor occupancy of emricasan & caspase 8 (39%). On the basis of these results it can be predicted that emricasan has better ligand receptor performance. It proves that emricasan has better affinity towards the caspase 8 hence follows necroptosis pathway. Emricasan may act as better leads and can be considered as novel effective treatment against metastatic cancer along with standard drugs.

Future scope

Necroptosis is genetically regulated form of necrotic cell death has emerged as an important pathway in human diseases like cancer. Our recent study demands tends to physiological evidence of the actions of the emricasan. On earlier studies emricasan was considered as cell injury propagation inhibiter but this could be drive as potent anticancer agents along with its standard congeners.

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