In silico prediction of some biologically active compounds against IBD

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Abstract

Drugs used in the treatment of inflammatory bowel diseases (IBD) without side have not developed yet. Based on this, one of the urgent problems is the identification of natural biologically active compounds without side effects that have the property of treating and preventing inflammatory bowel diseases. For the treatment of IBD such as ulcerative colitis and Crohn's disease, Janus kinase(JK) and 5-lipoxygenase (5LO) inhibitors are widely used, but unfortunately, when treated with these drugs, their side effect were observed. Based on this, one of the urgent problems is the development of therapeutic agents without side effects. This article aimed at studying the effect of some biologically active substances isolated from medicinal plants on the activity of 5LO and JK in order to identify drugs with anti-inflammatory properties in the treatment of IBD compared with synthetic drugs such as mesalazine, sulfasalazine, tofacitinib, using in silico methods. These studies show that rutin, dehydroquercetin, quercetin and beta caryophyllene have the highest activity in binding to the enzymes 5LO and JK compared to synthetic drugs.This resultsprovides a basis for further in vitro studies of these compounds to identify anti-inflammatory drugs against IBD.

Key words:quercetin, dehydroquercetin, rutin,α-pinene,β-caryophyllene, mesalazine,sulfasalazine, tofacitinib.

1.Introduction

Inflammatory bowel disease (IBD) is a state in which the intestines are inflamed. The exact cause of IBD is still unclear, but it is recognized that its etiopathology is multifactorial, including genetic predisposition, impaired mucosal function to certain lumen antigens, dysfunction of the mucosal barrier, immune or endogenous system, environmental and lifestyle factors that lead to chronic inflammation [1-5].

Synthetic drugs such as kinase inhibitors, lipoxygen (5-aminosalicylic acid) inhibitors, monoclonal antibodies, etc. are used to treat inflammatory bowel diseases.Sulfasalazine is one of the main agentsofcolitis. The composition of sulfasalazine includes mesalazine substances (5-aminosalicylic acid), which have an anti-inflammatory effect, and sulfapyridine, which ensures that mesalazine enters the colon. The mesalazine component suppresses inflammation by inhibiting the activity of neutrophil cell lipoxygenase and synthesis of arachidonic acid metabolites (prostaglandins and leukotrienes), which are mediators of inflammation. 5-lipoxygenase (5-LO) is the main enzyme of the arachidonic acid (AA) metabolic pathway, which leads to the production of pro-inflammatory mediators [6-7]. But unfortunately, patients are often exposed to serious side effects when treated with modern 5LO inhibitors.An example of kinase inhibitors thatare used in the treatment of ulcerative colitis, we can cite the drugs tofacitinib and yakvinus, which have retained the active ingredient tofacitinib. Tofacitinib is a drug that participates in several inflammations by inhibiting the enzymes Janus kinase (JK 1, -2, -3) prevents inflammation by blocking receptor signals that go to cytokines IL -2, -4, -7, -9, -12, -15, -21,-23. During treatment with this drug, many negative effects were also observed[8]. Harmlessdrugs used in the treatment of IBD have not yet been developed. Therefore, one of the urgent problems is the identification of natural biologically active compounds with no side effects that have the property of treating and preventing inflammatory bowel diseases. This study is aimed at studying the effect of certain biologically active substances on the activity of

lipoxygenase and Janus kinase by the in silico prediction method in order to identify drugs with anti-inflammatory properties in the treatment of IBD.

2. Materials and methods

2.1 Properties of molecules and drug

The pharmaceutical propertiesof each selected compound was learned by using Molinspiration WebME3.81 (https://molinspiration.com/cgi-bin/properties) and Molsoft L.L.C (molsoft.com/mprop/) programs methods.

2.2 Molecular docking

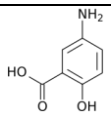
The study of molecular docking of substanceswas conducted using the website https://mcule.com/apps/1-click-docking/. For this, the PDB structures of the human enzymes Janus kinase 3 and arachidonate lipoxygenasewere used by downloading from the website. Also used in intestinal inflammation are synthetic preparations sulfasalazine, mesalazine, and tofacitinib, as well as natural chemical compounds found in food additives https://pubmed.ncbi.nlm.nih.gov the SMILE structure available on the website was used.

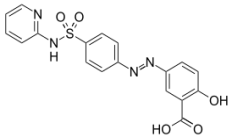
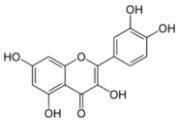
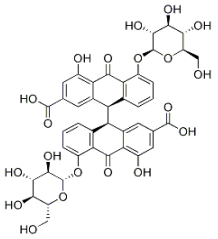
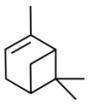
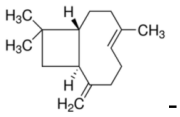
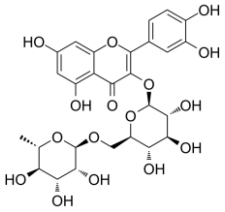
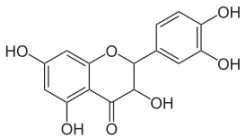
3. Results and discussion

3.1 Molecular properties

The atomic coordinates of these compounds were downloaded from the PubChem database to compare the binding affinity of quercetin, sennoside a, α-pinene, β - caryophyllene with mesalazine, sulfasalazine and tofacitinib. The structural data on these compounds are given in Table 1.

Table 1: Structure of synthetic and natural components used in intestinal inflammation

Components	***Pubchem CID***	***2D conformation***
Mesalazine	*4075*	

Sulfasalazine	*5353980*	
Quercetin	*5280343*	
Sennoside A	*73111.*	
α-pinene	*6654*	
β-caryophyllene	*5281515*	
Rutin	*5280805*	
Dehydroquercetine	*471*	

Comparison of the molecular properties of the isolated biologically active components with synthetic drugs was predictedusing the Molinspiration WebME program (Table 2).

Table 2: Molecular characteristics of natural and synthetic preparations

Indicators

Features	***Details***	***Mesalazine***	***Sulfasalazine***	***Tofacitinib***	***Rutin***	***Quercetin***	***Sennoside A***	***α-pinene***	***β-caryophyllene***	***Dehydroquercetin***
miLogP	octanol and water separation coefficient logP	**0,92**	**2.09**	**0.45**	**-1.06**	**1,68**	**0.86**	**3.54**	**5.17**	**0.71**
TPSA	Molecular surface area (PSA)	**83,55**	**137.82**	**88.91**	**269.43**	**131,35**	**347.96**	**0**	**0**	**127.44**
N atoms	Non-hydrogen atoms number	**11**	**28**	**23**	**43**	**22**	**62**	**10**	**15**	**22**
MW	Molecular mass	**153,14**	**398.40**	**312.38**	**610.52**	**302,24**	**862.75**	**136,24**	**204,36**	**304.25**
N ON	Hydrogen binding acceptors number (O and N atoms)	**4**	**9**	**7**	**16**	**7**	**20**	**0**	**0**	**7**
*n*OHNH	Hydrogen binding donors number(on and NH groups)	**4**	**3**	**1**	**10**	**5**	**12**	**0**	**0**	**5**
N violations	Number of five rules violations	**0**	**0**	**0**	**3**	**0**	**3**	**0**	**1**	**0**
n rotb	Number of rolling gardens	**1**	**6**	**3**	**6**	**1**	**9**	**0**	**0**	**1**
volume	Molecular volume	**130,35**	**320.77**	**389.26**	**496.07**	**240,08**	**698.52**	**151.81**	**229,95**	**246.32**

As can be seen from the prediction of molecular properties, the number of donors and acceptors of hydrogen bonds in rutin and quercetin exceeds the amount of mesalazine, sulfasalazine, and tofacitinib, which are synthetic drugs. According to the forecast, there are 20 hydrogen bond acceptors in sennoside A, 16 in rutin, and 7 hydrogen bond acceptors in quercetin, while sennoside A has 12 hydrogen bond donors, rutin 10, and quercetin 10 (table 2). In addition to the hydrogen bond state of quercetin, its molecular weight is 302.238 and its

logarithmic value is R 1.68 (table 2). Like mesalazine and sulfasalazine, quercetin, dehydroquercetin and Alpha pinene have not violated Lipinsky's "rule of five". This rule provides for the manifestation of features aimed at the oral administration of drugs.

They also demonstrated that sennoside A and rutin have a higher polar area compared to synthetic preparations such as sulfasalazine and mesalazine.

3.2 Drug likeness

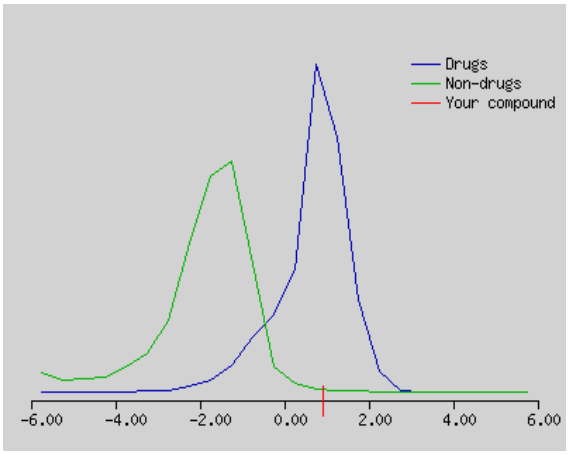
Drug substances usually affect concrete targets at the molecular level and then have a therapeutic effect by binding to a specific target in the cell. Before drug substances exert their pharmacodynamic effect on the body, before the reach the site of action of the drug in the body it must go through various obstacles. Pharmacokinetics here is studied to determine whether a drug reaches its target site from the gastrointestinal tract. That is, in general, this process can be determined by the following stages: absorption, transport, metabolic processes, and excretion. The first barrier to oral medication is normal absorption from the intestinal wall into the circulatory system. Once ingested, it is enters to the liver, where it can be modified by a panel of liver microsomal enzymes; some of these molecules can be metabolized and some excreted through bile. If a molecule of drugs survives this first transition metabolism, it enters the blood circulation and then spreads to the organs, including the target tissue. After the therapeutic response of this drug, it must be permanently eliminated from the body; otherwise, there may be a risk of bioaccumulation. Finally, the medicaments should not cause serious side effects when adding other medicines that the patient may be taking. Assessment of the similarity of the studied substance to drugs is a right-shifted distribution, which has reached its maximum level in the range of 0.8-1.2 on the scale of drug-likeness. The drug-like parameter is a method aimed at showing to what extent the substance is absorbed by the body and is suitable for treatment.

The drug-like rules concept was offered by Lipinsky, as a rule of five, which includes four different definitions of Physico-Chemical parameters (molecular mass≤ 500, log P ≤ 5, H-

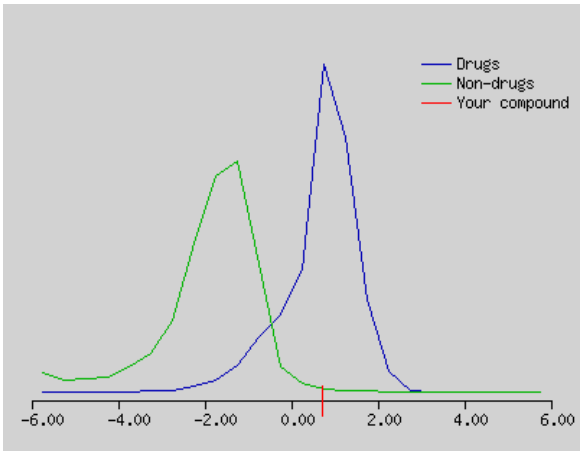
binder ≤ 5, H-binder ≤ 10 [9]. In 2012 based on the statistical distribution propertiesofselected from a set of 771 oral small molecule drugs and used to assess the drug suitability of a molecular target, Bikerton, Hopkins and others proposeda quantitative assessment of the drug similarity index [10].

Drug likeness parameters (https://molsoft.com/mprop/) was carried out using the program. The results are shown in Figure 1 below.

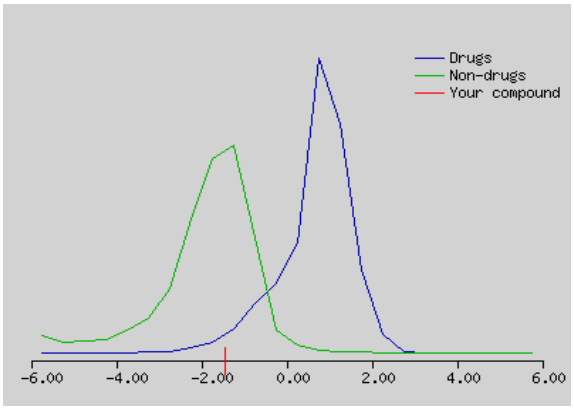
A)



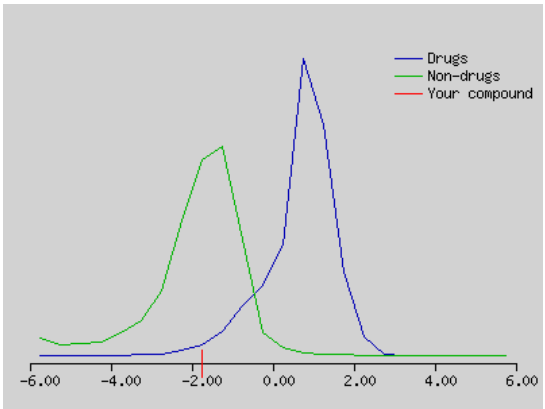
B)



C)



D)



E)

F)

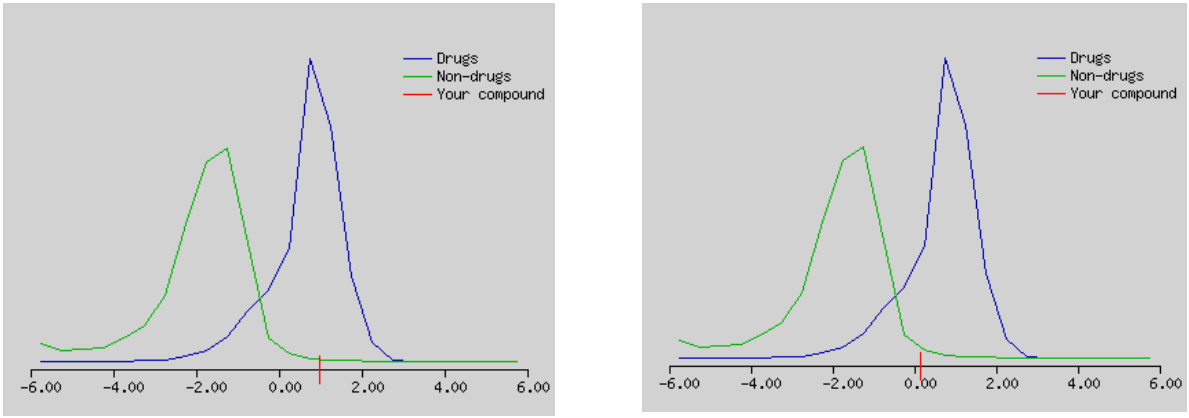


Figure 1: *Models of similarity of certain substances to drugs.*

*To assess the similarity with the drug, the Molsoft chemical fingerprint model was used, which consisted of 5 thousand sold drugs from World drug index as a positive result and carefully selected non-drug compounds as a negative control.*Drug-likeness model score of: A)*rutin*0.91 *; B*) *sennoside*0.71*; C)a-pinen* -1.45*; D) beta-caryophyllene*-1.74; *E) dehydroquercetin*1.00*; F) tofacitinib*0.15.

The above results found that the highest index of drug likeness was dehydroquercetin, rutin and sennoside A with 1.00 0.91 and 0.71 respectively. Sulfasalazine, quercetin, and tofacitinib substances with indicators of 0.69, 0.52 and 0.15. From this it can be seen that dihydroquercetin, sennoside A, and rutin, have high medicinal properties compared to synthetic drugs.

3.4 Molecular docking

The in Silicon molecular docking method is important in determining whether drugs can bind to enzymes involved in certain processes and protect the effect on them. Molecular docking is a program forthe prediction of the interaction of the protein with ligands molecules as Ligand-protein kilocalorie mole (kcal/mol) binding forces in accordance with transformations and free binding energies [11]. Molecular docking analysis creates virtual patterns of ligand-protein cooperation at the atomic scale, and the fact that it can be used in laboratory analyses in later stages applying traditional in vitro and in vivo analysis minimizes the period and expense of the drug detection process when creating a new drug [12].

A molecular docking forecast program was used in order to identify the peculiarities of the active substances extracted from the composition of food additives against intestinal inflammation and compare them with synthetic preparations. To do this, we studied the relationship of compounds such as sennoside A, β-caryophyllene, α-pinene, quercetin, dehydroquercetin, and rutin with synthetic drugs such as Mesalazine, sulfasalazine, and tofacitinib, which are inhibitors of lipoxygenase and Janus kinase used in intestinal inflammation.

Since the metabolic pathway of Janus kinase 3 (JK3) played an important role in causing inflammation, the development of JK3 inhibitors was carried out using molecular docking simulation of molecular dynamics and free-energy calculations to reveal selective binding mechanisms and find key structural features that apply to specific yak-3 inhibitors. Molecular docking analysis https://mcule.com/apps/1-click-docking / done online on the website. Crystal structure of the enzyme Janus kinase 3 for molecular docking PDB from code 1yvj (https://www.rcsb.org/) and Crystal structure of the enzyme arachidonat15-lipoxygenase PDB code 1lox was used (fig.2)

A) B)

Figure 2:*3D c*rystal structure of the enzymes: A) Janus kinase (PDB code 1yvj);B) arachidonat15-lipoxygenase (PDB code 1lox)

SMILE data of the chemical structure of sennoside A, α-pinene, β-caryophyllene, quercetin, dehydroquercetin, rutin, mesalazine, sulfosalazine and tofacitinib substances

https://pubchem.ncbi.nlm.nih.gov / retrieved from the website. The docking results are presented in Table 3.

As can be seen from the table below that relation to synthetic drugs as an inhibitor of Janus kinase, rutin is superior to sulfasalazine and tofacitinib drugs, which are synthetic with indicators of -9.9 (figure 3) and dehydroquercetin -8.4 kcal/mol.

Table 3: The energy of free binding of substances with enzymes

Name of substances	Janus kinase kcal/mol	Name of substances	Arachidonate lipoxygenase kcal/mol
Sulfasalazine	-8.9	Sulfasalazine	-6.6
Mesalazine	-5.2	Mesalazine	-5.8
Tofacitinib	-8.2	Tofacitinib	-5.5
Rutin	-9.9	Rutin	-5.7
Quercetin	-8.1	Quercetin	-6.5
dehydroquercetin	-8.4	dehydroquercetin	-8.2
α-pinen	-4.9	α-pinen	-5.2
β-caryophyllene	-6.9	β-caryophyllene	-6.3
Sennoside A	-6.7	Sennoside A	3.6

It has been clearly demonstrated that rutin has the peculiarity of taking a unit with the of the active site's amino acids of the enzyme Janus kinase in accordance with the above results (fig.4). β-caryophyllene and sennoside Awere found to be superior to the mesalazine drug with α -6.9 and -6.7 kcal/mol binding energies.

From the results of the analysis, it is obvious that rutin is considered the best Janus kinase inhibitor compared to sulfasalazine, and dehydroquercetin is considered superior compared to tofacitinib, while the other biologically active components have shown more activity compared to mesalazine

A) B)

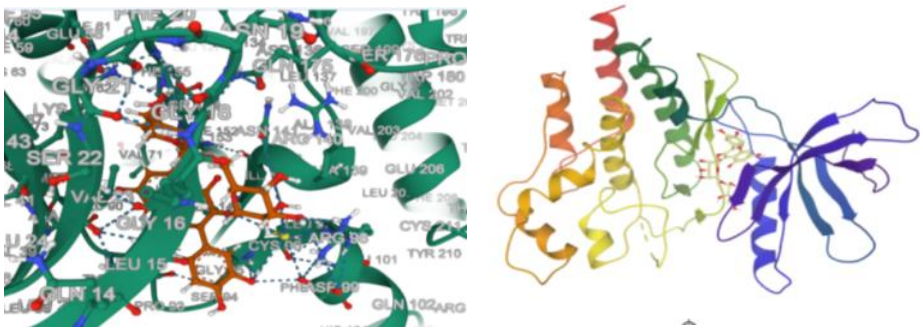
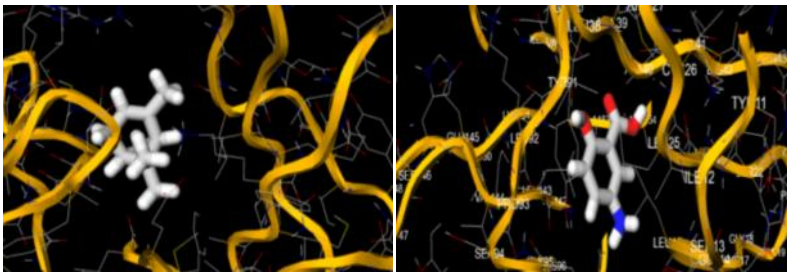


Figure 3: 3D interaction of ligand and protein: A)rutin andJanus kinase (JK) complex; B) 3D structure of JK.

A) B)

C) *D)*



E) F) G)

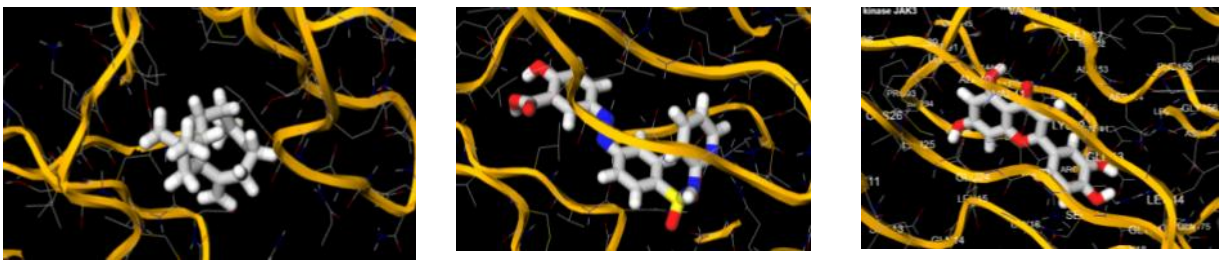


***Figure* 4:** Enzyme and ligand complex: A)tofacitinib with lipoxygenase; B) tofacitinib and Janus kinase; C) α-pinene with JK;D) mesalazine with JK; E)β-caryophyllene with JK; F) sulfasalazine with JK and G) Quercetin with JK.

Being a drug with the highest binding energy with the enzyme lipoxygenase was dehydroquercetin -8.2 kcal/mol then sulfasalazine, quercetin and β-caryophyllene with -6.6,-6.5, and -6.3 kcal/mol respectively.

It is obvious from the analysis data that dehydroquercetin has the highest activity with respect to lipoxygenase compared to all synthetic drugs whereas beta caryophyllene, quercetin and rutin were close to synthetic drugs. These studies clearly show that these biologically active compounds have the greatest activity against JK and LO enzymes which are involved in inflammatory diseases.

Conclusion

In conclusion, molecular complex interaction of these methods indicated that,the substances rutin, dehydroquercetin, quercetin, and beta caryophyllene, have the highest specific activity in binding with the enzymes lipoxygenase and Janus kinase active site. They could be potential harmless anti inflammation composition without side effect against IBD and can be used in the treatment of intestinal inflammations instead of synthetic drugs and that it is necessary to study them more deeply in the development of new natural anti-inflammatory drugs.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. Elson CO. Experimental models of intestinal inflammation: New insights into mechanisms of mucosal homeostasis In: Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR, eds. *Mucosal Immunology*, 2nd ed San Diego, CA: Academic Press; 1999:1007–1033. [Google Scholar]
2. German AJ, Hall EJ, Day MJ. Chronic intestinal inflammation and intestinal disease in dogs. *J Vet Intern Med* 2003;17:8–20. [PubMed] [Google Scholar]

3. Mizoguchi A, Mizoguchi E, Bhan AK. Immune networks in animal models of inflammatory bowel disease. *Inflamm Bowel Dis* 2003;9:246–59. [PubMed] [Google Scholar]
4. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet.* (2017) 389:1756–70. doi: 10.1016/S0140-6736(16)32126-2
5. PubMed Abstract | CrossRef Full Text | Google Scholar
6. Funk CD. Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science* 2001;294:1871–1875. [PubMed] [Google Scholar]
7. Charlier C, Michaux C. Dual inhibition of cyclooxygenase- 2 (COX- 2) and 5- lipoxygenase (5- LOX) as a new strategy to provide safer non steroidal anti- inflammatory drugs. *Eur J Med Chem* 2003;38:645–659. [PubMed] [Google Scholar]
8. https://medlineplus.gov/druginfo/meds/a613025.html
9. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004 Dec;1(4):337-41. doi: 10.1016/j.ddtec.2004.11.007. PMID: 24981612.
10. Bickerton GR, Paolini GV, Besnard J, Muresan S, Hopkins AL. Quantifying the chemical beauty of drugs. *Nat Chem*. 2012;4(2):90-98. Published 2012 Jan 24. doi:10.1038/nchem.1243
11. Forli S, Huey R, Pique M E, Sanner M, Goodsell D S and Olson A J 2016 Computational prote+in-ligand docking and virtual drug screening with the AutoDock suite Nat. Protoc. 11 905–19 [14]
12. Meng, X Y, Zhang H X, Mezei M and Cui M 2011 Molecular docking: a powerful approach for structure-based drug discovery Curr. Comput. Aided. Drug. Des. 7 146–5