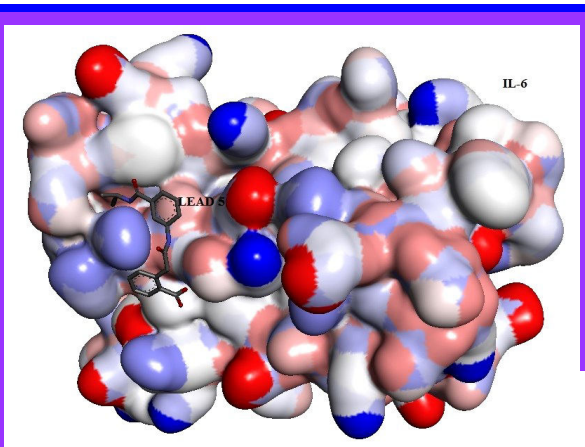

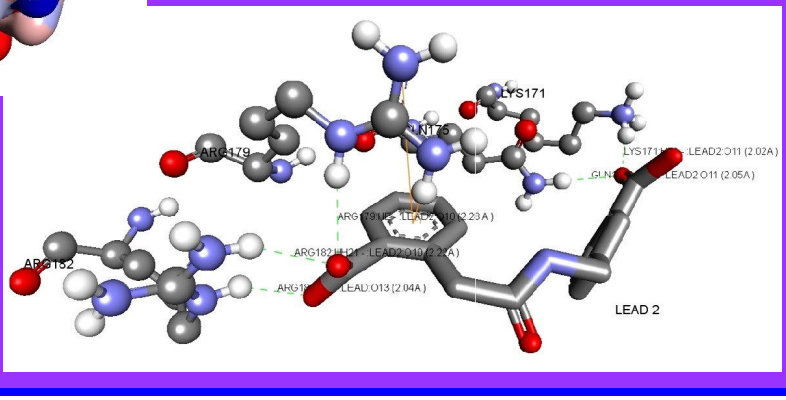

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In silico targeting of interleukin-6 by natural compounds

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Abstract

Rheumatoid arthritis an autoimmune disorder affecting many tissues and organs, is a challenging prospect in terms of treatment. Our work is looking into finding lead compound of natural origin against IL-6, an important cytokine having a profound role in pathogenesis of rheumatoid arthritis. For this purpose we have targeted the active site (ARG 179) of IL-6 using IBS database having 48531 natural compounds. Computer aided drug designing methods of virtual screening and *in silico* ADME/Tox helped us to limit our study of molecular docking to selected few for an atomic insight into their binding modes. The molecular docking analysis helped us to propose five possible drugs, out of which one compound (Chem. ID 10465) margaric acid is an edible fatty acid and exhibited good binding affinity with the active site of IL-6, thus making it a good starting point for developing drug for treatment of arthritis.

Introduction

Early mortality, severe disability and structural damages are the outcome of a chronic inflammatory autoimmune disorder, rheumatoid arthritis (Klareskog et al., 2009; Scott et al., 2010). Although a lot of progress has been achieved in controlling the disease activity by using drugs known as disease modifying anti-rheumatic drugs (DMARDs), but still challenges are there in early diagnosis and treatment (McInnes at al., 2011). The etiology of rheumatoid arthritis is a mystery till date and it is the pathogenesis that has been deciphered to a lot of extent (Misato et al., 2011).

IL-6 a highly up-regulated (Houssiau et al., 1988; Madhok et al., 1993) cytokine is a key molecule with a pivotal role in the pathogenesis of rheumatoid arthritis (Misato et al., 2011). Its role in synovitis (Maruotti et al., 2006) Joint damage (Gravallese et al., 2000), autoimmunity (Zhou et al., 2007), anemia (Hochberg et al., 1988) and hypolepidemia (Hashizume et al., 2010) makes it a potential target in treatment of rheumatoid arthritis. The argentine (ARG) at the 179th position of IL-6 plays an important role (Fontaine et al. 1992), targeting this particular site in IL-6 will impair its normal functioning. To achieve this we resorted to computer aided drug discovery, which has become an important tool in drug discovery (Alvarez, 2004; Chikan et al., 2013).

The present study targeted crystal structure of IL-6 bearing accession code of 1ALU (Somers et al., 1997), to obtain the selected few lead compounds of natural origin. To achieve our goal we used multistep structure-based virtual screening of around fifty thousand natural compounds obtained from Inter Bio Screen (IBS) database. Virtual screening which is incorporated in the major pipeline of drug discovery in most pharmaceutical companies has become an indispensible tool for iden -tifying active lead compounds. The Absorption, distribution, metabolism, elimination (ADME) and Toxicology (Tox) are also the properties that have been identified as the major cause of failure of major drug screening projects (Davis et al., 2004) therefore ADME/Tox data validation is an important aspect (Van et al., 2003).

Material and Methods

The crystal structure of human IL-6 protein (PDB ID: 1ALU) was obtained from protein databank (PDB).

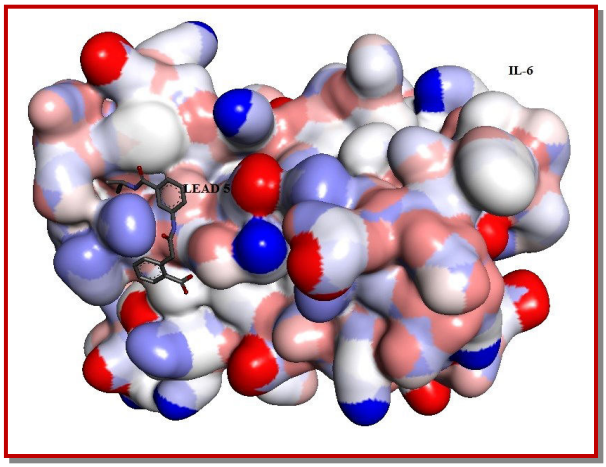

Figure 1: Step wise illustration of the Modus operandi in use

SPDB viewer at the default cut off RMSD (Root Mean Square Deviation) value of 0.5 Å using OPLS 2001 force field was used for energy minimization of the PDB structure. The modus operandi that followed this step is illustrated in Figure 1.

Virtual screening (VS)

Virtual screening has become a necessary tool in cutting short lead compounds and has fitted itself in the pipeline of drug discovery in most pharmaceutical companies. We used ArgusLab (Thompson et al., 2004) to perform the VS of the ligand dataset. The software was set to high precision screening using ArgusDock docking engine. The ligands were set to flexible mode and were targeted against the amino acid ARG at 179 position with a grid box of diameter 15 X 15 X 15 Å. Natural compounds from IBS Database, having 48531 natural compounds were used for the VS of IL-6 inhibitors. From the initial dataset, a total of 500 compounds were selected based on their calculated binding affinity with the protein IL-6 for further *in silico* ADME/Tox analysis.

Rule of Five and ADME/T screening

The subset of 500 compounds was screened for Lipinski Rule of five violations (Lipinski, 2004) with the objective of increasing the success rate of compounds reaching further stages of the development. The subset of compounds was also subjected to *in silico* prediction (Muanz et al., 2013). A total of fifty top compounds which satisfied the criteria were chosen for further analysis.

Molecular docking

AutoDock 4.2 is used for molecular docking (Morris et al., 2009). The software was used to find the best binding mode using binding free energy evaluations and number of physical interactions. AutoDock calculates the energy values by the characterization of internal energy of ligand, torsional free energy and intermolecular energy consisting of van der Walls energy, hydrogen bonding energy, desolvation energy, and electrostatic energy.

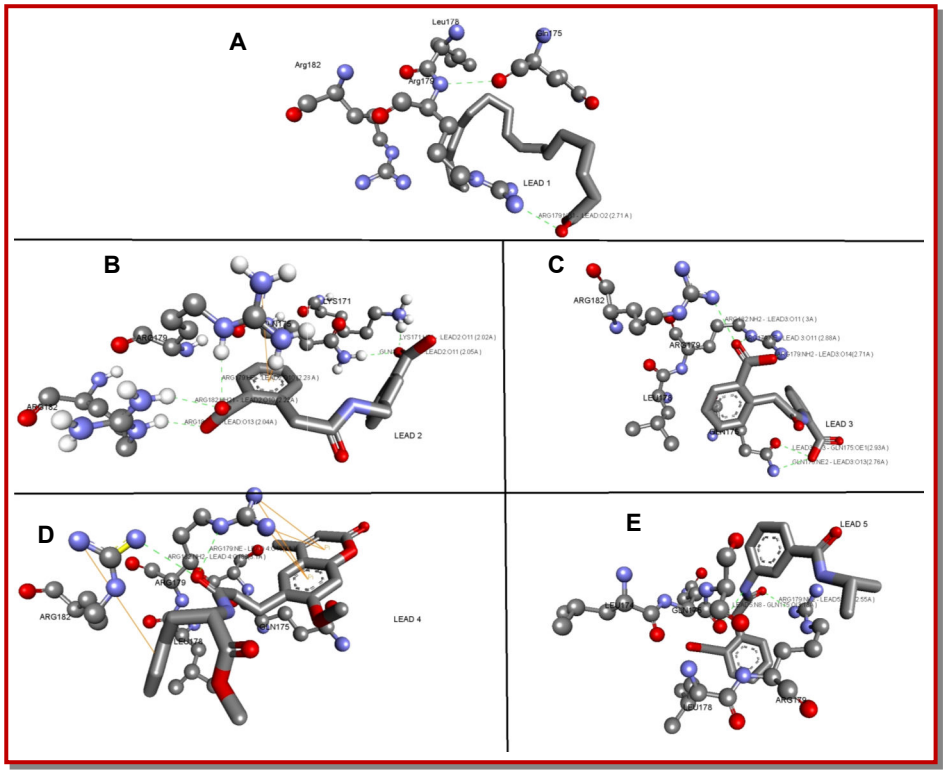

Figure 2: The solid surface structure of Il-6 with lead 5 binding pocket

Table I

Lipinski Rule of five and toxicolgy report

Name	ChemId	MW (Dalton)	clogp	HBD	HBA	Mutagenicity	Carcinogenecity
Lead 1	10465	270.5	4.7	1	2	No	No
Lead 2	Not found	313.3	1.9	3	6	No	No
Lead 3	10518237	299.3	2.8	5	6	No	No
Lead 4	Not found	423.5	3.1	1	7	No	No
Lead 5	Not found	340.4	2.0	3	6	No	No

Table II

AutoDock analysis of five lead natural products

Name	IUPAC Name	ΔG Kcal/mol	Ligand binding pocket	Interactions
Lead 1	Heptadecanoic acid	-1.5	GLN175,LEU178,ARG179, ARG182	1 Hydrogen bond
Lead 2	2-(2-((3-carboxybenzyl)amino)-2-oxoethyl)benzoic acid	-5.4	LYS171, GLN175,ARG179, ARG182	5 Hydrogen bond 2 Pi bonds
Lead 3	2-(2-(2-carboxyphenyl)acetamido) benzoic acid	-5.4	GLN175,LEU178,ARG179, ARG182	4 Hydrogen bond
Lead 4	(S)-methyl 2-(3-(7-methoxy-4-methyl-2-oxo-2H-chromen-6-yl) propanamido)-3-phenylpropanoate	-3.9	ASP26, ARG30, LYS171, GLN175, SER176, LEU178, ARG179, ARG182	2 Hydrogen bond 5 Pi bonds
Lead 5	2-(2-((3-(isopropylcarbamoyl)phenyl) amino)-2-oxoethyl)benzoic acid	-5.2	LEU33, CYS73, PHE74, GLN75, LEU174, GLN175, SER176, LEU178, ARG 179, ARG 182.	2 Hydrogen bond 1 Pi bonds

The ligand binding pocket and the hydrogen bond formation was calculated using Discovery Studio 3.5 software. The bold amino acids represent the one which are involved in forming interaction with the ligand

Result and Discussion

Virtual screening followed by *in silico* ADME/Tox analysis of IL-6 and IBS database was successful in limit -ing our search to top 50 compounds. From the initial data set of around fifty thousand natural products selected for the study, subsets of five hundred lead-like natural products were selected using ArgusLab based on the feature of binding energy. The VS was followed by ADME/Tox analysis using Lazar Toxicity Predictions online server to further limit the size of initial data -set. The tool was pivotal in checking their mutagenic and carcinogenic property, the shortlisted fifty compound were selected on their drug likeness (Lipinski et al., 2012) and their observance of ADME and related properties of typical drugs (Zhao et al., 2001, van de Waterbeemd et al., 1998). For drug likeness molecular weight (MW), number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA) and total polar surface area (tpsa) ,which all fall under Lipinski's rule of five (RO5) were calculated. Table I shows the RO5 data of top five compounds. These properties have an important role in the final outcome and analyzing them using by *in silico* means not only saves time but also is economically more viable. The permissible range of RO5 are MW≤500 Dalton, clog p≤4.5, HBD ≤5, HBA ≤10, which is associated with 90% of orally active drugs that have achieved phase II clinical status (Lipinski, 2004). Our lead compounds are in compliance with the RO5 properties se by Lipinski.

The initial database contained 48,531 natural products selected for the virtual screening was condensed based on binding free energy obtained by ArgusLab, the initial dataset was shortened almost 99% of the initial size to mere five hundred compounds. Further screening ensured that only 0.1% of total compounds i.e. fifty compounds of natural origin were available for further *in silico* studies using AutoDock 4.2 tool. The proposed target site was optimized for performing the docking studies. Among the top 50 compounds only 5 compounds were found to physically interact with Arg179.

The final shortlisted natural compounds obtained by using AutoDock 4.2 tool into the optimized target site (Figure 2) of the protein were analyzed for interaction using Discovery Studio 3.5 client software, the interactions are described in Table II. All five lead compounds were found to form hydrogen bond with ARG179. The interactions (covalent or non covalent), the number of hydrogen bonds and number of *Pi-Pi* interactions has been displayed in Figure 3 for all the selected lead compounds.

The first lead compound is famous as Margaric acid

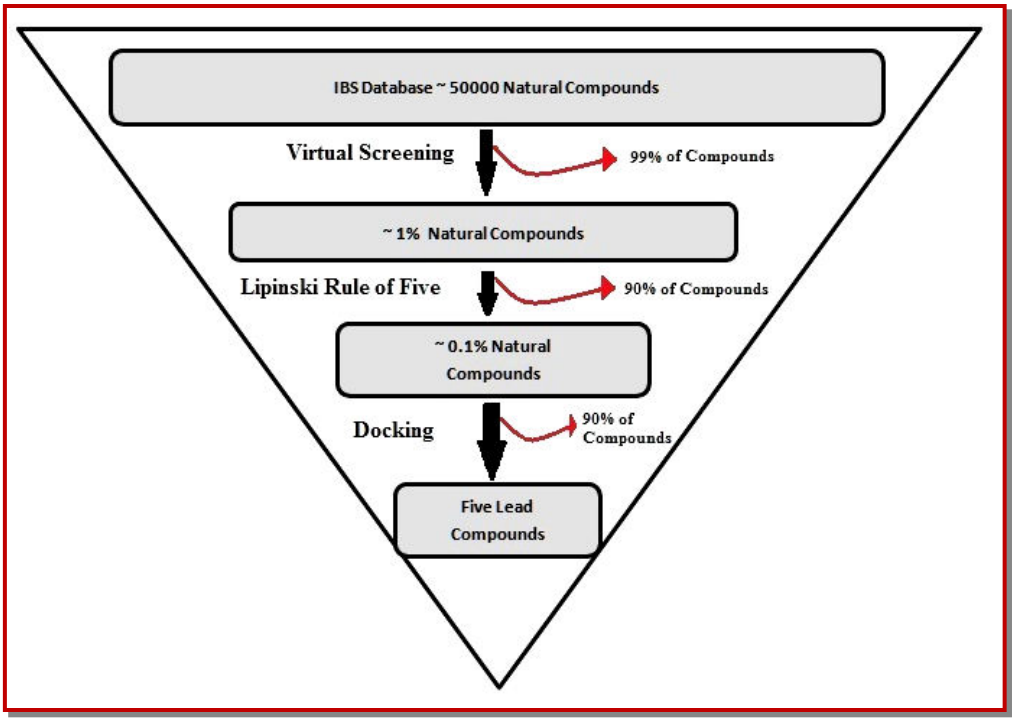

Figure 3: 2D docked conformation of five lead compounds with IL-6

having PubChem ID 10465 showed lowest binding energy of -1.5 kcal/mol. The complex exhibited a lone hydrogen bond (Figure 4A) with the key residue ARG179 of IL-6.The hydrogen bond interaction of 2.72 Å is between the O2 of the Lead 1 and N1 atom of ARG179. The binding pocket of this complex constitutes of GLN175, LEU178, ARG179 and ARG182.

The second lead interacting with targeted site of IL-6 has IBS ID: 77198. Its binding pocket comprises of LYS171, GLN175, ARG179 and ARG182. The complex of lead 2 and IL-6 is the most stable having ΔG of -5.4 Kcal/mol with five hydrogen bonds and two *Pi-Pi* interactions. The interaction of our interest here is that of ARG179 and ligands O10 position. The distance between the two is 2.28 Å. The two *Pi-Pi* interactions are with NH1 and NH2 of ARG179 with a distance of 5.5 Å and 3.72 Å respectively, Figure 4B shows the mapping of complex 2.

With a ΔG of -5.4 Kcal/mol, four hydrogen bonds and no *Pi-Pi* interaction, lead 3 is showing that the compound is second to lead 2. From Figure 4C we can point out that out of the four interactions two are of our interest as they involve ARG179. The first being between O11 of the lead 3 and NE of ARG 179 having distance of 2.88 Å and the second interaction of 2.71 Å is between O14 of lead and NH of ARG179. The binding pocket of Lead 3 comprises of GLN175, LEY178, ARG179 and ARG182.

The complex between lead 4 and IL-6 is rich in *Pi-Pi* interactions (Figure 4D), four of the six *Pi-Pi* interactions is between the lead 4 and ARG179 with a distance ranging from 3 Å to 5 Å. The ΔG of -3.9 Kcal/mol makes it the forth least free energy complex forming two hydrogen bond interaction , the interaction with ARG179 is of 3 Å between O18 of lead and NE of ARG179. The binding groove consists of ASP26, ARG30, LYS171, GLN175, SER176, LEU178, ARG179 and ARG182.

The final complex to form an interaction with ARG179 is Lead 5, with ΔG of -5.2 Kcal/mol the complex is third in line in terms of free energy. The complex is forming both hydrogen bond and *Pi-Pi* interactions. The NH2 of ARG179 is forming a hydrogen bond with O14 of lead compound with a distance of 2.55 Å. The lone *Pi-Pi* interaction is between PHE74 of IL-6 and lead 5. The binding pocked of the lead comprises of LEU33, CYS73, PHE74, GLN75, LEU174, GLN175, SER176, LEU178, ARG 179 and ARG 182.

All the five compounds are of natural origin having tremendous potential in blocking IL-6 activity and are providing an opportunity for further *in vitro* and *in vivo* analysis. Our approach of computer aided drug designing has helped us in limiting our focus to mere 0.01% of the total volume of Inter Bio Screen database of around fifty thousand compounds (Figure 5).

Out of the five compounds only lead 1 source could be confirmed, this particular compound is known as margaric acid a fatty acid found in fat and milk fat of ruminants (Cooke et al., 1957). Margeric acid is also found in the contents of imitation butter famously known as

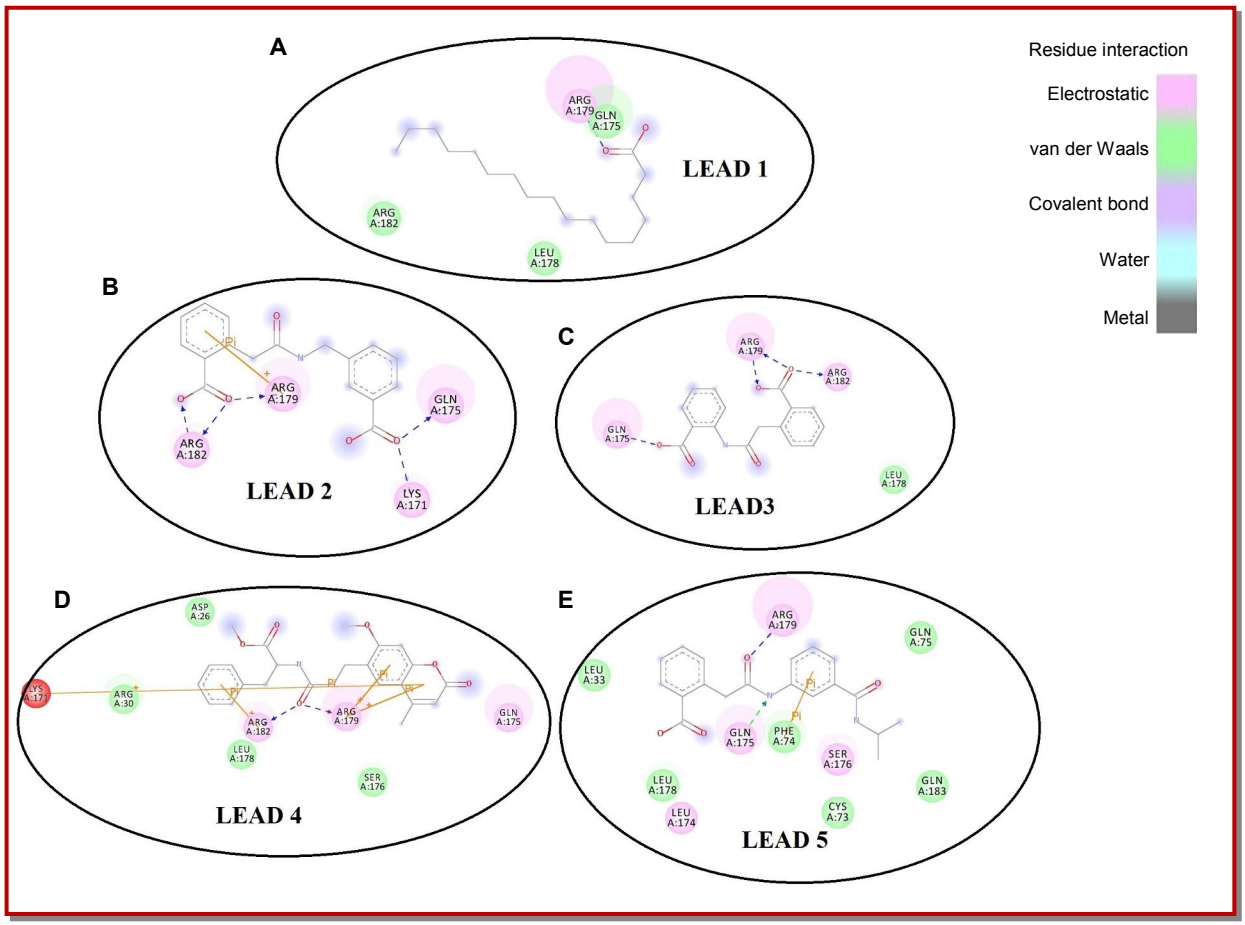

Figure 4: 3D mapping of lead compounds with their respective hits where (A, B, C and E) represent the lead compounds in order

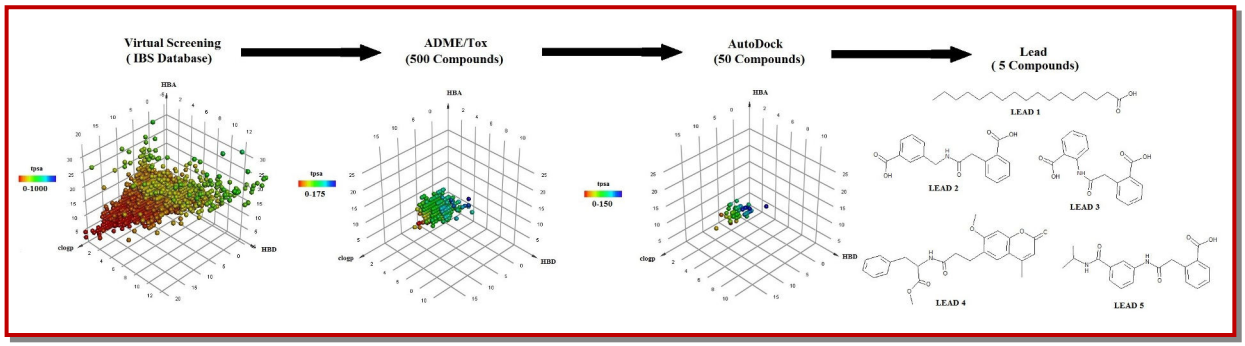

Figure 5: 3D point plot of four properties (HBA, HBD, clogP, and tpsa) of the compounds at different stages of drug discovery, showing the journey from ~ 50000 compounds to top five in accordance with Lipinski's parameters

margarine. As fatty acids are already reported to have anti inflammatory effects (Khalil et al., 2000) makes lead 1 the most significant of the five shortlisted compounds. This particular selected natural compound we feel holds an edge over the selected four other compounds and can be crucial in blocking considerably elevated Il-6 in the serum of rheumatoid arthritis patients.

Contribution

First two authors contributed equally in this study.

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