REVIEW ON STRATEGIES IN THE RATIONAL DRUG DESIGN

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Abstract:-

Rational design has played a pivotal role in the discovery of novel lead drugs, with its rapid development primarily driven by significant advancements in computer science, statistics, molecular biology, biophysics, biochemistry, medicinal chemistry, pharmacokinetics, and pharmacodynamics over recent decades. The promising aspect that characterizes the application of rational drug design is its use of all available theoretical and experimental knowledge of the system under study to develop potential leads in drug discovery. The utilization of molecular knowledge of these systems ultimately aims to reduce human labor costs, save laboratory expenses, and improve efficiency in drug discovery. In this review, various strategies applied to systems will be analyzed, including

1. absence of knowledge of the receptor active site,

2. knowledge of a homology model of a receptor,

3. knowledge of the experimentally determined (i.e., X-ray crystallography, NMR spectroscopy) coordinates of the active site of the protein in absence, and

4. presence of the ligands

keywords: Rational drug design, cannabinoid, fullerene, stileukemic steroids, molecular docking, MD simulations, 3D QSAR.

Introduction:-

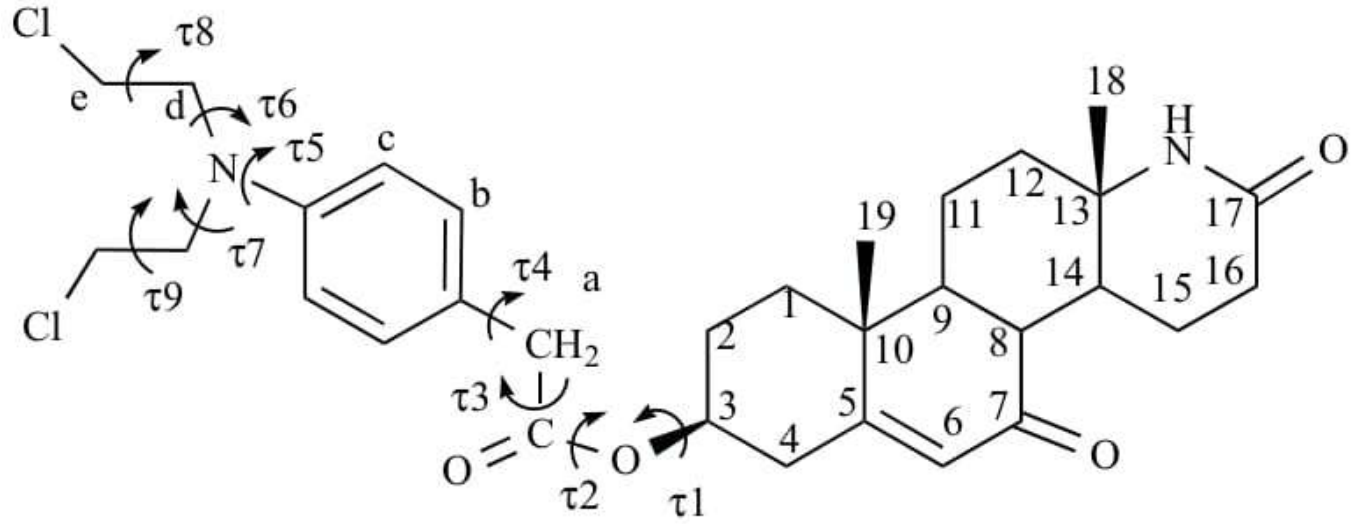
Quantitative Structure-Activity Relationship (QSAR) modeling has gained prominence in rational drug design, gradually replacing the traditional approach where medicinal chemists mainly relied on chemical intuition to create new compounds. Several factors account for the shift towards rational design over classical methods: (i) advancements in computer science have led to the development of powerful, user-friendly computing systems; (ii) statistical software capable of utilizing both theoretical and experimental datasets enables QSAR applications; (iii) new experimental methods, such as X-ray crystallography and NMR spectroscopy, facilitate the characterization of proteins and biological targets; (iv) novel theoretical methodologies, including Molecular Mechanics (MM), semi-empirical, and Quantum Mechanics (QM), now support geometry optimization and total energy calculations; (v) progress in enzymology and molecular biology has significantly advanced our understanding of drug mechanisms, particularly in fields like pharmacokinetics and pharmacodynamics; (vi) conformational search methodologies, such as Molecular Dynamics (MD) and Monte Carlo (MC) simulations, have become increasingly popular; (vii) ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) simulations now play a crucial role in refining drug development. key features of rational drug design include the comprehensive use of all available knowledge about the system under study to formulate strategies for potential lead identification, aiming to reduce costs related to manpower, time, and laboratory resources. Both theoretical and experimental

foundations are essential, and early unsuccessful attempts often provide crucial feedback for the development of new drug candidates.

In essence, the system presents a challenge for the drug designer, similar to an opponent in a game of chess, where each piece—such as the queen, bishop, knight, and pawns—represents different facets like flexibility, the absence of crystallographic data, or limitations of a homology model. Rational design doesn't follow a single strategy; instead, it is shaped by the researcher's experience and teamwork. Some strategies used in our research efforts address challenges based on the level of information available. The first case involves a system with no data on the receptor site (designated as system-1), where neither ligand nor receptor site coordinates are known. The second scenario, where X-ray or NMR-defined targets are unavailable, uses homology models based on data from similar prototype proteins (system-2). Another example includes cases with only the protein's X-ray structure (system-3), or where both the protein structure and ligand binding measurements are available (system-4). These four cases have been thoroughly examined and compared in detail.

STRATEGY APPLIED FOR SYSTEM-1:-

A study was conducted on 38 new steroidal mustard ester compounds exhibiting a broad spectrum of antileukemic activity, classified as system-1 type. A 3D QSAR analysis was performed on these 38 steroidal esters, which possess flexible nitrogen mustard groups, to determine the relationship between their structures and activities. The study's most active compound was further examined using 2D NOESY NMR spectroscopy alongside theoretical calculations, revealing a possible bioactive conformation of the molecule. This conformation was then used as a reference, and the other compounds were superimposed onto it (Fig. 1). The strategy for designing new, more potent compounds is shown in Scheme.



Due to the presence of multiple rotatable bonds in the alkylating agent, the template compound is highly flexible. A thorough conformational analysis was performed on this template using molecular modeling and experimental methods. After superimposing the compounds, statistical analyses like partial least squares (PLS) were applied, producing a reliable model developed with CoMFA and CoMSIA methods. Theresulting data was presented in graphical form for bettervisualization. Guided by the 3D QSAR model, de novo drug design studies (Leapfrog) were initiated.

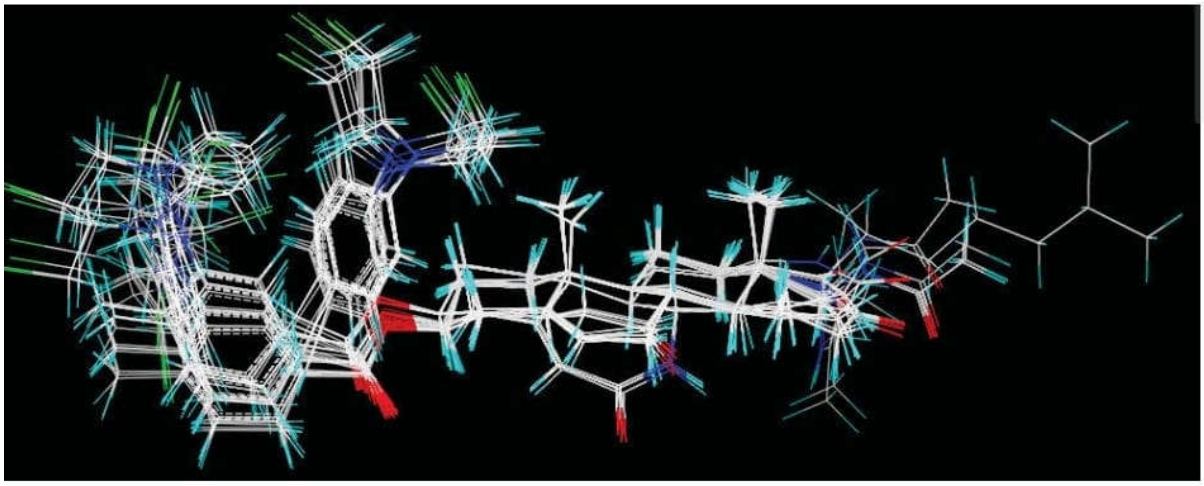
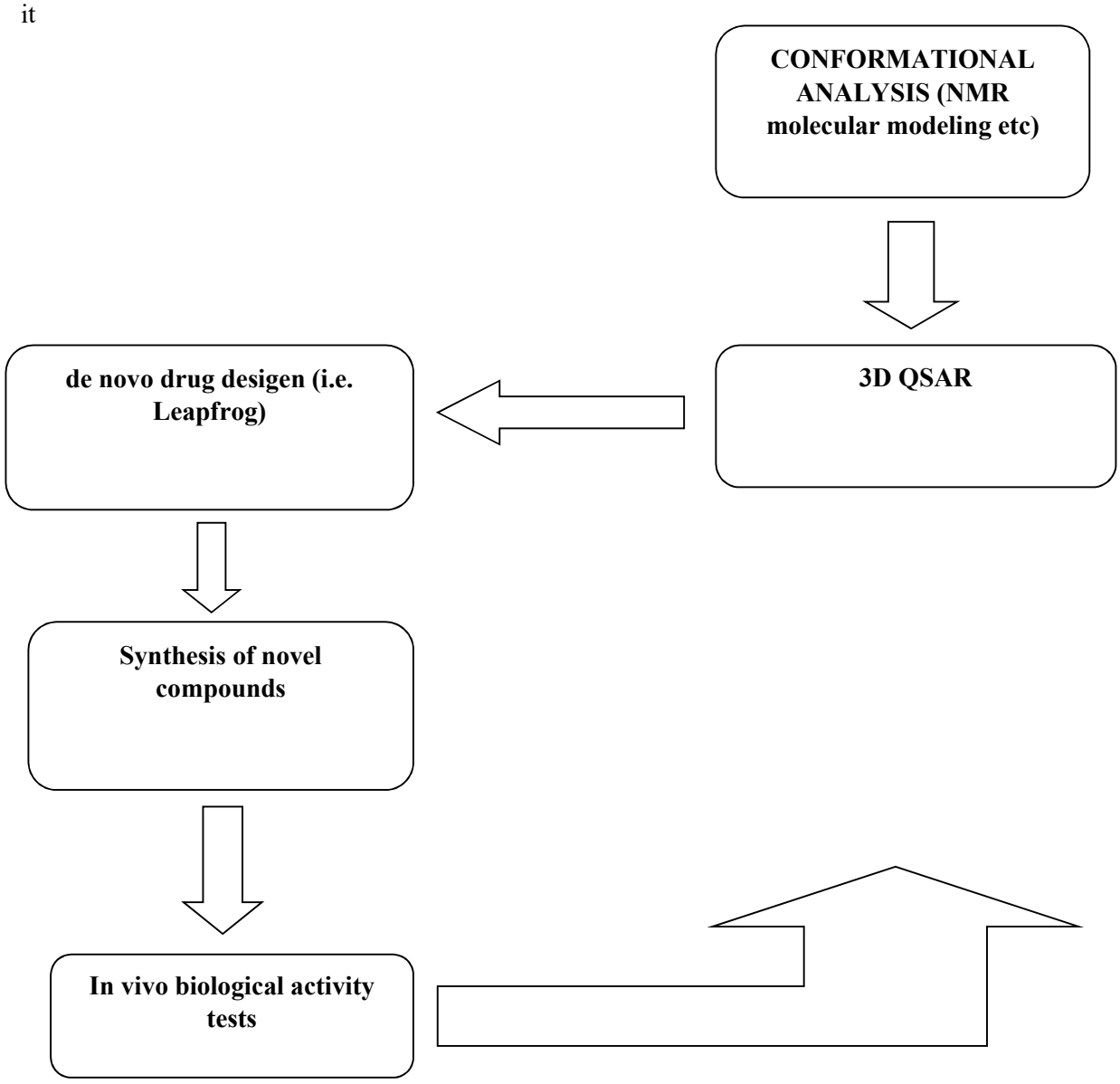


Fig no :1 The molecules used as a template and a rest 37 molecule used for the study superimposed on it



Scheme 1: strategy used to develop more potant bioactive molecules acting on a system-1 type.

The Leapfrog analysis generated several compounds with enhanced activity compared to the original template (see Table 1). Two of these newly identified compounds were synthesized through this approach, and experimental data confirmed the predictions made by the 3D QSAR studies (see Table 2). This demonstrates the successful integration of experimental NMR spectroscopy, theoretical modeling, and 3D QSAR/Leapfrog methods, ultimately resulting in molecules with improved bioactivity

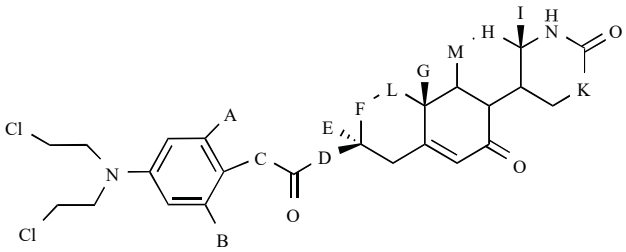
STRATEGY APPLIED FOR SYSTEM-II:-

The second case focuses on cannabinoids (CBs), which interact with two receptors: CB1 and CB2. Both receptors are G protein-coupled receptors (GPCRs) characterized by seven transmembrane (TM) helices. Due to the absence of X-ray structures for these receptors, models were created using human rhodopsin and β-adrenergic receptors as templates, given their shared GPCR family membership. The approach for designing new leads included several methods: (a) theoretical calculations combined with 2D NMR spectroscopy; (b) in silico docking studies; (c) molecular dynamics (MD) simulations in different environments; (d) biophysical analyses; and (e) 3D QSAR analyses (see Scheme 2) [91].

A representative molecule in the cannabinoid class is the C-1-dithiolane A-tetrahydrocannabinol (A-THC) analogue, (-)-2-(1a, 7, 10, 10a-tetrahydro-6,6,9-trimethylhydroxy-6H-dibenzo[b,d]pyranyl)-2-hexyl-1,3-dithiolane, also known as AMG3 (Fig. 2). This compound is among the most potent synthetic cannabinoid ligands produced by Dr. Papahadjis's group, with Ki values of 0.32 nM for the CB1 receptor and 0.52 nM for the CB2 receptor. However, CB receptors, whether free or ligand-bound, have yet to be crystallized, so their ligand binding sites are not definitively known. Instead, these binding sites have been inferred using receptor binding studies of various CB derivatives with both wild-type and mutant receptors, supported by molecular modeling and 3D QSAR research.

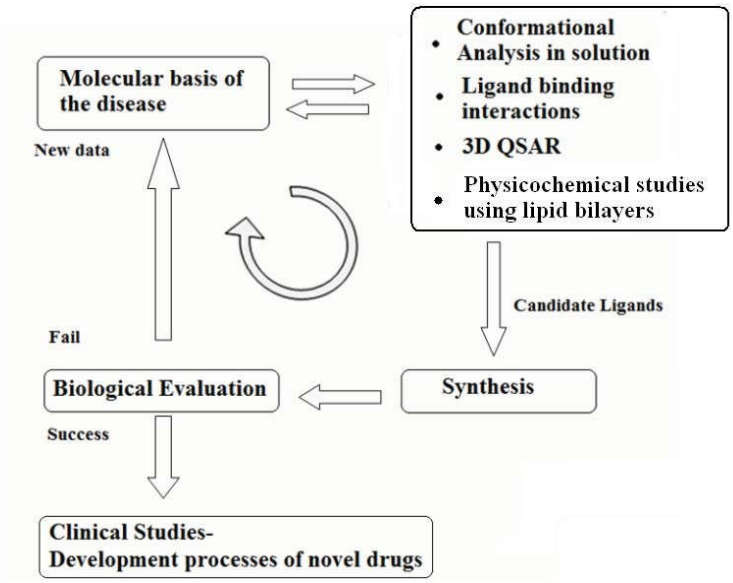
Table 1. Results Obtained from Leapfrog Analysis. The Compounds Shown are Only those that Possess Higher Bioactivity in Comparison to the Template Molecule

Compounds	B	C	E	F	G	H	K	L	M	Pred.*
12	H	CH_2	H	CH_2	CH_3	CH_2	CH_2	CH_2	CH_2	343
LF21	H	CH_2	H	CH_2	Cl	CH_2	CH_2	CH_2	CH_2	369
LF22	H	NH	$NHCH_3$	CH_2	CN	CH_2	CH_2	CH_2	CH_2	369
LF23	H	NH	H	NH	Cl	CH_2	NH	CH_2	CH_2	367
LF24	H	NH	H	NH	CN	CH_2	NH	CH_2	CH_2	366
LF25	H	NH	H	CH_2	CN	CH_2	NH	CH_2	CH_2	366
LF26	H	CH_2	H	CH_2	CH_3	CH_2	$CHCF_3$	CH_2	NH	366
LF27	CHO	CH_2	H	$CHCH_3$	CH_3	NH	CH_2	CH_2	CH_2	366
LF28	CHO	CH_2	H	$CHCH_3$	CH_3	CH_2	CH_2	NH	NH	366
LF29	H	NH	H	CH_2	CN	CH_2	NH	NH	CH_2	364
LF30	CHO	CH_2	H	$CHCH_3$	CH_3	NH	NH	CH_2	CH_2	364
LF31	CHO	CH_2	H	CH_2	CH_3	NH	NH	CH_2	CH_2	363
LF32	H	$CHCH_2OH$	H	CH_2	Cl	CH_2	NH	CH_2	CH_2	363
LF33	H	CH_2	H	CH_2	CN	CH_2	NH	CH_2	NH	362
LF34	H	NH	H	CH_2	CN	CH_2	CH_2	CH_2	CH_2	361
LF35	H	CH_2	H	NH	CN	CH_2	NH	CH_2	CH_2	360
LF36	H	$NCOCH_3$	H	CH_2	CH_3	CH_2	CH_2	CH_2	CH_2	349



The values shown in the column represent the predicted T/C relatively to the experimentally determined value 343 of compound **12.** T/C is an indicator of antileukemic efficiency and designates the quotient of the mean time of survival of the animals under therapy. T (in days)% to the mean time of survival of the control animals (c)X100. **Table 2. Experimental Versus Theoretical Calculations of Two of the Proposed Molecules for Synthesis**

Samples	T/C (calculated)	T/C (experimental
LF 36	350.3	349
LF 21	365.2	369



Scheme 2. Strategy used for designing new leads in the system-II.

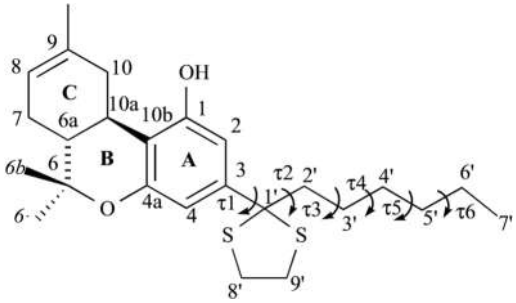


Fig. (2). Chemical structure of AMG3.

Structure-activity relationship (SAR) and 3D QSAR studies on classical cannabinoids indicate that the alkyl side chain is the primary structural feature for receptor activation. Low-energy conformations of A-THC analogues, obtained through NMR spectroscopy and molecular modeling in solution, revealed a range of low-energy states. Identifying the lowest-energy conformation as the bioactive form of the template molecule, and superimposing all compounds onto this template, are fundamental steps in 3D QSAR, particularly within CoMFA and CoMSIA frameworks. Comparative MD simulations of the ligand in solution and at the receptor's binding site helped clarify its favored conformation at the receptor, improving the alignment process. Consequently, MD simulations in both contexts provide more statistically robust models. Because MD simulations have been

conducted for both CB1 and CB2 receptors, these insights can guide synthetic chemists toward developing more selective cannabinoid compounds.

The positioning of AMG within the binding sites of CB1 and CB2 is significant. It aligns within the core region of TM3 to TM7, consistent with earlier biophysical, crystallographic, and modeling studies. MD studies in solution showed that the dihedral angle between AMG13's aromatic ring and dithiolane ring is more resistant to torsional shifts, while the angle adjacent to the dithiolane ring in the alkyl chain remains flexible, allowing both gauche and trans configurations. The remaining dihedral angles in the alkyl chain consistently adopt a trans orientation.

Stategies Applied of system III:

Fullerene analogs functioning as anti-HIV I PR analogs are the subject of the third example (system-III). In this instance, the HIV-1 PR receptor's X-ray structure is known, and biological ligand binding information for a few analogues with distinct biological activities is accessible (Fig. 7). Schome illustrates the tactic used in this instancesMany fullerene derivatives have been created recently by various experimental groups worldwide, but only a small number of them have undergone bioaffinity testing against HIV-1 PR. Our team just announced a stable QSAR model.. 3D QSAR/CMFA data based on various template ligand AMG1 conformations were used to create cantour graphs. Molecular dynamics simulations at the active site and (lett) 2D NMR spectroscopy in conjunction with computational analysis were used to determine the low energy conformers of AMG 3.

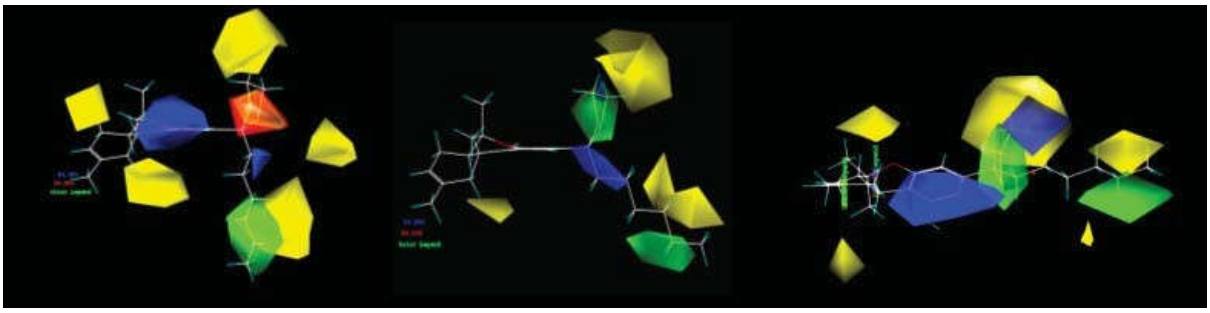


Fig. (3). Contour plots derived using 3D QSAR/CoMFA results based on different conformations of template ligand AMG3. The low energy conformers of AMG 3 were obtained using (left) 2D NMR spectroscopy combined with computational analysis; (middle) *in silico*docking results; (right) molecular dynamics simulations at the active site.

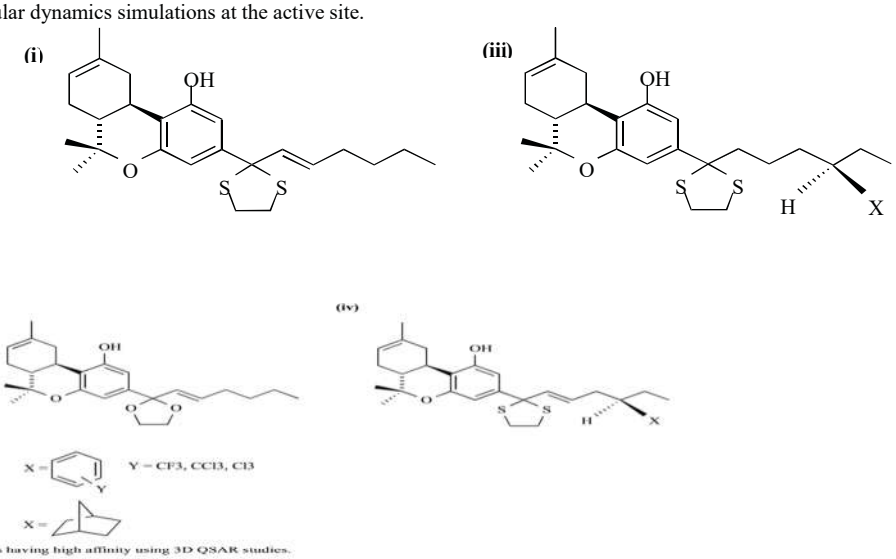
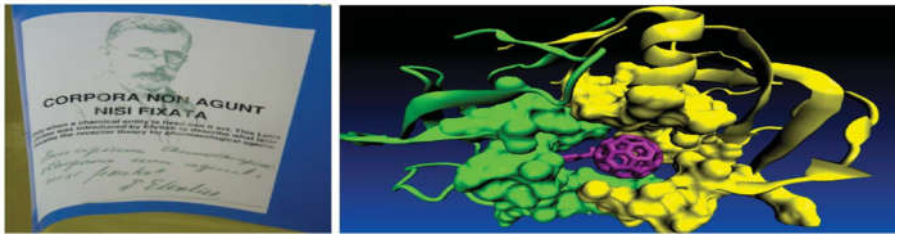
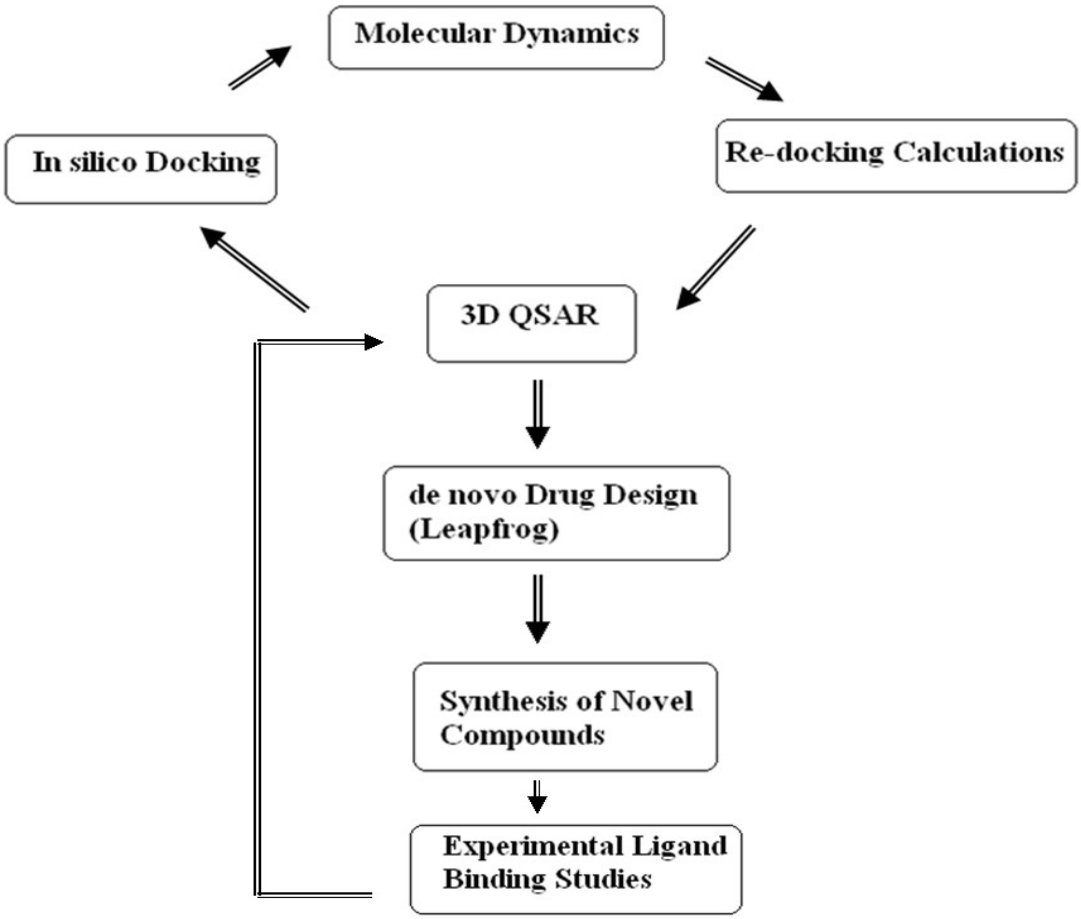
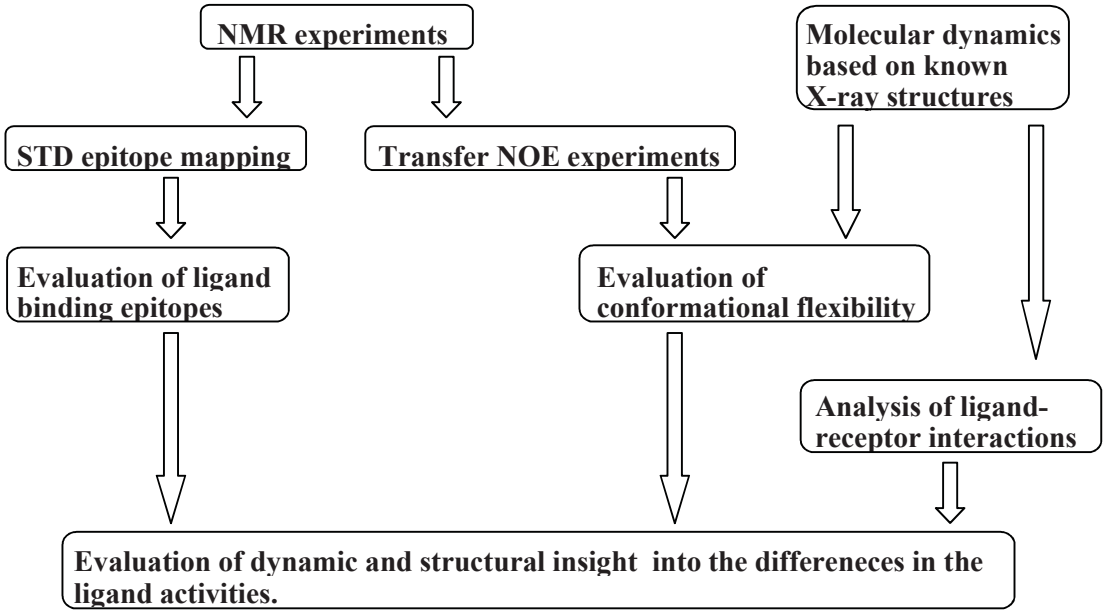


Fig. (6). Analogs proposed as having high affinity using 3D QSAR studies.





Scheme 3. Strategy used for developing new fullerene analogs as anti HIV inhibitors.



Group with these synthetic fullerene counterparts that have been physiologically assessed. It goes without saying that the stability of the built model and its output should rise as the number of compounds in the data set increases [117-118]. However, because there is very little variance in the bioaffinity of the measured fullerene compounds and only a small number of experimentally studied fullerene compounds at HIV-1 PR inhibition, it is challenging to expand the number of compounds in this specific scenario. Docking simulations were used to analyze the

binding energy results of biologically evaluated fullerenes in IIIV 1 PR cavities, and a correlation between experimental and theoretical values was discovered.

As a result of this discovery, the data set was expanded to include the structures.From substances that have been computationally designed

Their actions in building QSAR models from the docking simulations. To create new fullerene compounds with increased activity, the binding interactions of fullerene inhibitors at the HIV-1 PR active site were examined using MD simulations and molecular docking 3D 10 OSAR combination. Despite appearing huge for a therapeutic candidate, the [60] fullerene's size is comparable to that of numerous tiny pharmaceutical compounds (-10 A).Both the ligand-free and ligand-bound HIV-1 PRs were subjected to MD simulations. In addition to providing coreet enzyme input coordinates for the docking investigations, MID simulations made a significant contribution to our understanding of the structural alterations at the flap and antalytic regions for these two distinct systems. The unbound and bound systems of the HIV-1 PR binding cavity have been revealed to differ structurally

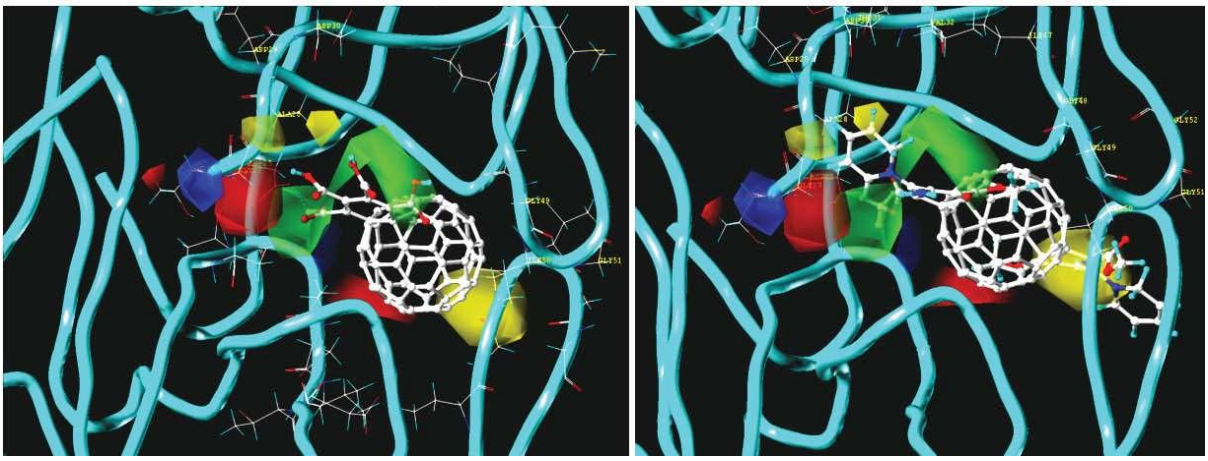


Fig no 4: CoMSIA steric/electrostatic contour maps of templet compound and compound that has worst binding affinity in trening set.

The structure of the free HIV-1 PR takes on a semi-open conformation with flaps moved away from the dual Asp25 catalytic site, whereas the inhibitor-bound forms have flaps brought in near the bottom of the active sue the closed form. Additionally, MD simulations demonstrated that ap, catalytic, and terminolateralUsing molecular docking studies, a number of fullerene derivatives have been created, and their binding energies with HIV-1 PR have been calculated. Using the COMSIA method of 3D QSAR, the relationship between structures and binding affinities has been investigated. The significance of van der Waals interactions with the non-polar HIV-1 PR surface in fullerene activity is confirmed by the high relative contributions of steric fields from derived contour maps of COMSIA models (Fig. 8). Leapfrog de novo drug design studies and contour maps from built 3D QSAR/ COMSIA models helped to suggest new fullerene compounds with higher anticipated potency. Table 3 provides a few illustrative cases. Initial biological findings seem to support these hypotheses

Strategies Applied for system IV ;

Recent NMR and MD studies of the binding mechanism of derivatives of naphthalene N sulfonyl-D-glutamic acid as novel MurD ligase inhibitors are cited in the fourth example [119]. Intracellular enzymes called mur ligases are involved in the manufacture of cytosolic peptidoglycan precursors [120-121] and are appealing targets for the creation of new antibacterial drugs. In this instance, the sonse ligand-MurD complexes' X-ray structures are known. Nevertheless, it was not possible to determine the crystal structures of a number of new derivatives in complex with MurD. One of the main causes of the numerous failures in the design of effective muramyl ligase inhibitors is the absence of three-dimensional complex structures. Additionally, our research has demonstrated the impact of bound ligand conformational dynamicsThe conformational flexibility .Bound ligands is demonstrated byTransfer NOE tests combined with MID. It is widely acknowledged that conformational dynamics have a substantial impact on the ligand-receptor complex's stability and, consequently, ligand activity. Using NMR saturation transfer difference experiments, ligand epitope maps are produced. The crucial relationships and structural prerequisites for activity can be seen in these maps. As a result, they can support the

drag design of new, pharmacologically improved analogue. displays the new inhibitors together with their nontrivial NOE connectivities with matching distances in A determined from transferred 2D NOESY spectra. Mutually exclusive NOEs between molecular segments suggest that the ligands at the enzyme binding site have conformational dynamics that X-ray diffractive analysis is unable to investigate.

According to MD analyses, the degree of conformational flexibility can be linked to variations in ligand activities and is dependent on the specificity of the ligand molecular structure and adaptability within the hinding site. The L-Glubutoxy derivative 20 (Fig. 93), which has the lowest activity among the substituted derivatives, has the most noticeable conformational flexibility in naphthalene ring rotations around its C2-Ch axis. The L-Glu moiety's electrostatic interactions with the enzyme are significantly impacted by these rotations (Fig. 10). However, the hydrogen bonding of the D-Glu moiety is unaffected by the less noticeable naphthalene ring totations of D-Glubutoxy derivatives 17e and 38.During 2 ns of simulation, the naphthalene ring of the most active D-Gin arylaškynxy derivative 171 is by far the most accessible (Fig. 91). These findings greatly improve the drug design studies that were based only on the crystal structures. Flexibility is only seen in the Có substituent of 171, i.e., the rotation in the alkyoxymeicty coupled with the flip of the cyanobenzyl ring is observed (Fig. 9). This does not affect its hydrophobic contacts and possibly its interaction with the enzyme (21). For instance, despite the three-fold difference in their IC values, only minor variations in the binding mode of ® and (S)-5-hutoxy derivatives 20 and 17e could be seen in the crystal structures of MurDcomplexes.The significance of hydrophobic interactions and the molecular segments in charge of them were demonstrated by the ligand epitope maps produced by STD NMR. For Instance, the form of the naphthalene 6-substituent-a lengthy spacer chain with an aromatic ring that increases hydrophobic interactions and decreases the ICs- has been discovered to play a critical role. Furthermore, the interaction between polar and hydrophobic contacts that was discovered and its potential impact on ligand affinity suggest that suitable hydrophobic moieties that also contain sites for hydrogen bond formation must be designed.

Engineering Strategies:-

Protein and peptide engineering primarily rely on two broad strategies: rational design and combinatorial engineering (Figure 1). Both methods are used in antifreeze protein (AFP) design to enhance the properties of existing AFPs.

Rational Design:-

Rational design is a protein engineering approach that involves modifying amino acid sequences based on known structural or functional information about the peptide. This method usually results in a small library of peptide variants, which are chemically synthesized and tested using low-throughput assays to evaluate characteristics or activities, such as amino acid substitution or sequence length. Rational design has been widely used to enhance the properties of antifreeze proteins (AFPs), such as antifungal efficacy, proteolytic stability, and thermal resilience.

Typically, rational design for AFPs begins by analyzing the structure-function relationships of the peptides, which helps in predicting sequences with desired properties. For example, Gu et al. used this approach to improve cremycin-5 by comparing it with related peptides to identify residues critical for antifungal activity [22]. They pinpointed important sites using alanine scanning mutagenesis, which guided the creation of variants with improved fungicidal effectiveness, thermal stability, and serum stability. In our lab, we also applied rational design to enhance the proteolytic stability and antifungal activity of histatin 5 [23,24]. By observing that histatin 5 was cleaved at lysine residues by secreted aspartic proteases from *Candida albicans* [16], we engineered variants with amino acid substitutions at these positions. These modified peptides demonstrated minimal to no proteolytic degradation in the presence of these proteases, along with increased antifungal activity [24,25]. These cases illustrate how insights into peptide structure and function can lead to the rational design of peptides with improved properties.

Combination Engineering:-

Computational tools are valuable in facilitating rational AFP design. Online tools that analyze and predict the physicochemical attributes of peptides can guide engineering efforts for improved AFPs or support the evaluation of newly designed peptides. For instance, using ProtParam [26], Li et al. predicted the properties of modified CGA-N46 peptides—derived from human chromogranin A—and discovered that a variant, CGA-N12, exhibited reduced hemolytic activity along with enhanced antifungal potency [27]. Molecular dynamics (MD)

simulations are also instrumental in rational peptide design, though they require specialized expertise. MD simulations can model critical peptide-membrane interactions, which play a significant role in AFP activity. For example, Pandey et al. used MD simulations to examine the radish defensin peptide RsAFP2, which is active against *C. albicans* [28], as well as two variants (Gly9Arg and Val39Arg) [29]. The simulations revealed that these substitutions improved conformational stability and membrane deformation, with the variants targeting specific lipids in the fungal cell membrane. By incorporating computational tools to inform rational design and interpret experimental results, this approach becomes a powerful strategy to optimize AFP

Combinatorial engineering of peptides creates a large library of diverse peptides, enabling a more comprehensive exploration of "sequence space" when screening for desired traits. For peptide engineering, these libraries can consist of fully or partially random peptide sequences of specified lengths, commonly synthesized on polymer resins through solid-phase synthesis [30,31]. Although combinatorial peptide libraries are not yet widely used for designing antifreeze proteins (AFPs) and antimicrobial peptides (AMPs), they can also be synthesized recombinantly by introducing genes encoding the peptide library into host cells

An essential part of combinatorial peptide engineering is the screening method, which must have high enough throughput to effectively handle the library's size. AMP screening typically takes place in multi-well plate assays, where each well contains a unique peptide. There are two frequently used screening assays: the liposome leakage assay and the microbial sterilization method. In the liposome leakage assay (Figure 2A), liposomes filled with a fluorescent dye are combined with peptides in the wells, and dye leakage is monitored by measuring fluorescence intensity [33,34]. This assay is commonly used to screen AMP libraries [35–37] and has identified peptides with antifungal properties [36]. The microbial sterilization assay (Figure 2B) involves exposing microbial cells to individual peptides, incubating the mixture, adding growth medium for overnight incubation, and observing which peptides inhibit cell growth [38]. Though often used for antibacterial peptides, this method could be adapted to identify antifungal peptides.

If libraries undergo repeated rounds of generation and screening to improve peptide properties iteratively, the process resembles natural selection and is known as directed evolution. For example, the Wimley lab designed and synthesized a library of 16,384 peptides on polymer beads with a photocleavable linker. They screened the library for liposome leakage and antimicrobial effects, including activity against *Cryptococcus neoformans*, and sequenced promising peptides [38,39]. Two peptides were selected as "parents" for a second-generation library of 28,800 members, which was screened for antibacterial activity and low hemolytic potential, resulting in five promising peptides [40]. One of these peptides was effective in reducing infection in a mouse surgical wound model, demonstrating the potential of directed evolution in AMP engineering.

Surface display is another technique for library screening where peptide variants are presented on the surface of a cell or phage particle (Figure 2C), with yeast surface display [41] and phage display [42] being the most common types. Here, each cell or particle displays a unique peptide variant encoded by a plasmid, which allows for easy recovery of the peptide sequence. This method has been used in AMP engineering to identify peptides that bind bacterial or fungal cell surfaces [43–48], and this approach could be extended to identify antifungal peptides.

Though computational strategies are more commonly used with rational design, they also have applications in combinatorial engineering. Yoshida et al., for instance, used a genetic algorithm (introducing variation into a parent sequence), machine learning, and experimental testing to evolve an AMP [49]. Starting with 93 peptides similar to a moderately effective temporin peptide, they trained a model to predict the effects of amino acid changes on antibacterial activity. This fitness matrix informed the synthesis and testing of 90 new peptides, with the matrix updated after each round. After three optimization cycles, they produced peptides with up to a 160-fold increase in antibacterial activity compared to the starting peptide, showcasing the power of combining computational diversity with experimental testing. Further work is needed to adapt these strategies to AFPs.

Conclusion;

Examples of logical design techniques used to suggest new, powerful analogs in case-covering systems have been given. Where,

(i) there is either insufficient information about the receptor or

(ii) the receptor is represented using a homology model of a crystalline receptor.

(iii)just the receptor's x-ray structure is known;

(iv) the receptor-ligand complex's x-ray structure is known. Using antileukemic analogs as an example, we have demonstrated that the rational design did, in fact, result in more effective and desirable counterparts. Ongoing research on fullerene analogs demonstrates that predictions are accurate and that stronger analogs can be created. We demonstrated that prospective analogues in the realm of cannabinoids, It can be synthesized. The validity of these forecasts needs to be tested. However, it is certain that these concepts will create new opportunities for the synthetic

This review paper concludes by demonstrating that rational design still has a long way to go and that there is plenty of room for fresh, innovative ideas that will undoubtedly result in the development of useful medications for the human well-being.

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