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DRUG LIKE FILTERS AND MOLECULAR DYNAMICS AS A TOOL IN RATIONAL DRUG DESIGN: APPLICATION TOWARDS BIOACTIVE ANALGESIC PEPTIDE LEADS

*Kandasamy Nagarajan[1], Nitesh Chauhan[2], Dhananjay Sharma[3], Nishant Chaudhary[4], Manoj Kumar[5], Pritesh Kumar Rai[6], L.K. Ghosh[7]

[1]Dr. Kandasamy Nagarajan, Professor & Head, Research Laboratory of Drug Discovery & Central Instrumentation Frontiers, Department of Pharmaceutical Chemistry, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad-Meerut Road, Ghaziabad–201206, India
[2]Mr. Nitesh Chauhan, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad Meerut Road, Ghaziabad–201206, India
[3]Mr. Dhananjay Sharma, PG Research Scholar, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad Meerut Road, Ghaziabad–201206, India
[4]Mr. Nishant Chaudhary, PG Research Scholar, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad Meerut Road, Ghaziabad–201206, India
[5]Mr. Manoj Kumar, PG Research Scholar, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad Meerut Road, Ghaziabad–201206, India
[6]Mr. Pritesh Kumar Rai, PG Research Scholar, KIET School of Pharmacy, 13 Km. Stone, Ghaziabad Meerut Road, Ghaziabad–201206, India
[7]Dr. L.K. Ghosh, Professor & Head, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.
*e-mail: rknagaraaja@gmail.com; mobile: 09997628670

Abstract

The main objective of our rational drug design is to predict the best novel shorter chain peptide leads for analgesic effect from the designed set of templates with various descriptors such as Boman index, lipinski rules and molecular dynamics simulations. We built sixty five candidate molecules (20 tripeptides; 45 tetrapeptides) from chemical templates and subjected to Boman index through online antimicrobial peptide database. The best resulting eighteen templates (3 tripeptides; 15 tetrapeptides) derived from this database were passed them through empirical lipinski filters to assess drug like properties using various molecular descriptors namely molecular mass, partition coefficient, Hydrogen bond donors and Hydrogen bond acceptors respectively. Later, we conducted molecular dynamics on the best resulting templates with twenty nano second simulations of total atoms at an average temperature 298.15K in hundred steps with applying constraints to all bonds in pure water. Finally, the conformational stability of the candidates were analyzed with measurement of Total potential energy, Total Kinetic energy, Bond stretch energy, Bond angle bending energy, Torsion angle energy, Restraining torsional energy and Hydrogen bond energy. In addition, the protein–ligand binding was tested with Lennard jones energy and Electrostatic energy for all the best templates. Among the results obtained with molecular dynamics for various non–bonded interactions, we identified Phe-Lys-Gln-Tyr (FKQY), Phe-Trp-Lys-Tyr (FWKY), Phe-His-Arg-Tyr (FHRY) and Met-Met-Phe-Tyr (MMFY) as potent tetrapeptide leads with maximum conformational stability for analgesic effect among the selected sixty five template sets.

Key words: Tetrapeptides; Boman index; Lipinski rules; Molecular dynamics; Analgesics; Rational drug design.

Introduction

Lead molecular discovery and development is an expensive and lengthy process. For the Pharmaceutical Industry, the number of years to bring a drug from discovery to market is approximately 14 years, costing up to US$880 million per individual drug (Mathieu, 2001). Given the vast size of organic chemical space (>10^{18} compounds) (Walters *et al.*, 1988), drug discovery cannot be reduced to a simple "synthesize and test" drudgery. There is an urgent need particularly for life threatening diseases to identify and design lead like molecules from the vast expense of what could be Synthesized.

Peptides are key regulators in cellular and intercellular physiological responses and possess enormous promise for the treatment of pathological conditions. Opioid peptide activity within the central nervous system is of particular interest for the treatment of pain owing to the elevated potency of peptides and the centrally mediated actions of pain processes (Witt and Davis, 2006). The development of new drugs with pharmacological efficacy and clinical utility is now a major activity in the chemical and biological sciences. Successes in mid–century have led to a level of confidence in our ability to make some predictions in the design of molecules with desirable characteristics. Today, we are on the threshold of rational drug design based on the occasional ability to recreate and model the molecular level scene of action of a ligand molecule and an effector using computer graphic simulations. From such models, variations in structure may be made with the objective of improving the drug–receptor encounters leading to a better drug (Kier and Hall, 2002).

Molecular dynamics (MD) simulations can be used to model the motions of molecular systems, including proteins, cell membranes and DNA, at an atomic level of detail. A sufficiently long and accurate MD simulation could allow scientists and drug designers to visualize for the first time many critically important biochemical phenomena that cannot currently be observed in laboratory experiments, including the "folding" of proteins in to their native three–dimensional structures, the structural changes that underlie protein function, and the interactions between two proteins or between a protein and a candidate drug molecule (Brooks and Csae, 1993; Frenkel and Smit, 2001). Such simulations could answer, some of the most important open questions in the fields of Biology and Chemistry and have the potential to make substantial contributions to the process of drug development (Karplus and McCammon, 2002; Schlick *et al.*, 1999).

Using MD simulations, the thermodynamic properties and time–dependent phenomena (i.e. Kinetic) can be studied and this allows an understanding of various dynamic aspects of biomolecular structure, recognition and function. The major strengths of MD simulations are the possibility of its combination with statistical mechanics which connects the microscopic simulation with macroscopic observables. Statistical mechanics can provide a rigorous framework of mathematical expressions that can relate the distribution and motion of atoms with macroscopic observables such as temperature, pressure, heat–capacity and free energies.

In this way, we are able to predict changes in the binding free energy of a particular drug candidate or the mechanisms and energetic consequences of conformational changes in a protein. Other aspects which can be studied by the aim of MD are macromolecular stability (Tiana *et al.*, 2004), the role of dynamics in enzyme activity (Wang et al., 2001; Warshel, 2003), molecular recognition and the properties of complexes (Brooijmans and Kuntz, 2003) and small molecule transport (Roux, 2002; Bond and Sansom, 2004).

Three analogues of β-endorphin have been synthesized and tested for analgesic activities, in which all three peptides at high doses exhibit either no or much weaker analgesic activity than β-endorphin (Li *et al.*, 1978). A synthetic combinatorial library containing 52, 128, 400 D-aminoacid hexapeptides was used to identify a ligand for the mu

opioid receptor. The peptide Ac-rfwink-NH2, bears no resemblance to any known opioid peptide. Simulations using molecular dynamics showed that three aminoacid moieties have the same spatial orientation as the corresponding pharmacophoric groups of the opioid peptide PLO17 and was shown to be a potent agonist at the mu receptor and induced long-lasting analgesia in mice (Dooley *et al*., 1994). An invention provided a novel chimeric peptide containing an opioid peptide moiety and a nociceptive peptide moiety for producing analgesia (Carr *et al*., 2004).

Cell based therapy for neuropathic pain could provide analgesics to local pain modulatory regions in a sustained, renewable fashion and transplantable cells may be engineered to produce increased levels of analgesic peptides using a potential supplementary analgesic peptide, (Ser^1)-histogranin (SHG), the stable synthetic derivative of a naturally occurring peptide with N-methyl D-aspartate (NMDA) antagonistic properties. Transplantation of such cells provided modest analgesia in a rodent pain model consistent with low levels of SHG peptide in the cerebrospinal fluid (Gajavelli *et al*., 2008).

The synthesis of bioactive peptides in multiple antigen peptide (MAP) dendrimeric form can result in increased half life, due to acquired resistance to protease and peptidase activity. The *in vitro* and *in vivo* efficiency of dendrimeric peptides like MAPs is generally ascribed to their multimeric nature, which enables polyvalent interactions (Bracci *et al*., 2003). The conotoxin peptides, about 10-20 residues in length are naturally available in minute amounts in the venom of the cone snails have analgesic activity and are thus useful for treating or preventing pain (Mcintosh *et al*., 2008).

Six analogues of (Hyp6) DM and (Pro6) DM of dermorphins (DM) were synthesized and their analgesic activity was assessed in mice using Eddy's hot plate method and tail-flick method. Peptides I, II and III (Substitution of Ser at position 7; Gly at position 4 in (Hyp6) DM; and (Pro6, Sar7) DM) possessed considerable analgesic activity (Sivanandaiah *et al*., 1997). The methanolic extract of seeds of *Citrullus lanatus* has good analgesic potential in a dose of 200 mg/kg and found significant at $p<0.05$ as compared to diclofenac sodium (Gill *et al*., 2010). As a novel unified theory of the structure activity relationship of opioids and opioid peptides is hypothesized, it was observed that the first 3 amino acid sequences of beta endorphin (l-tyr-gly-gly) and the active opioid dipeptide, l-tyr-pro form a virtual piperazine like ring which is smaller in size, shape and location to the heterocyclic rings of morphine, meperidine and methadone and could be important for future analgesic drug design (Goldberg, 2010).

Certain analogue compounds of analgesic peptides derived from the venom of *Crotalus durissus* terrificus snakes are used for the treatment, diagnosis and prevention of painful conditions or mediated by opioid receptors with their corresponding pharmaceutical compositions (Cury *et al*., 2009). The Pharmaceutical potential of natural analgesic peptides to cross the blood brain barrier with increasing peptide cell membrane affinity through drug design was carried out by grafting ibuprofen to kyotorphin (l-Tyr-l-Arg, KTP), an analgesic neuropeptide unable to cross Blood Brain Barrier. Two new KTP derivatives, IBP-KTP (IbKTP-OH) and IBP-KTP-amide (IbKTP-NH(2)) were synthesized and characterized for membrane interaction, analgesic activity and mechanism of action. Ibuprofen enhanced peptide membrane interaction, endowing a specificity for anionic fluid bilayers and IBP-KTP-amide (IbKTP-NH(2)) was identified as the most potent analgesics in a dose of 25 $\mu mol.kg^{-1}$(Ribeiro *et al*., 2011).

Hence our main objective is to predict the best novel tripeptide or tetrapeptide candidate from the designed set of 65 test analogues for analgesic activity using certain molecular descriptors as empirical filters, Boman index for protein binding potential and molecular dynamics for conformational stability with various energy levels involving numerous atoms within the designed molecule.

Materials & Methods

Materials

For all the selected test analogues, we have calculated the accessible topological descriptors such as Lipinski rules as selective empirical drug filters for correlating their biopotency (Ivanciuc, 2002). The above study was carried out at Supercomputing facility for Bioinformatics and Computational Biology, IIT Delhi, Boman index of the test set analogues was calculated directly from the online Peptide Database (http://aps.unmc.edu/AP/main.php). Molecular dynamics was performed with Schrodinger Suite 2011, Germany, supported by Windows 7, 32 bit operating system with Intel (R) Core (TM) 2 Duo CPU E 4600 @ 2.40 GHZ and 1 GB RAM in HP 7540.

System Efficiency

We balance our design very differently from a general–purpose supercomputer architecture. Relative to other high performance computing applications, Molecular dynamics uses much communication and computation but surprisingly little memory. Consider an MD simulation of 25,000 particles. If each particle requires 64 bytes of storage, then the entire architectural state is just 1.6 MB. Divided among the 512 nodes of a typical machine, this is only 3.2 KB per node, which would fit handily in to the L1 cache of any modern processor. We exploit this property by using only 1GB RAM and small L1 caches on our ASIC, with all code and data fitting on–chip in normal operation. Rather than spending silicon area on large caches and aggressive memory hierarchies, we instead dedicate it to communication and computation.

Methodology

1. Template Library

Chemical templates are conceived as building blocks/structural frameworks for assembly and generation of various tripeptide & tetrapeptide analogue leads. We have built a structure based template library for the design of tripeptide & tetrapeptide leads and created a set of 65 templates using 2D viewer in Schrodinger Suite 2011. The selected test set are given subsequently in Table 1.

2. Protein Binding Potential for Preliminary Analgesic Screening

All the 65 inbuilt templates, in which 20 have an input of 3 amino acid residues and the rest have an input of 4 aminoacid residues each. The Boman index estimates the potential for a protein to bind to other proteins (Boman, 2003). In other words, a high Boman index value indicates that the designed lead will be multifunctional or play a variety of different roles within the cell due to its ability to interact with a wide range of proteins. All the 65 shorter chain peptide leads were subjected to Boman index according to the online Peptide Database (http://aps.unmc.edu/AP/main.php), which are believed to be important for establishing analgesic activity. Following their design, peptide leads were selected for further empirical drug like lipinski filters by eliminating wrong candidates through screening.

3. Molecular descriptors and drug like filters

A successfully lead discovery strategy must ensure bioavailability from the very start in generating leads while eliminating wrong candidates from considerations. Lipinski rules of five (Lipinsky *et al*., 1997) helps in distinguishing between drug like and non–drug like molecules. The Lipinski rules predicts the high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules:–

1. Molecular mass less than 500 Dalton.

2. High Lipophilicity (Log p less than 5).

3. Less than 5 Hydrogen bond donors.

4. Less than 10 Hydrogen bond acceptors.

5. Molar refractivity should be between 40–130.

We have introduced only 18 template leads to this stage which were already screened through Boman

index from the 65 templates and few of the the structure inbuilt as for example are given in Fig. 1. The selected 18 templates, viz., MKY, MVY, MIY, MCY, MRY, MALY, MMFY, MFWY, MWKY, MICY, MHRY, FALY, FLMY, FWKY, FKQY, FVIP, FHRY and TRRH was subjected to some empirical computational Lipinski filters based on drug like properties/molecular descriptors.

The above mentioned molecular descriptors could act as computational filters based upon their accepted limits to screen the candidate compounds. Introduction of filters facilitates computational tractability by restricting the chemical space for potential candidates, saving much time and cost in new lead discovery.

4. Molecular Dynamics

In rational drug design, one of the most important problems to be solved is the description of the molecular aspects and the related energetics which are at the basis of the interactions between proteins or more general macromolecular receptors, and molecules which could be either endogenous natural ligands or a drug.

Given the structure of a biomolecular system i.e. the coordinates of the constituent atoms, there are various computational methods able to investigate the dynamics of the molecular system. All the dynamics methodologies employed are highly dependent upon the description of a suitable potential energy function to describe the energy of the system with respect to the molecular degrees of freedom.

In particular, the choice of an appropriate energy function for describing the intermolecular and intramolecular interactions is critical for a successfull MD simulation. In conventional MD, the energy function is calculated by means of molecular mechanics methods, thus considering the atomic motion only from a nuclear point of view according to the Born–oppheneimer approximation (Born and Oppenheimer, 1927).

An MD computation simulates the motion of a collection of atoms (the chemical system) over a period of time. The chemical system may consist of a peptide and its surrounding environment (solvent, usually water), and may also include other types of molecules, such as lipids, carbohydrates, nucleic acid or drug molecules. This MD computation breaks time in to a series of discrete time steps, each representing a few femtoseconols of simulated time. A time step has two major phases. Force calculation computes the force on each particle due to other particles in the system. Integration uses the net force on each particle to update that particle's position and velocity.

Methodology

In recent years, molecular dynamics (MD) simulations have been increasingly used to understand the complex conformational equilibria of polypeptides in solution and to predict structural preferences (Roccatano *et al*., 1999; Colombo *et al*., 2002; Garcia and Sanbonmatsu, 2001).

Eighteen systems were simulated with molecular dynamics. For each tripeptide & tetrapeptide, 20–nano second simulations for total atoms at an average temperature 298.15K in 100 steps with applying constraints to all bonds in pure water was performed.

All MD simulations were performed in the constant surface area ensemble with the NAMD2 program (Philips *et al*., 2005) and the CHARMM 27 force field (Mackerrell *et al*., 1998). A short–range cutoff of 12A° was used for non–bonded interactions, and long–range electrostatic interactions were treated with the Particle Mesh Ewald method (Darden *et al*., 1993). During the run, translational and rotational motions for the system are removed and the whole simulations were integrated with verlet integrator.

The structural and dynamical properties of the resulting ensemble of structures were analysed accordingly with Schrodinger Suite 2011 in order to explore the conformational space of the candidate molecule by measuring the total energy of system, total potential energy, total kinetic energy, bond

stretch energy, angle bending energy, torsion angle energy, restraining energy for torsions, H–bond energy and various types of Lennard Jones energy and electrostatic energy. Finally, the conformational stability of all the 18 tripeptide & tetrapeptide leads are to be compared and a suitable template lead having maximum stability and analgesic activity should be identified by this MD simulation techniques.

Results & Discussion

1. Boman Index

The results of Boman index and total net charge of subjected 20 tripeptide & 45 tetrapeptide leads were summarized in Table1.

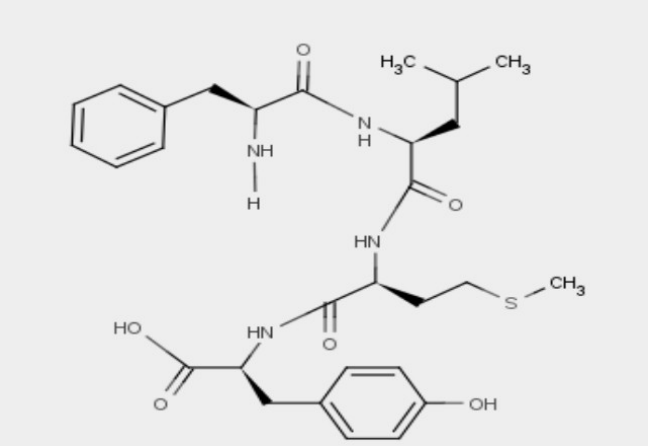
see Table 1.

The above results from Table 1 clearly indicated that the templates subjected to drug design have shown very low to high penetration power with their corresponding total net charge ranging from –1 to +3 respectively. In addition, the Boman index values in K.cal/mole for the above 65 templates showed wide margin of interaction with proteins and narrow margin of interaction with proteins. Some of the templates doesn't show satisfactory interaction with proteins.

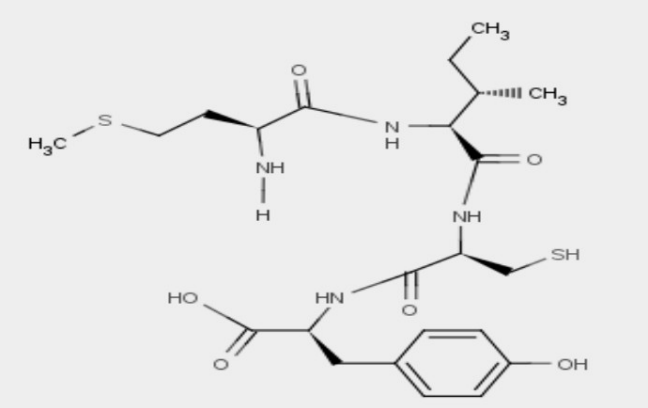
Among the 65 templates subjected to Boman index, only 18 template analogues are selected with the rational drug design approach as far as the analgesic potency is concerned and these selected analogues along with their corresponding hydrophobic ratios are depicted as final outcome with Table 2 with their structure inbuilt as few for example from Fig. 1.

see Table 2.

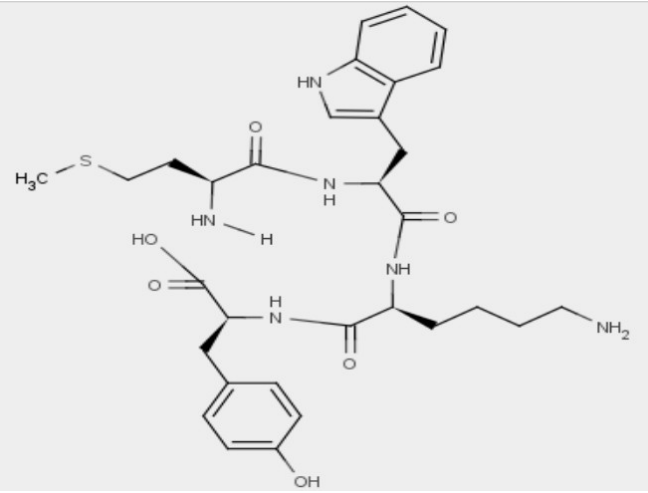
Fig. 1. Inbuilt structure of selected tripeptide & tetrapeptide leads from their best predicted total net charge and Boman index values.



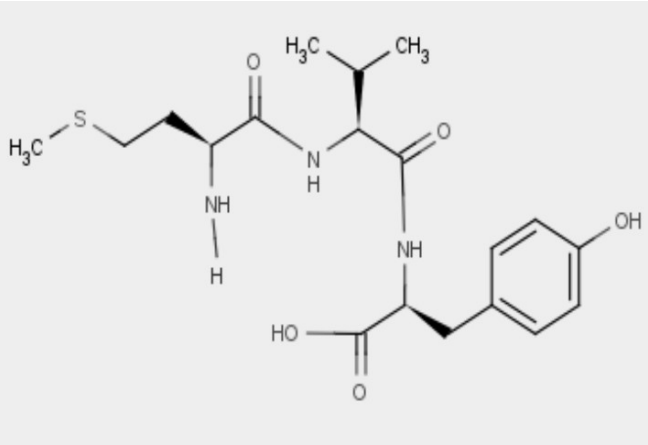
Phe-Trp-Lys-Tyr (FWKY)



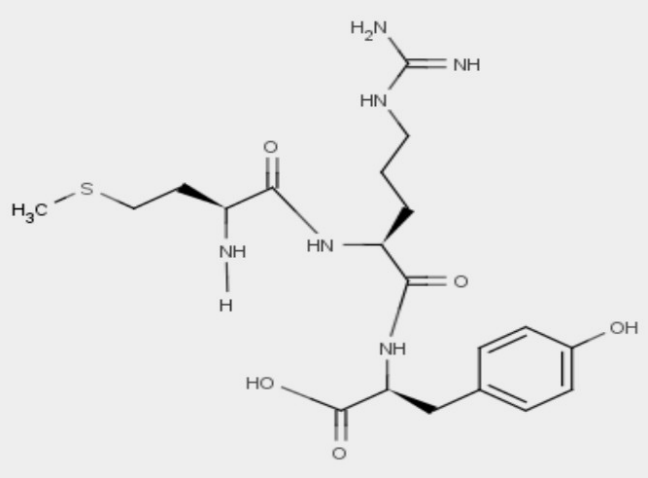
Met-Ile-Cys-Tyr (MICY)



Met-Trp-Lys-Tyr (MWKY)



Met-Val-Tyr (MVY)



Met-Arg-Tyr (MRY)

As from the results of Table 2, we observed that certain tripeptide & tetrapeptide leads fulfill the designed characteristic descriptors, viz., Hydrophobic ratio, total net charge and Boman index. Hence, it is concluded that the designed tripeptide & tetrapeptide leads, viz., Met-Arg-Tyr (MRY), Met-His-Arg-Tyr (MHRY), Phe-Lys-Gln-Tyr (FKQY), Phe-His-Arg-Tyr (FHRY) and Thr-Arg-Arg-His (TRRH) are found to have theoretical active analgesic effect and has to be further tested invivo to predict their analgesic effect after synthesizing the same corresponding leads.

2. Molecular descriptors and drug like filters

The results of Lipinski rules for the 18 template leads are indicated in Table 3. As per Lipinski rules, we observed that 4 out of 18 test sets are found to be drug like molecule, which clearly indicated that the drug design what we have performed is a rational drug design. Among the test set of 18 analogues, Met-Lys-Tyr (MKY), Mat-Val-Tyr (MVY), Met-Ile-Tyr (MIY) and Met-Arg-Tyr (MRY) were fulfilling all the molecular descriptors, viz., H–bond donors, H–bond acceptors, Molecular mass and Log P tested with empirical Lipinski filter. The remaining 14 test analogues namely MCY, MALY,MMFY, MFWY, MWKY, MICY, MHRY, FALY, FLMY, FWKY, FKQY, FVIP, FHRY and TRRH exhibited partial drug like characteristics as screened through empirical Lipinski drug filter, since they doesn't fulfill all the molecular descriptors used in the study.

see Table 3.

3. Molecular dynamics

All the 18 templates are subjected to molecular dynamics simulations and their corresponding final energy reports are summarized in Table 4.

see Table 4.

Computation of absolute binding free energies from atomic level descriptors of the systems is a formidable task (Kalra *et al*., 2001; Beveridge and Dicapua, 1989; Jayaram *et al*., 2002). In a phenomenon logical view, the net binding free energy may be considered to be a sum of the free energy changes due to the following contributions, (i.e.), the Vander Waals interactions between the protein and the inhibitor indicating the influence of shape complementarities and packing effects and net electrostatics which includes interactions between partial or full charges, hydrogen bonds and electrostatics of desolvation upon binding and added salt effects.

Molecular dynamics simulations were configured on the unbound species (free protein and free ligand), with explicit solvent under ambient temperature and pressure conditions. In a nutshell, the electrostatic contribution to the interaction energy

is computed via Coulomb's law with a sigmoidal dielectric function. The vander waals interactions are modeled (Novotny *et al*., 1989; Ajay and Murcko, 1995) using a Lennard-Jones potential between the atoms of the protein and ligand.

The energy function described in Table 4 enables evaluation of the total non-bonded interaction energy of a protein-ligand complex in aqueous environment from the cartesian coordinates of all the atoms.

All the total atoms present in the tripeptide & tetrapeptide were subjected to molecular dynamics simulations. The lower the potential energy of molecule, the more stability of the lead. Among the 18 templates simulated, only 6 tetrapeptide leads have shown minimum potential energy, viz., TRRH (–8.735 K. cal/mol); MHRY (–7.347 K. cal/mol); FKQY(–6.875 K. cal/mol); MMFY (– 5.982 K. cal/mol); MICY (– 5.864 K. cal/mol); FALY (– 5.464 K. cal/mol) and proved to have maximum conformational stability.

Bond energies represent a state of potential chemical energy. Bond energy must be introduced to break a bond. The more bond energy, the more stability has enhanced for the lead. This has been confirmed with the Bond stretch energy obtained for following template leads, viz., MIY(9.580 K. cal/mol); FWKY (2.073 K. cal/mol); FKQY (2.053 K. cal/mol); FHRY (2.011 K. cal/mol) and MFWY & MWKY (1.979 K. cal/mol) respectively.

Higher torsion angle energy leads to distorted local conformation. The low torsion angle energy was observed with MRY(1.426 K. cal/mol); MWKY (1.443 K. cal/mol); MMFY (1.548 K. cal/mol); MVY (1.621 K. cal/mol); FWKY (1.637 K. cal/mol) and FKQY (1.647K. cal/mol) correspondingly. Without a Bond angle bending energy, a stable configuration cannot be found. The maximum bond angle energy was observed with FVIP (4.091 K. cal/mol); FHRY (3.621 K. cal/mol); MALY (3.553 K. cal/mol); MICY (3.427 K. cal/mol); FWKY (3.198 K. cal/mol) and FKQY (3.046 K. cal/mol) respectively.

The loss in surface area from the minimum number of atoms (61) to maximum number of atoms (96) among the 18 tri & tetrapeptide leads indicating that the net loss in surface area is favourable for binding. The net contribution of Vanderwaals component with electrostatic interactions indicating higher negative values in TRRH (–1.920 K. cal/mol); FHRY (– 3.102 K. cal/mol); FLMY (–3.601 K. cal/mol); MMFY (–3.639 K. cal/mol); FALY (–3.678 K. cal/mol) and MRY(– 3.729 K. cal/mol), which are responsible for the proper binding of ligand and protein atoms. This was further observed with the non-bonded count for the above template leads as seen from Table 4. All the above datas have shown the binding affinity prediction of protein-ligand design. The Lennard-Jones parameters adopted for the system have improved the sensitivity of binding affinity between the protein and ligand.

Conclusion

The discovery of new pharamceuticals via computer modeling is one of the key challenges in modern medicine. Computational methods are anticipated to play a pivotal role in exploiting the structural and functional information to understand specific molecular recognition events of the target peptide molecule with candidate hits leading ultimately to the design of improved leads for the target. In this article, we sketch a realization of the various stages in the pathway proposed with our own research to demonstrate the way in which an iterative process of computer based rational drug design can aid in developing potent tetrapeptide leads such as Phe-Lys-Gln-Tyr (FKQY), Phe-Trp-Lys-Tyr (FWKY), Phe-His-Arg-Tyr (FHRY) and Met-Met-Phe-Tyr (MMFY) with maximum conformational stability by MD simulations for potent analgesic effect as final outcome from the selected 65 inbuild templates.

In analyzing all the most active analgesic peptides identified from Schrodinger suite, the aminoacid present in all the combination of tetra peptides, atleast possess either Lysine & tyrosine; Arginine & tyrosine or Tyrosine alone as evidenced with Phe-**Lys**-Gln-**Tyr** (FKQY), Phe-Trp-**Lys**-**Tyr** (FWKY), Phe-

His-**Arg**-**Tyr** (FHRY) and Met-Met-Phe-**Tyr** (MMFY) respectively. This clearly shows that our identified test leads are well correlated with tri peptide beta endorphin (l-tyr-gly-gly) and di peptide kyotorphin (l-tyr-l-arg) containing aminoacid moieties.

Multiple antigen peptides have a peptidyl core of radially branched lysine residues (Bracci *et al.*, 2003), which is very well correlated with our most potent analgesic target lead containing lysine residue with Phe-Lys-Gln-Tyr (FKQY), identified by the above experimental computer aided drug design approach. All the previous research done showed aminoacids like serine, glycine and proline as one of the substituent derivative in their combination of short chain peptides for the analgesic action, whereas our rational drug design approach with identification of lead moieties does not contain any of these residues and they possess the structural resemblance of natural mu opioid receptor analgesic beta endorphin and analgesic neuro peptide kyotorphin. This is the first evidence based report derived from computer aided drug design and being a rationale approach for the identification of target leads for analgesic potency, which has to be synthesized & further tested for in vivo potency also.

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References

1. Ajay, Murcko MA. (1995) Computational Methods to predict binding free energy in ligand-receptor complexes. J. Med. Chem. 38, 4953-4967.
2. Beveridge DL, Dicapua FM. (1989) Free energy via molecular simulation: Applications to chemical and biological systems. Annu. Rev. Biophys. Biophys. Chem. 18, 431.
3. Boman H. (2003) Antibacterial Peptides: basic facts and emerging concepts. J.Int. Med. 254, 197-215.
4. Bond PJ, Sansom MSP. (2004) The Simulation approach to bacterial outer membrane proteins. Mol. Membr. Biol. 21, 151-161.
5. Born M, Oppenheimer JR. (1927) Zur Quantentheorie der Molekeln. Ann. Phys. 84, 457-484.
6. Bracci L, Falciani C, Lelli B, Lozzi L, Runci Y, Pini A, De Montis MG, Tagliamonte A, Neri P. (2003) Synthetic peptides in the form of dendrimers become resistant to protease activity. Journal of Biological Chemistry. 278, 46590-46595.
7. Brooijmans N, Kuntz ID. (2003) Molecular recognition and docking algorithms. Ann. Rev. Biophys. Biomol. Struct. 32, 355–373.
8. Brooks CL, Case DA. (1993) Simulations of Peptide Conformational Dynamics and Thermodynamics. Chem. Rev. 93, 2487–2502.
9. Carr, Daniel B, Lipkowski, Andrzej W, Kream, Richard, Misicka-kesik, Aleksandra. (2004) Chimeric analgesic peptides. United States Patent 6759520. Application Number:09/428692.
10. Colombo G, Roccatano D, Mark AE. (2002) Folding and Stability of the three-stranded b-sheet peptide betanova: Insights from molecular dynamics simulations. Proteins Struct. Funct. Genet. 46, 380-392.
11. Cury Y, Picolo G, Konno K, Giorgi R, Brigatte P, Gutierrez V, Camargo A. (2009) Analogue compounds of analgesic peptides derived from the venom of Crotalus durissus terrificus snakes, their uses, compositions, methods of preparation and purification. United States Patent 51414. Application Number:20090203618.
12. Darden T, York D, Pedersen L. (1993) Particle mesh Ewald: An N log (N) method for Ewald Sums in large systems. J. Chem. Phys. 98, 10089-10092.
13. Dooley CT, Chung NN, Wilkes BC, Schiller PW, Bidlack JM, Pasternak GW, Houghten RA. (1994) An all D-amino acid opioid peptide with central analgesic activity from a combinatorial library. Science. 266 (5193), 2019-2022.
14. Frenkel D, Smit B. (2001) Understanding Molecular Simulation: From Algorithms to Applications, 2nd Edn., Academic Press, London.
15. Gajavelli S, Castellanos DA, Furmanski O, Schiller PC, Sagen J. (2008) Sustained analgesic peptide secretion and cell labeling using a novel genetic modification. Cell Transplant. 17 (4), 445-455.
16. Garcia AE, Sanbonmatsu KY. (2001) Exploring the energy landscape of a b-hairpin in explicit solvent. Proteins Struct. Funct. Genet. 42, 345-354.
17. Gill NS, Bansal RK, Garg M, Sood S, Muthuraman A, Bali M. (2010) Evaluation of antioxidant, anti-inflammatory and analgesic potential of Citrullus lanatus seed extract in rodent model. The Internet Journal of Nutrition and Wellness. 9 (2), 10.5580
18. Goldberg JS. (2010) Stereochemical basis for a unified structure activity theory of aromatic and heterocyclic rings in selected opioids and opioid peptides. Perspectives in Medicinal Chemistry. 4, 1-10.
19. Ivanciuc O. (2002) Rev. Roum. Chim. 47, 675-686.
20. Jayaram B, Mc Connell KJ, Dixit SB, Das A, Beveridge DL. (2002) Free-energy component analysis of 40 protein-DNA complexes: A consensus view on the thermodynamics of binding at the molecular level. J. Comput. Chem. 23, 1-14.
21. Kalra P, Reddy V, Jayaram B. (2001) Free energy component analysis for drug design: A case study of HIV-1 protease-inhibitor binding. J. Med. Chem. 44, 4325–4338.
22. Karplus M, McCammon JA. (2002) Molecular Dynamics Simulations of Biomolecules. Nat. Struct. Bio. 9(9), 646–652.
23. Kier LB, Hall LH. (2002) The Meaning of Molecular Connectivity: A Bimolecular Accessibility Model. Croatica Chemica Acta. 75(2), 371-382.

24. Li CH, Yamashiro D, Tseng LF, Loh HH. (1978) β-Endorphin: Synthesis and Biological activity of shortened peptide chains. International Journal of Peptide and Protein Research. 11 (2), 154-158.
25. Lipinsky CA, Lombardo F, Dominy BW, Feeney PJ. (1997) Experimental and Computational approaches to estimate solubility and permeability in drug discovery and development. Adv. Drug Del. Rev. 23, 3-25.
26. Mackerrell AD, Bashford D, Bellott M, Dunbrack RL, Evanseck JD, et al. (1998) All atom empirical potential for molecular modelling and dynamics studies of proteins. J. Phys. Chem. B. 102, 3586-3616.
27. Mathieu MP. (2001) PAREXEL's Pharmaceutical R & D Statistical Sourcebook, pp. 96, Blackwell Publishers, UK.
28. Mcintosh, Michael J, Olivera, Baldomero M, Cruz, Lourdes J, Corpuz, Gloria P, Jones, Robert M, Garrett, James E. (2008) Conotoxin peptides. United States Patent 7368432. Application Number:10/893300.
29. Novotny J, Bruccoleri RE, Saul FA. (1989) On the attribution of binding energy in antigen-antibody complexes Mc PC603, D 1.3, and HyHEL-5. Biochemistry. 28, 4735-4749.
30. Philips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, et al. (2005) Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781-1802.
31. Ribeiro MM, Pinto AR, Domingues MM, Serrano I, Heras M, Bardaji ER, Tavares I, Castanho MA. (2011) Chemical conjugation of the neuropeptide kyotorphin and ibuprofen enhances brain targeting and analgesia. Molecular Pharmaceutics. 8 (5), 1929-1940.
32. Roccatano D, Amadei A, Di Nola A, Berendsen HJC. (1999) A molecular dynamics study of the 41-56 b-hairpin from B1 domain of protein G. Protein Sci. 8, 2130-2143.
33. Roux B. (2002) Computational studies of the gramicidin channel. Acc. Chem. Res. 35, 366-375.
34. Schlick T, Skeel RD, Brunger AT, Kale LV, Board JA, Hermans J, Schulten K. (1999) Algorithmic Challenges in Computational Molecular Biophysics. J. Comput. Phys. 151(1), 9–48.
35. Sivanandaiah KM, Suresh BVV, Shankaramma SC, Lakshmana M. (1997) Pharmacological activities of some synthetic peptides related to dermorphin. Indian Journal of Pharmacology. 29 (2), 92-98.
36. Tiana G, Simona F, De Mori GMS, Broglia RA, Colombo G. (2004) Understanding the determinants of stability and folding of small globular proteins from their energetic. Protein Sci. 13, 113–123.
37. Walters PW, Stahl MT, Murcko MA. (1988) Virtual Screening – An Overview. Drug Discov. Today. 3,160.
38. Wang A, Donini O, Reyes CM, Kollman PA. (2001) Biomolecular Simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. Ann. Rev. Biophys. Biomol. Struct. 30, 211–220.
39. Warshel A. (2003) Computer Simulations of enzyme catalysis: methods, progress and insights. Ann. Rev. Biophys. Biomol. Struct. 32, 425-443.
40. Witt KA, Davis TP. (2006) CNS drug delivery: Opioid peptides and Blood brain barrier. AAPS Journal. 8 (1), E76-E88.

S.No.	Test Set	Total Net Charge	Membrane Penetration Power for Bioactivity	Boman Index (K.cal/Mol)	Margin ofInteraction with Proteins
1.	MKY	+1	M	1.11	LS
2.	MVY	0	L	-2.08	NS
3.	MIY	0	L	-2.37	NS
4.	MCY	0	L	-1.16	NS
5.	MRY	+1	M	4.23	W
6.	MALY	0	L	-2.23	NS
7.	MMFY	0	L	-1.88	NS
8.	MFWY	0	L	-1.88	NS
9.	MWKY	+1	M	0.25	LS
10.	MICY	0	L	-2.1	NS
11.	MHRY	+2	H	4.34	W
12.	FALY	0	L	-2.39	NS
13.	FLMY	0	L	-2.52	NS
14.	FWKY	+1	M	0.09	LS
15.	FKQY	+1	M	2.06	N
16.	FVIP	0	L	-2.98	NS
17.	FHRY	+2	H	4.18	W
18.	TRRH	+3	H	9.26	W
19.	MAY	0	L	-1.34	NS
20.	MLY	0	L	-2.37	NS
21.	MMY	0	L	-1.52	NS
22.	MFY	0	L	-1.73	NS
23.	MWY	0	L	-1.51	NS
24.	MQY	0	L	1.11	LS
25.	MEY	-1	VL	1.53	N
26.	MSY	0	L	0.39	LS
27.	MPY	0	L	-0.73	NS
28.	MYY	0	L	-0.69	NS
29.	MHY	+1	M	0.81	LS
30.	MNY	0	L	1.47	LS
31.	MDY	−1	VL	2.17	N
32.	MTY	0	L	0.11	LS
33.	MFGY	0	L	-1.53	NS
34.	MGAY	0	L	-1.24	NS
35.	MLNY	0	L	-0.12	NS
36.	MKQY	+1	M	2.22	N
37.	MQEY	-1	VL	2.53	N
38.	MESY	-1	VL	1.99	N
39.	MSPY	0	L	0.29	LS
40.	MPVY	0	L	-1.56	NS
41.	MCYY	0	L	-0.83	NS
42.	MYHY	+1	M	0.64	LS
43.	MRNY	+1	M	4.83	W
44.	MNDY	-1	VL	3.28	N
45.	MDTY	-1	VL	2.27	N
46.	MTGY	0	L	-0.14	NS
47.	FGGY	0	L	-1.18	NS
48.	FGAY	0	L	-1.39	NS
49.	FMFY	0	L	-2.04	NS
50.	FFWY	0	L	-2.03	NS
51.	FQEY	-1	VL	2.37	N
52.	FESY	-1	VL	1.84	N
53.	FSPY	0	L	0.13	LS
54.	FPVY	0	L	-1.72	NS
55.	FICY	0	L	-2.25	NS
56.	FCYY	0	L	-0.99	NS
57.	FYHY	+1	M	0.49	LS
58.	FRNY	+1	M	4.68	W
59.	FNDY	-1	VL	3.13	N
60.	FDTY	-1	VL	2.11	N
61.	FEGY	-1	VL	0.75	LS
62.	TRRH	+3	H	6.92	W
63.	THRH	+3	H	6.7	W
64.	MGY	0	L	-1.05	NS
65.	MVIP	0	L	-2.82	NS

Table 1. Boman index and total net charge for template library analogues designed for initial Analgesic Screening.

L	-->Low Penetration Power	1.5-4	-->N
M	-->Moderate Penetration Power	1≤	-->LS
H	-->High Penetration Power	0≤	-->NS
W	-->Wide margin of interaction	N	-->Narrow margin of interaction
LS	-->Less Satisfactory margin of interaction	NS	-->No satisfactory interaction

S.No.	Selected Lead Set	Hydrophobic Ratio (%)	Total Net Change	Boman Index (K.cal/Mol)
1.	MKY	33	+1	1.11
2.	MVY	66	0	-2.08
3.	MIY	66	0	-2.37
4.	MCY	66	0	-1.16
5.	MRY	33	+1	4.23
6.	MALY	75	0	-2.23
7.	MMFY	75	0	-1.88
8.	MFWY	75	0	-1.88
9.	MWKY	50	+1	0.25
10.	MICY	75	0	-2.1
11.	MHRY	25	+2	4.34
12.	FALY	75	0	-2.39
13.	FLMY	75	0	-2.52
14.	FWKY	50	+1	0.09
15.	FKQY	25	+1	2.06
16.	FVIP	75	0	-2.98
17.	FHRY	25	+2	4.18
18.	TRRH	0	+3	9.26

Table 2. Rational Drug Design outcome for Analgesic peptide leads with their Hydrophobicity ratios.

S. No.	Test Set	Observed H-bonds		Drug like fulfillment with respect to		Molecular Mass	Log P	Molar Refra c-tivity	Drug like fulfillment with respect to			Final Remark
		Number of H-bond donor	Number of H-bond acceptor	H-bond donor	H-bond acceptor				Mol. Mass	Log P	Molar Refrac-tivity	
1.	MKY	0	4	S	S	409	-0.241	89.65	S	S	S	DL
2.	MVY	0	4	S	S	383	-0.056	85.17	S	S	S	DL
3.	MIY	0	4	S	S	395	0.025	88.42	S	S	S	DL
4.	MCY	0	3	S	S	391	0	0	S	S	NS	PDL
5.	MRY	0	4	S	S	437	-0.988	93.02	S	S	S	DL
6.	MALY	0	6	S	S	461	0	0	S	S	NS	PDL
7.	MMFY	0	5	S	S	553	0	0	NS	S	NS	PDL
8.	MFWY	0	6	S	S	607	0	0	NS	S	NS	PDL
9.	MWKY	0	5	S	S	585	0	0	NS	S	NS	PDL
10.	MICY	0	5	S	S	493	0	0	S	S	NS	PDL
11.	MHRY	0	5	S	S	567	0	0	NS	S	NS	PDL
12.	FALY	0	5	S	S	477	0	0	S	S	NS	PDL
13.	FLMY	0	5	S	S	533	0	0	NS	S	NS	PDL
14.	FWKY	0	5	S	S	601	0	0	NS	S	NS	PDL
15.	FKQY	0	6	S	S	545	0	0	NS	S	NS	PDL
16.	FVIP	0	4	S	S	437	0	0	S	S	NS	PDL
17.	FHRY	0	5	S	S	583	0	0	NS	S	NS	PDL
18.	TRRH	0	6	S	S	529	-3.426	111.4	NS	S	S	PDL

Table 3. Molecular descriptors of test data set analogues by Lipinski rules.

S=Satisfied; NS=Not satisfied; DL=Drug like characteristics; PDL=Partial Drug like characteristics.

S. No.	Test Set	Total No. of Atoms & System Temperature (°K)	Total Energy of System (K.cal/ Mol)	Total Potential Energy (K. cal/ mol)	Total Kinetic Energy (K. cal/ mol)	Bond Stretch Energy (K. cal/ mol)	Angle Bending Energy (K. cal/ mol)	Torsion Angle Energy (K. cal/ mol)	Restraining Energy for Torsion	1, 4 Lennard Jones Energy (K. cal/ mol)	1, 4 Electro Static Energy (K. cal/ mol)	Lennard Jones Energy (K. cal/ mol)	Electro Static Energy (K. cal/ mol)	H–bond Energy (K. cal/ mol)	Non–bonded Count
1.	FALY	82 (302.528)	-7.446 +00	-5.464 +01	4.719 +01	1.858 -11	2.126 +01	2.548 +01	0 +00	3.406 +01	1.192 +02	-1.258 +01	-2.420 +02	0 +00	2894
2.	FHRY	94 (308.493)	8.985 +00	-4.619 +01	5.517 +01	2.011 -11	3.621 +01	2.152 +01	0 +00	3.083 +01	-1.037 +02	-1.384 +01	-1.718 +01	0 +00	3886
3.	FKQY	92 (287.576)	-1.818 +01	-6.875 +01	5.058 +01	2.053 -11	3.046 +01	1.647 +01	0 +00	3.784 +01	1.068 +02	-1.345 +01	-2.468 +02	0 +00	3703
4.	FLMY	89 (288.712)	-2.833 +00	-5.189 +01	4.905 +01	1.734 -11	2.761 +01	2.018 +01	0 +00	3.411 +01	1.153 +02	-1.233 +01	-2.368 +02	0 +00	3454
5.	FVIP	81 (364.149)	6.533 +01	9.247 +00	5.608 +01	1.669 -11	4.091 +01	2.384 +01	0 +00	2.740 +01	1.061 +02	-8.868 +00	-1.801 +02	0 +00	2801
6.	FWKY	99 (287.799)	2.167 +01	-3.238 +01	5.405 +01	2.073 -11	3.198 +01	1.637 +01	0 +00	4.123 +01	1.320 +02	-1.469 +01	-2.393 +02	0 +00	4325
7.	MALY	79 (300.720)	-7.932 +00	-5.335 +01	4.542 +01	1.780 -11	3.553 +01	1.998 +01	0 +00	2.534 +01	1.206 +02	-8.624 +00	-2.462 +02	0 +00	2675
8.	MCY	61 (294.900)	-1.309 +01	-4.708 +01	3.399 +01	1.314 -11	1.854 +01	6.872 +00	0 +00	2.090 +01	8.898 +01	-7.669 +00	-1.747 +02	0 +00	1526
9.	MFWY	94 (292.770)	2.882 +00	-4.919 +01	5.207 +01	1.979 -11	2.825 +01	1.677 +01	0 +00	3.900 +01	1.313 +02	-1.405 +01	-2.505 +02	0 +00	3882
10.	MHRY	91 (320.462)	-1.775 +01	-7.347 +01	5.572 +01	1.776 -11	2.653 +01	2.470 +01	0 +00	3.078 +01	-8.205 +01	-1.114 +01	-6.229 +01	0 +00	3631
11.	MICY	80 (312.682)	-1.079 +01	-5.864 +01	4.784 +01	1.175 -11	3.427 +01	1.810 +01	0 +00	2.463 +01	1.273 +02	-1.469 +01	-2.482 +02	0 +00	2749
12.	MIY	69 (295.180)	8.700 +00	-3.001 +01	3.871 +01	9.580 -12	2.545 +01	1.820 +01	0 +00	2.305 +01	8.916 +01	-9.261 +00	-1.766 +02	0 +00	1993
13.	MKY	72 (290.368)	1.916 +01	-2.066 +01	3.981 +01	1.065 -11	2.325 +01	1.763 +01	0 +00	2.230 +01	8.976 +01	-8.687 +00	-1.649 +02	0 +00	2185
14.	MMFY	87 (315.053)	-7.548 +00	-5.982 +01	5.228 +01	1.820 -11	2.178 +01	1.548 +01	0 +00	3.664 +01	1.246 +02	-1.173 +01	-2.466 +02	0 +00	3298
15.	MRY	74 (288.153)	-7.148 +00	-4.780 +01	4.066 +01	1.672 -11	2.546 +01	1.426 +01	0 +00	2.138 +01	-1.233 +02	-1.145 +01	2.584 +01	0 +00	2328
16.	MVY	66 (296.363)	-2.161 +00	-3.926 +01	3.710 +01	1.338 -11	2.185 +01	1.621 +01	0 +00	2.584 +01	9.208 +01	-9.178 +00	-1.861 +02	0 +00	1810
17.	MWKY	96 (327.319)	1.091 +01	-4.893 +01	5.984 +01	1.979 -11	2.830 +01	1.443 +01	0 +00	3.360 +01	1.387 +02	-1.396 +01	-2.500 +02	0 +00	4055
18.	TRRH	91 (291.493)	4.224 +01	-8.735 +00	5.097 +01	1.504 -11	3.001 +01	3.722 +01	0 +00	2.864 +01	-3.016 +02	-3.935 +00	2.010 +02	0 +00	3629

Table 4. Final energy reports of Tripeptide & Tetrapeptide template leads for conformational stability using MD simulations.

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