ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Virtual Screening Approach to Identify Potential ALK Inhibitor from Traditional Chinese Medicine Database.

Anish Kumar and K Ramanathan *

Associate Professor, Industrial Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

ABSTRACT

Anaplastic Lymphoma Kinase has been in the spotlight since it was found to be a potential target for the treatment of Non-Small Cell Lung Cancer and as a landmark discovery till recent days, the most promising drug considered against it was crizotinib. Since the identification of certain mutations in ALK gene, there has been a reported decrease in the therapeutic efficacy of crizotinib due to the most common noted reason of drug resistance. Thus, it has been common strive among scientists to find a drug more potent and therapeutically effective than Crizotinib, while at the same time convincing to pose the least chances of having a side-effect. In this study, a novel class of lead molecule was identified from the traditional Chinese medicine database using virtual screening approach. All collected the compounds from TCM Database on the basis of structural similarity and checked their ADME properties using the Lipinski's rule of five. Following this, the toxicity of the screened compounds was analyzed. Finally the short listed compounds were employed under molecular docking study. Thus, the obtained results indicate that Graveoline can become a promising lead compound against NSCLC and prove effective against the treatment of crizotinib resistant ALK mutations.

Keywords: Crizotinib resistance, Traditional Chinese medicine database (TCMD), Virtual screening, Molecular docking.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Cancer is one of the most devastating diseases in the world and only a modest improvement has been seen in the period of the last 5 years with the help of 'new and improved' therapies [1]. It has been seen one out of every four deaths are resulted from cancer, and most of them who are diagnosed with the disease manage to carry on for further five years [2]. The statistics have proven that among all the cancers, lung cancer ranks second in the incidence and first in mortality [3]. The prevalence of lung cancer is second only to that of prostate cancer in men and breast cancer in women [4]. It is a global problem and the incidence of lung cancer is increasing at 0.5% every year. But the prognosis of lung cancer is very poor and nearly 80% of patients die within 1 year of diagnosis [5]. There are 2 main types of lung cancer: small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC makes up to 85% of all lung cancer [6]. Currently there is no effective method for screening NSCLC and most patients are diagnosed at an advanced stage, hence surgery is not an option for the majority of the patients. Hence platinum-based chemotherapy was used as the disease management tool in the first line setting. But the prognosis remained poor [7]. But with the growing knowledge of molecular oncology, several therapeutic targets have been identified and this made personalized medicine come into the limelight, which targeted tumor specific protein or gene mutations [8]. These drugs have a significant single-agent activity and increase progression free survival rate [9].

In 2007, a fusion gene was identified in some of the NSCLC patients, between echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene that had a transforming activity and lead to the promotion of cellular growth and inhibition of apoptosis [10]. This ALK fusion usually takes place at ALK exon 20 and is also known as oncogenic driver in tumors [4]. Later it was found that almost 3%-5% of NSCLC patients have tumors that are positive for ALK fusion gene [6] and is more common in patients who have never smoked or are light smokers and also in the younger patients (<65 years) [4]. Hence this discovery lead to the invention of the drug Crizotinib, which is an ALK inhibitor and was recently, approved by FDA for the treatment of patients with locally advanced or metastatic NSCLC that is ALK-positive, only after 4 years of clinical experiments [6]. The high response ratio made crizotinib as one of the most successful drugs for the treatment of lung cancer [11]. But eventually cancer cells developed resistance to this drug. This happened as the cancer cells gradually developed mutation in them against the drug, thus making the drug ineffective against the disease. Some of the invivo resistance mutations in ALK are L1196M, C1156Y, F1174L, L1152R, G1269A, G1202R (resistance mutation), S1206Y (resistance mutation) and 1151Tins (an insertion mutation).

All these mutations make the drug ineffective via different mechanisms. L1196M and G1269A mutations occur at the ATP-binding pocket of ALK, hence hindering the binding of crizotinib to the ALK domain due to the substitution of a smaller amino acid by a larger one. F1174L mutation enhances the affinity of ATP for the mutated ALK. The insertion mutation 1151Tins disrupts the H-bond between T1151 and E1129 thus changing the affinity of ATP for ALK. G1202R and S1206Y mutations have bulky residues that affect the affinity of crizotinib for ALK. The mutations L1152R and C1156Y occur at a distant location from crizotinib-binding domain but do confer resistance to crizotinib. The mutation L1152R also modify the activity of the ALK tyrosine kinase, thus making it more easily been activated while the mutation C1156Y triggers a conformation change in the P-loop, Beta sheet and alpha C-helix [11]. Hence, the main aim of the present study is to screen a virtual library of crizotinib analogues and successfully identify them which are more effective against the L1196M mutation and show lesser side effects, with the help of Traditional Chinese Medicine Database (TCMD). Traditional Chinese Medicine (TCM) plays an important role in medical diagnostics and treatments in Eastern Asia since thousands of years and with the help of proper understanding and systematic investigations, it has gained recognition even in the western society. The data and compounds in TCM database are mostly derived from natural herbs, minerals and animal products and are found to be effective against cancer, viral infections and inflammations. Hence, in this work molecular docking studies have been applied to screen a potent molecule from TCM against the listed mutations by virtual screening and using crizotinib as a query. Thus, certain compounds have been proposed in the following article which has proven to be effective against the lung cancer with the least side-effects.

ISSN: 0975-8585

MATERIALS AND METHODS

Datasets

A single native and a mutant (L1196M) of Lung Cancer were selected from RCSB Protein Data Bank. Their corresponding PDB codes were 3L9P and 2YFX respectively [12]. The respective resolutions of the structures were 1.80 Å and 1.70 Å, which were solved experimentally by X-Ray Diffraction method and had 367 (only the catalytic domain) residues, while mutant had 327 residues complex with crizotinib, which were made free from crizotinib during docking by manually removing the VGH atoms. To avoid error in the results hetero-atoms were also removed from the mutant structures. The query molecule used in the experiment was crizotinib. The SMILES strings were collected from PubChem, a database maintained in NCBI [13] and submitted to Molinspiration program for the calculation of molecular properties and prediction of bioactivity of the molecules.

Virtual Screening

Virtual screening [15] (VS) is a computational technique analogous to biological screening, which is used in drug discovery, whose main purpose is to identify those structures, molecules or compounds which are most likely to bind to a drug target, typically a protein receptor or enzyme in pharmaceutical research. It automatically evaluates very large libraries of compounds using computer programs. It focuses largely on how to reduce the enormous chemical space of over 1060 conceivable compounds to a manageable number that can be synthesized and tested in the laboratory [16]. We have obtained the canonical SMILES of crizotinib from PubChem database and submitted it in the TCM database by using advanced search and by choosing the similarity option in order to get the structures similar to crizotinib [17].

ADME

ADME is an abbreviation in pharmacokinetics for Adsorption, Distribution, Metabolism and Excretion and is an important criterion to test the drug-likeliness of ligands. This criterion is applied using molecular properties such as membrane permeability and bioavailability associated with some basic molecular descriptors such as LogP (partition coefficient), molecular weight (MW) and number of hydrogen bonds acceptors and donors in a molecule [18]. These molecular properties are used in formulating “Lipinski’s rule of five”. The rule states that most molecules with good membrane permeability have Molecular Weight ≤ 500 amu, calculated octanol–water partition coefficient, Q log P(oct/wat) ≤ 5, hydrogen bond donors ≤5 and acceptors ≤ 10 [19]. An orally active compound/drug should have no more than one violation of these rules. An orally active compound/drug should have no more than one violation of these rules. All the test compounds with the violation of ‘0’ passed the Lipinski screening test. Poor absorption of permeation is more likely when a ligand molecule violates Lipinski’s rule of 5 [20]. In the present study, these molecular properties for all the lead compounds were estimated by using MOLINSPIRATION program [14].

Toxicity

To successfully discover a drug candidate, such lead structures are required that are not only of superior quality in their properties but also possess some significant traits that increase their likelihood of being converted into a drug substance [21]. And to achieve this, features like toxicity and poor pharmacokinetics should be eliminated in the early stages of drug discovery. Hence, the hits were further screened using drug likeliness, drug score and toxicity characteristics. These physico-chemical properties were therefore calculated for the filtered set of hits using the OSIRIS programs [22]. The OSIRIS program calculates the drug likeliness based on a list of about 5,300 distinct substructure fragments created by 3,300 traded drugs as well as 15,000 commercially available chemicals yielding a complete list of all available fragments with associated drug likeliness. The drug score combines drug-likeliness, cLogP, logS, molecular weight, and toxicity risks as a total value which may be used to judge the compound’s overall potential to qualify for a drug.

Computation of Docking Energy

The lead compounds obtained from the virtual screening analysis were used in the docking calculation. Efficient docking was performed using algorithm PatchDock [23]. PatchDock is a geometry-based

molecular docking algorithm. Its main aim is to find those docking transformations that yield good molecular shape complementarity and when such transformations are applied it induces both wide interface areas and small amounts of steric clashes. The PatchDock algorithm divides the Connolly dot surface representation [24] [25] of the molecules into concave, convex and flat patches. Then, complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by a scoring function that considers both geometric fit and atomic desolvation energy [26]. Finally, an RMSD (root mean square deviation) clustering is applied to the candidate solutions to discard redundant solutions. The 3D coordinates of native and mutant were submitted in PDB format with default parameters.

RESULTS AND DISCUSSION

Virtual screening and Bioactivity analysis

A total of 14 lead compounds was identified on the basis of structural similarity from the TCMD when the current most promising drug i.e. crizotinib was used as a template. Most of the compounds that fail during clinical trials are due to the reasons of poor pharmacokinetics and toxicity issues. Hence, to give better and favorable results, the drugs are first evaluated on the basis of their characteristics to show the property of drug-likeliness, which is done individually and in the absence of the target. The molecular properties and bioactivity for the compounds was predicted using Molinspiration program (www.molinspiration.com). The molecular properties that were unveiled via Molinspiration were Log P (also known as octanol/water partition coefficient) [27] [28] which are used to measure the molecular hydrophobicity of the compound, as hydrophobicity affects the drug absorption and bioavailability of the molecules [29], along with Molecular Polar Surface Area, number of atoms, molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, volume, number of rotatable bonds and number of violations. The results showed that 7 molecules have zero violations of the Rule of 5 which suggest that these molecules likely to have good bioavailability (Table 1). Since the canonical SMILES for Salsilinol is not available, hence the test cannot be performed for that particular compound. Thus, the subsequent analysis was performed with the help of these screened molecules.

Table 1: **Calculations of molecular properties of crizotinib and lead compound using Molinspiration.**

Compound	miLogP	TPSA	Number of atoms	Molecular weight	Number of Hydrogen Bond Acceptors	Number of Hydrogen Bond Donors	Number of violations	Number of rotatable bonds	Volume
Crizotinib	**4.006**	**78.002**	**30.0**	**450.345**	**6**	**3**	**0**	**5**	**375.175**
Salsilinol	**1.056**	**52.483**	**13.0**	**179.219**	**3**	**3**	**0**	**0**	**168.632**
Oxyberberine	**3.314**	**58.941**	**26.0**	**351.358**	**6**	**0**	**0**	**2**	**301.577**
1,7-Diphenylhept-4-en-3-one	**4.645**	**17.071**	**20.0**	**264.368**	**1**	**0**	**0**	**7**	**269.06**
Kupitengester 3	4.509	140.759	41.0	574.623	11	0	2	12	517.153
Nicotine	**1.091**	**16.13**	**12.0**	**162.236**	**2**	**0**	**0**	**1**	**165.623**
Glycyrrhizic acid	1.967	267.044	58.0	822.942	16	8	3	7	741.927
Benzyl acetate	**1.979**	**26.305**	**11.0**	**150.177**	**2**	**0**	**0**	**3**	**145.375**
3,4-Benzopyrene	6.011	0.0	20.0	252.316	0	0	1	0	232.594
Kaempferol	**2.172**	**111.123**	**21.0**	**286.239**	**6**	**4**	**0**	**1**	**232.067**
Graveoline	**2.503**	**40.473**	**21.0**	**279.295**	**4**	**0**	**0**	**1**	**244.285**
Delta-Guaiene	5.071	0.0	15.0	204.357	0	0	1	1	230.274
1,6-Dimethyl-cis-cyclohexane	**3.218**	**0.0**	**8.0**	**112.216**	**0**	**0**	**0**	**0**	**135.778**
Arctiin	0.207	153.387	38.0	534.558	11	4	2	10	473.416

Bold indicates ADME screened compounds based on Lipinski rule of 5

ISSN: 0975-8585

Toxicity and physicochemical properties

Since the main cause behind the failure of the compound to become a potent drug is the issues related to pharmacokinetics and toxicity, hence these two reasons have become important evaluation parameters in today's drug discovery program and in our paper we have evaluated these two parameters using OSIRIS program. The pharmacokinetic property of a lead compound can be analyzed using variables cLogP and logS. cLogP is a well-established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high log P values may cause poor absorption or permeation. It has been shown for compounds have a reasonable probability of being well absorb their logP value must not be greater than 5.0. On this basis, Graveoline have log P value in the acceptable criteria [30].

Table 3: Molecular Docking Results predicted using PatchDock and further refined using FireDock Solution.

Docked Complexes	Binding Energy by FireDock Solution [kcal/mol]
Native (3L9P)- Crizotinib	-32.97
Mutant (2YFX))- Crizotinib	-13.70
Native (3L9P)- Salsilinol	-19.23
Mutant (2YFX))- Salsilinol	-16.26
Native (3L9P)- Graveoline	**-33.99**
Mutant (2YFX)- Graveoline	**-26.86**
Native (3L9P)- Oxyberberine	-24.95
Mutant(2YFX))- Oxyberberine	37.79
Native (3L9P)- Arctiin	-39.72
Mutant (2YFX))- Arctiin	-31.39
Native (3L9P)- 1,3-Diphenylpropane-1,2-diol-3-one	-29.97
Mutant (2YFX)- 1,3-Diphenylpropane-1,2-diol-3-one	-28.65
Native (3L9P)- 1,6-Dimethyl-cis-cyclohexane	-12.28
Mutant (2YFX)- 1,6-Dimethyl-cis-cyclohexane	-11.85
Native (3L9P)- 1,7-Diphenylhept-4-en-3-one	-35.89
Mutant (2YFX)- 1,7-Diphenylhept-4-en-3-one	-33.96
Native (3L9P)- Nicotine	-19.93
Mutant (2YFX)- Nicotine	-15.89
Native (3L9P)- Kaempferol	-29.08
Mutant (2YFX)- Kaempferol	-32.02
Native (3L9P)- Kupitengester 3	-37.41
Mutant (2YFX)- Kupitengester 3	-21.42
Native (3L9P)- Glycyrrhizic acid	-49.87
Mutant (2YFX)- Glycyrrhizic acid	-29.64
Native (3L9P)- Delta-Guaiene	-18.58
Mutant (2YFX)- Delta-Guaiene	-37.09
Native (3L9P)-Benzyl acetate	-17.74
Mutant (2YFX)- Benzyl acetate	-10.59
Native (3L9P)- 3,4-Benzopyrene	-31.47
Mutant(2YFX)- 3,4-Benzopyrene	-27.19

Bold indicates PatchDock and FireDock screened compound.

Drug solubility is typically affects the absorption and distribution characteristics of a compound and it has been proven that low solubility goes along with bad absorption [19]. Our estimated log S value is a unit stripped logarithm (base 10) of a compound's solubility measured in mol/liter. There are more than 80% of the drugs on the market have an (estimated) log S value greater than −4. Table 3 shows solubility of Crizotinib and other virtual compounds. It is clear from the table that the solubility of Graveoline was found in the comparable zone with that of standard drugs to fulfill the requirements of solubility and could be considered as a candidate drug for oral absorption.

ISSN: 0975-8585

Drug likeness

Yet one of the other qualitative concepts used in drug design is drug likeliness, and it is so because it is this criterion that confirms a molecule to be a drug if it shows favorable results to the tests conducted on absorption, distribution, metabolism, excretion, toxicological (ADMET) parameters. Currently, there are many approaches to assess a compound drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness structure keys or other properties such as clog P and molecular weight [31]. In this work, Osiris program was used for calculating the fragment-based drug-likeness of crizotinib and other virtually screened compounds [30]. The drug likeness value of Nicotine, Oxyberberine and Graveoline were found to be in acceptable criteria (Table 2).

Toxicity

The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified that are mutagenic, tumorigenic, irritant and reproductive effect [30]. From the data evaluated in Table 2 it is indicated that Salsilinol, Graveoline, Arctiin, Delta-Guaiene,1,7-Diphenylhept-4-en-3-one and Glycyrrhizic acid are non-mutagenic, non-irritating with no tumorigenic effects or adverse reproductive effect when run through the mutagenicity assessment system compared with standard drug used.

Drug score

We have also examined the overall drug score (DS) for all the compounds and compared with that of Crizotinib. The drug score combines drug likeness, miLogP, log S, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug [30]. The result is shown in Table 2. Salsilinol, Graveoline and Arctiin showed good DS as compared with other lead molecules and standard drug used. The toxicity, drug-likeness and drug-score results for the Graveoline were illustrated in Figure 1.

Molecular docking studies

The main aim of molecular docking study after testing the toxicity of the screened compounds is to find such a molecule that that shows a good binding affinity towards the target protein or molecule as well as forms a stable complex. And thus, the strength of association between the two molecules can be predicted using scoring functions. The higher the score the more stable the complex. Hence molecular docking can be considered as an optimization problem that describes which ligand which shows the 'best fit' orientation towards the target protein. Here, we used Patch-Dock, a very efficient algorithm for protein–ligand docking for analysis. The PDB format of the two molecules and the receptor binding sites were uploaded into the server. The FireDock solution 1 of native-crizotinib complex is -32.97 and of mutant-crizotinib complex is -13.70, while the FireDock solution 1 of native-graveoline complex is -46.63 and of mutant-graveoline complex is -26.86. It was interesting to note that the docking score of native structure is higher than the mutant structure. Since the results showed a higher FireDock score for Graveoline, hence it can be interpreted that Graveoline has a better binding affinity for both the native and the mutant protein as compared to the crizotinib.

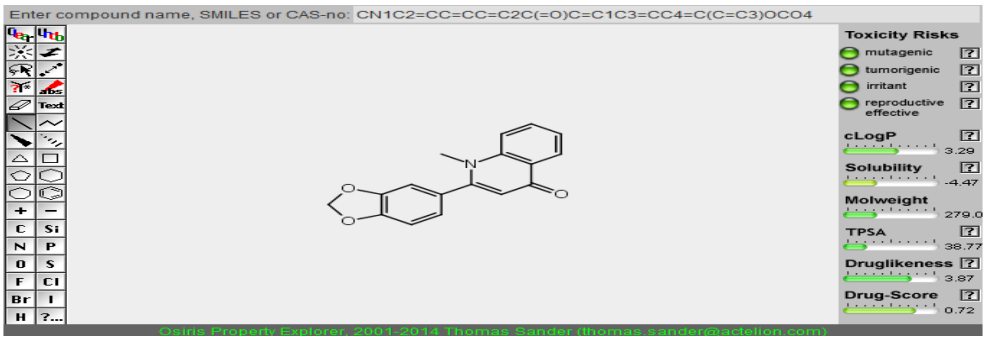

Figure 1: Osiris property explorer showing drug likeness properties of Graveoline.

ISSN: 0975-8585

Table 2: Toxicity risks and physicochemical properties of crizotinib and virtual compounds predicted by OSIRIS property explorer.

Compound	Mutagenic	Tumorigenic	Irritant	Reproductive Effective	cLogP	Solubility	Molecular Weight	TPSA	Drug Likeness	Drug Score
Crizotinib	No Risk	No Risk	No Risk	No Risk	3.54	-5.26	449.0	77.99	3.12	0.52
Salsilinol	High Risk	No Risk	No Risk	No Risk	1.27	-1.32	179.0	52.49	1.93	0.54
Oxyberberine	No Risk	No Risk	No Risk	High Risk	3.57	-4.38	351.0	57.23	4.75	0.41
1,7-Diphenylhept-4-en-3-one	No Risk	No Risk	No Risk	No Risk	4.83	-4.03	264.0	17.07	-4.55	0.33
Kupitengester 3	Medium Risk	No Risk	No Risk	Medium Risk	2.9	-4.72	574.0	140.7	-1.44	0.18
Nicotine	High Risk	No Risk	No Risk	High Risk	1.17	-0.79	162.0	16.13	5.07	0.35
Glycyrrhizic acid	No Risk	No Risk	No Risk	No Risk	0.98	-5.14	822.0	267.0	-4.29	0.19
Benzyl acetate	High Risk	High Risk	High Risk	No Risk	1.55	-1.91	150.0	26.3	-10.64	0.1
3,4-Benzopyrene	High Risk	High Risk	High Risk	High Risk	5.88	-7.68	252.0	0.0	-2.33	0.02
Kaempferol	High Risk	No Risk	No Risk	No Risk	1.84	-2.79	286.0	107.2	0.9	0.46
Graveoline	**No Risk**	**No Risk**	**No Risk**	**No Risk**	**3.29**	**-4.47**	**279.0**	**38.77**	**3.87**	**0.72**
Delta-Guaiene	No Risk	No Risk	No Risk	No Risk	4.73	-3.61	204	0.0	-20.56	0.35
1,6-Dimethyl-cis-cyclohexane	No Risk	No Risk	Medium Risk	No Risk	2.59	-2.47	112.0	0.0	-7.86	0.37
Arctiin	**No Risk**	**No Risk**	**No Risk**	**No Risk**	**0.86**	**-3.21**	**534.0**	**153.3**	**0.61**	**0.53**

Bold indicates OSIRIS screened compounds based on Toxicity and Drug Score.

From the results obtained via screening and docking studies, it could easily be interpreted that Graveoline is the most suitable compound that could be further taken into consideration to be used as a drug against ALK mutation in NSCLC. Our research is also supported via theoretical evidence obtained for Graveoline against cancer. It has been proved that Graveoline can be used for the treatment of skin cancer as it induces both apoptotic and autophagic cell death in the skin cancer cells [32]. Graveoline has also been found to have cytotoxic activity HeLa cancerous cells [33].

CONCLUSION

Using TCM Database, we were successful in screening an effective compound Graveoline as a potent inhibitor of the ALK mutation in NSCLC. Graveoline was found to be more drug like as it efficiently passed through the parameters of pharmacokinetics and toxicity. Also the docking study proved that, Graveoline has a higher binding affinity as compared to Crizotinib to the native as well as the mutations in ALK. Since Graveoline is obtained from the natural source, i.e. extracted from the plant Ruta Graveolens (whose extracts have proven to show cytotoxic activity), hence it is expected to have a lesser undesirable side effects as compared to the other synthetic drugs. Moreover, through literature study it has been found that Graveoline has shown positive results against skin cancer and HeLa cells via the process of apoptosis and autophagy. We believed that the findings reported here might provide useful clues for designing powerful drugs against drug resistant target of lung cancer.

ISSN: 0975-8585

ACKNOWLEDGMENT

The authors express a deep sense of gratitude to the management of Vellore Institute of Technology for all the support, assistance and constant encouragements to carry out this work.

REFERENCES

[1] Sarah Shigdar, et al. Cancer Lett 2014; 345: 271–278.
[2] Michael L, et al. Cancer Lett 2014; 344: 180–187.
[3] Asal Mohamadi Johnson, Robert B. Hines, James Allen Johnson III, A. Rana Bayakly. Lung Cancer 2014; 83: 401– 407.
[4] Solange Petersa, Miquel Taronb, Lukas Bubendorfc, Fiona Blackhalld, Rolf Stahele. Lung Cancer 2013; 81:145–154.
[5] Jozef Skarda1, Marian Hajdúch, Vítezslav Kolek. Cancer Ther 2008; 6: 377-388.
[6] Sam Abdelghany and Kejal Patel. Formulary J 2011.
[7] Schiller JH, Harrington D, Belani CP. N Engl J Med 2002; 346: 92–98.
[8] Mok TS. Nat Rev Clin Oncol 2011; 32.
[9] Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT. N Engl J Med 2009; 361: 947–957.
[10] Soda M, Choi YL, Enomoto M. Nature 2007; 448: 561–566.
[11] Huiyong Sun, Youyong Li, Dan Li, Tingjun Hou. J Chem Inf Model 2012; 53(9): 2376–2389.
[12] Berman HM,Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. Nucleic Acids Res 2000; 28: 235–242.
[13] Feldman J, Snyder KA, Ticoll A, Pintilie G, Hogue CW. FEBS Lett 2006; 580: 1649–1653.
[14] Molinspiration. http://www.molinspiration.com. 2008.
[15] Brian K. Stoichet. Nature 2004; 432: 862-865.
[16] Tondi D, Slomczynska U, Costi MP, Watterson DM, Ghelli S, Shoichet BK. Chem Biol. 1999; 6: 319–331.
[17] Chen CYC. PLoS One 2011; 6:e15939.
[18] Ertl P, Rohde B, Selzer P. J Med Chem 2000; 43: 3714–3717.
[19] Muegge I. Med Res Rev 2003; 23:302–321.
[20] Saravana Kumar N, Pradeep T, Jani G, Divya Silpa, Vijaya Kumar B. J Adv Pharm Technol Res 2012; 3: 57-61.
[21] Proudfoot. Bioorg Med Chem Lett 2002; 12: 1647–1650.
[22] http://www.organic-chemistry.org/prog/peo/. 2001.
[23] Schneidman D, Inbar Y, Nussinov R,Wolfson HJ. Nucleic Acids Res 2006; 33 : 363–367.
[24] Connolly ML. Science 1983; 221:709–713.
[25] Connolly ML. J Appl Crystallogr 1983; 16: 548–558.
[26] Zhang C, Vasmatzis G, Cornette JL, DeLisi C. J Mol Biol 1997; 267: 707–726.
[27] Remko M. J Mol Struct (THEOCHEM) 2009; 897: 73–82.
[28] Wang R, Fu Y, Lai L. J Chem Inf Comput Sci 1997; 37: 615–621.
[29] Clark DE. J Pharm Sci 1999; 88: 807–814.
[30] Vasudevan Karthick and Karuppasamy Ramanathan. Springer Plus 2013; 2: 115.
[31] Tetko. Drug Discovery Today 2005; 10: 1497–1500.
[32] Ghosh S, Bishayee K, Khuda-Bukhsh AR. Phytother Res 2014; 28(8): 1153–1162.
[33] Tian-Shung Wu, Li-Shian Shi, Jhi-Joung Wang, Song-Chou Iou, Hsien-Chang Chang, Yuh-Pan Chenc,Yao-Haur Kuo, Ya-Ling Chang and Che-Ming Teng. J Chinese Chem Soc 2003; 50: 171-178.
[34] Khalda Fadlalla, Angela Watson, Teshome Yehualaeshet, Timothy Trner, Temesgen Samuel. Anticancer Res 2011; 31: 233-242.