Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 12, 2015

Original Article

SYNTHESIS OF NOVEL CYCLOHEXANONE DERIVATIVES AS BCR-ABL T1351 INHIBITORS

SULAIMAN ALI MUHAMMAD, SUBASH SUGANYA, SUBBAN RAVI*, SENNIAPPAN VENKATACHALAPATHI

Department of Chemistry, Karpagam Academy of Higher Education, Coimbatore 641021 Tamilnadu, India
Email: ravisubban@rediffmail.com

Received: 11 Sep 2015 Revised and Accepted: 27 Oct 2015

ABSTRACT

Objective: Several 3(rd) generation inhibitors are being developed for the treatment of patients with Chronic myelogenous leukemia (CML). The present work mainly aims to discover novel small molecular inhibitors against important molecular target T3151 ABL mutant involved in leukemia.

Methods: Docking study was carried out and the binding affinity of the proteins with the phenothiazine compounds 3a-h and 7a-c was measured. The docking scores of the N-acylated compounds 7a-c are higher than 3a-h. The drug likeliness of these compounds was tested by the Lipinski's rule of five. The phenothiazine compounds with good docking scores and 7a-c were synthesized and screened by *in-vitro* methods for inducing antiproliferative effect by trypan blue and MTT assay and induction of apoptosis in K562 cells.

Results: All the N-acylated compounds and, in particular, 7c with a chloro substituent in the para position of the phenyl ring appeared to be most potent molecule with an IC_{50} value of 32.44 and 24.01(µg/ml) by trypan blue and MTT assay respectively. Further, a dose-dependent increase in LDH release was observed, confirming the antiproliferative potential of the compounds.

Conclusion: The compounds 7a-c was tested for antiproliferative effect against K562 cell lines by MTT assay LDH assay and Trypan blue assay. All the compounds 7a-h behaves as 3(rd) generation inhibitors for the treatment of patients with Chronic myelogenous leukemia (CML). These can act as a template for the further development and optimization studies.

Keywords: Synthesis, Phenothiazine, Cyclohexanone derivatives, Chronic myelogenous leukemia, Molecular docking, Anti-proliferative activity.

INTRODUCTION

In the post-genomic era, anti-cancer drug discovery aims to discover small molecules that modulate the activity of key therapeutic targets responsible for carcinogenesis. Computer-aided *design* [1-6] is being utilized to expedite and facilitate the lead molecule identification [7-12]. It reduces the size of chemical space and thereby allows to focus on more promising candidates for lead discovery and optimization [13-17].

Chronic myelogenous leukemia (CML) is a hematological stem cell disorder caused by increased and unregulated growth of myeloid cells in the bone marrow [18,19], and the accumulation of excessive white blood cells. Abelson tyrosine kinase (ABL) [20] is a non-receptor tyrosine kinase involved in cell growth and proliferation and is usually under tight control. However, it was reported that majority of CML patients have the ABL gene from chromosome 9 fused with the breakpoint cluster (BCR) gene from chromosome [21], resulting in a short chromosome known as the Philadelphia chromosome [22-25]. This Philadelphia chromosome is responsible for the production of BCR-ABL, a constitutively active tyrosine kinase that causes uncontrolled cellular proliferation [26, 27]. An ABL inhibitor, imatinib, is used at present as first line therapy. However, a high percentage of clinical relapse has been observed due to long-term treatment with imatinib [28]. A majority of these relapsed patients have several point mutations at and around the ATP binding pocket of the ABL kinase domain in BCR-ABL [29]. In order to address the resistance of mutated BCR-ABL to imatinib [30], 2(nd) generation inhibitors such as dasatinib, and nilotinib [31] were developed [32,33]. All of the BCR-ABL mutants are inhibited by the 2(nd) generation inhibitors with the exception of the T315I mutant. Several 3(rd) generation inhibitors are being developed to target the T315I mutation [34].

This prompted us to try some of the phenothiazine derivatives which are synthesized in our laboratory in order to address the resistance of mutated BCR-ABL to imatinib and 2(nd) generation inhibitors such as dasatinib, and nilotinib. If the present work is encouraging it is anticipated that additional drugs will be available for the treatment of patients with the mutated BCR-ABL-T315I. The success of these inhibitors will have greater implication not only in CML, but also in other diseases driven by kinases where the mutated gatekeeper residue plays a major role.

MATERIALS AND METHODS

Docking studies

Active site prediction

The catalytic sites of tyrosine kinase Receptor along with area and volume of binding pocket was carried out with Meta Pocket 2.0 Finder program [35]. In the binding pocket the Active site residues of T3151 ABL mutant are Ala350, Ala365, Ala366, Asn297, Arg483, Asp381, Asp421, Asp482, Cys305, Cys369, Gln477, Gly303, Gly372, Gly383, Gly426, Ile315, Ile347, Leu302, Leu364, Met458, Ser265, Thr267, Trp405, Tyr320 and Val299.

Preparation of the protein and the compounds

The three-dimensional crystal structure of the T3151 ABL mutant in complex with the aura kinase inhibitor was retrieved from the Protein Data Bank [36]. The complexes bound to the receptor molecule, all the heteroatoms, and the non-essential water molecules were removed and finally hydrogen atoms were merged to the target receptor molecule using Argus Lab [11]. The series of phenothiazine compounds synthesized in our laboratory 7a-h forms the database. The compound structures were drawn using ChemDraw software and converted to mol format with the standard settings and further used for docking studies.

Molecular docking

The molecular docking program Aurgus Lab 4.0 software which is most commonly available software was used to perform the virtual screening. The receptor was defined from the crystallographic coordinates of the ligand. The binding site atoms were further defined from a file listing the cavity atoms. Dockings were performed under 'Standard default settings' mode. All the parameters used in Argus lab docking were selected by default. Calculation type was set to "dock" mode and "flexible mode" was selected for the ligand. The docking results were analyzed using PYMOL (TM) software, which allows visualization of the protein-ligand docking and calculation of several descriptors such as feasible hydrogen bonding between the protein and the ligand. The scores were calculated and presented in the table 1. Least energy indicated the easy binding character of ligand and receptor.

Synthesis of the compounds 7a-h

Chalcones and their cyclohexonones were prepared as per the procedure we have already reported [37] and characterised. 0.001 mole of 2-acetyl phenothiazine 1 was dissolved in 25 ml methanol and 0. 001 moles of different substituted benzaldehydes 2a-h were added, followed by the addition of 5 ml aqueous solution of NaOH (5%) and heated for 6 h with constant stirring in a magnetic stirrer. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water, neutralized with con. HCl and left overnight in a refrigerator. The resulted precipitate was filtered, dried and the purity of the compounds was checked by TLC using chloroform as the solvent. The compounds were purified by column chromatography using silica gel (60-120 mesh).

Cell culture

K562 cell line purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37 °c in 5 % CO_2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025% Trypsin in PBS/0.5 mM EDTA solution (Himedia)) for 2 min and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at concentrations of 6.25 µg, 12.5µg,25 µg,50 µg and 100µg from a stock of 1 mg/ml 0.1% DMSO and incubated for 24 h. The % difference in viability was determined by standard MTT assay after 24 h of incubation [37].

Cell viability by Trypan blue exclusion assay

The effect of compound 7a-c on the viability of cancer cells (K562) was determined by trypan blue dye exclusion assay [38]. Briefly, the cells were plated at a density of $1x10^5$in six-well plates followed by addition of different concentrations of compound (6.25, 12.5, 50 and 100 µg in 1 ml of DMSO) or untreated cell. After incubation for every 24 h, cells were collected and diluted in an equal volume of media and mixed with 20 ml of trypan blue. Cells were counted under the microscope using hemocytometer. All the experiments were performed in triplicate and yielded similar results [38].

Trypan blue is a vital stain used to colour selectively dead tissues or cells blue. The live cells possess intact cell membranes that exclude trypan blue, whereas dead cells do not. So a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. A 1:1 dilution of the cell culture suspension with a 0.4% trypan blue solution (1;1 dilution in PBS) was charged on the counting chamber of a haemocytometer and counted at 40x (Olympus CH 20). Stained cells and total cells were counted to the percentage of viable cells was calculated. Control was also treated in the same manner fig. 2.

$$\% \text{ of viability} = [A/(A+B) \times 100$$

A = number of viable cells

A+B = total number of viable and dead cell

Cell proliferation by MTT assay

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product was measured at 570 nm. Since the reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells [38].

The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT-5 mg/ml dissolved in PBS). It was then incubated at 37 °C for 3 h. MTT was removed by washing with 1x PBS and 200 µlof DMSO was added to the culture. Incubation was done at room temperature for 30 min until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. Optical density was read at 570 nm using DMSO as blank in an ELISA reader (LISASCAN, Erba). All the experiments were performed in triplicate and yielded similar results fig. 3.

LDH release assay

The lactate dehydrogenase (LDH) release is an indicator of membrane integrity and thus cell injury [39, 40]. LDH assay was performed to evaluate the LDH release to the media following treatment with the 7a-h (6.25, 12.5, 25, 50 and 100 µg) on K562 for 24 h and it was measured using standard protocols. The intracellular LDH was determined after lysing the cells by rapid freezing and thawing in liquid nitrogen. The LDH release was measured at an absorbance of 490 nm. The percentage of LDH release was calculated as: (LDH activity in media)/(LDH activity in media þ intracellular LDH activity) X100%. Results are presented as a percentage of LDH release subtracting the control values from treated ones. All the experiments were performed in triplicate fig. 4.

RESULTS AND DISCUSSION

From medicinal chemistry perspective, phenothiazines are important groups of condensed three ring heterocycles. Phenothiazine derivatives and their hetero analogues containing 1, 4-thiazine structural fragment, show diverse biological activities. Phenothiazine derivatives that contain aminoalkyl substituent at the thiazine nitrogen atom used as antipsychotic and antihistamine drugs. In the present work to start with a series of phenothiazine derivatives 7a-h were selected.

K562 cell line protein structures were derived from PDB and used a target for docking simulation. To investigate if these compounds have a similar mechanism as BCR-ABL kinase inhibitors, docking was performed using Argus lab software. The crystal structure of protein T3151 mutated BCR-ABL kinase was refined from the crude PDB structure and then saved as mole file to be used for docking simulation. Compounds 7a-h was constructed on chemdraw 8.0. Structure and the 2D structure of the selected compounds were converted to their 3D from, and then energy minimized and saved as mol. The observed negative value for the docking score energy (table 2) indicate the binding affinity of these compounds into T3151 mutated BCR-ABL kinase, this may give a reasonABLe explanation for their high activity.

These detections of ligand-binding sites are often the starting point for protein function identification and drug discovery. In our study, Meta pocket active site finder predicted the active site of the K562 cell line protein. The active sites of K562 cell protein comprise of amino acids which are hydrophobic and become the main contributors to the receptor-ligand interaction.

In the binding pocket the Active site residues of T3151 ABL mutant are Ala350, Ala365, Ala366, Asn297, Arg483, Asp381, Asp421, Asp482, Cys305, Cys369, Gln477, Gly303, Gly372, Gly383, Gly426, Ile315, Ile347, Leu302, Leu364, Met458, Ser265, Thr267, Trp405, Tyr320 and Val299.

Energy analysis in table 1 demonstrated the lower binding energy of inhibitors 7a-h with T3151 mutated BCR-ABL kinase under the participation of Van der Walls force, hydrogen bonds and hydrophobic interaction during the process of enzyme-inhibitor interaction. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. The binding affinity of the K562 cell line protein with the compounds 7a-h was measured by kcal/mol.

The fig. 1 shows the hydrophilic and hydrophobic interactions of the ligands with the protein. From the table 1 it was learned that compounds 7a, 7b and 7c with H, chloro and bromo substituent respectively exhibited a reasonably good scores-12.716, 12.3456 and 14.665.0186 k cal/mol. These three compounds were selected for further *in-vitro* studies.

Table 1: Docking scores, of the 7a-h ligands with tyrosine kinase inhibitors

S. No.	Compound	Score
1	7a	-12.751
2	7b	-12.363
3	7c	-14.663
4	7d	-12.469
5	7e	-11.454
6	7f	-11.236
7	7g	-12.690
8	7h	-12.536

All the cyclohexanone derivatives of phenothiazine chalcones (7a-h) showed good docking scores. The table 2 shows the docking scores, H-bonded interactions and the amino acids surrounding the ligand.

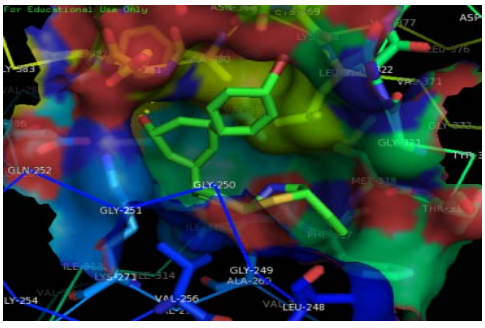
Table 2: Docking scores, H-bond interactions' and surroundings amino acids of the 7a-c ligands with tyrosine kinase inhibitors

Compound	Score	H-bond interaction	Surrounding amino acid
7a	-12.7515	No H-Bonding	GLU-466,459,462,GLY-463, 426, PRO-465,461, CYS-464, ARG-460, 473, LEU-429, MET-458,472, TYR-469
7b	-12.363	VAL-268	ALA-269,ASP-233,ILE-315, 314, LEU-301, 266, 302, GLN-300, GLU-236, GLY-303, VAL-268, 304, THR-267, TYR-264, SER-265,
7c	-14.6632	LYS-298, ALA-380	ALA-380, PHE-382, 317 LEU-298, 378, 372,VAL-299, GLY-250, 321, GLU-316, ILE-315, MET-318, THR-319

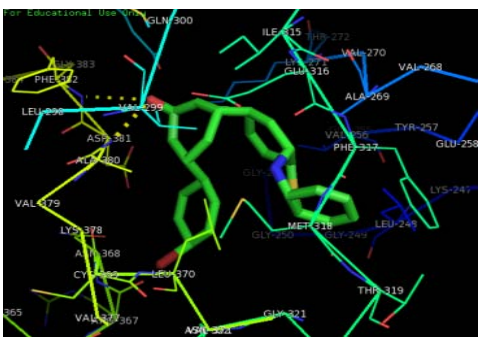
Table 3: Lipinski's rule of five parameters for 7a-h

Compound	Molecular weight	Molecular formula	H-bond donor	H-bonding acceptor	Rotatable bonds	Log p
7a	383.51	$C_{25}H_{21}NOS$	1	1	2	4.94
7b	382.5	$C_{25}H20ClNOS$	1	1	2	5.5
7c	462.4	$C_{25}H_{20}BrNOS$	1	1	2	5.77
7d	397.53	$C_{26}H_{23}NOS$	1	1	2	5.43
7e	411.52	$C_{26}H_{21}NO_2S$	1	2	2	4.69
7f	401.5	$C_{25}H_{20}FNOS$	1	2	2	5.1
7g	428.5	$C_{25}H_{20}N_2O_3S$	1	3	2	-
7h	428.57	$C_{27}H_{26}NO_2S$	1	1	2	4.81

To identify whether the above-tested compounds are drug like molecules the Lipinski's rule of five parameters like molecular weight, molecular formulae, a number of H-bond donor, Number of H-bond acceptors, Number of rotatable bonds and the Log P value were calculated for the compounds 7a-h and the results were shown in the table 3. All the parameters are well within the limits for 7a, 7b, 7e and 7h.

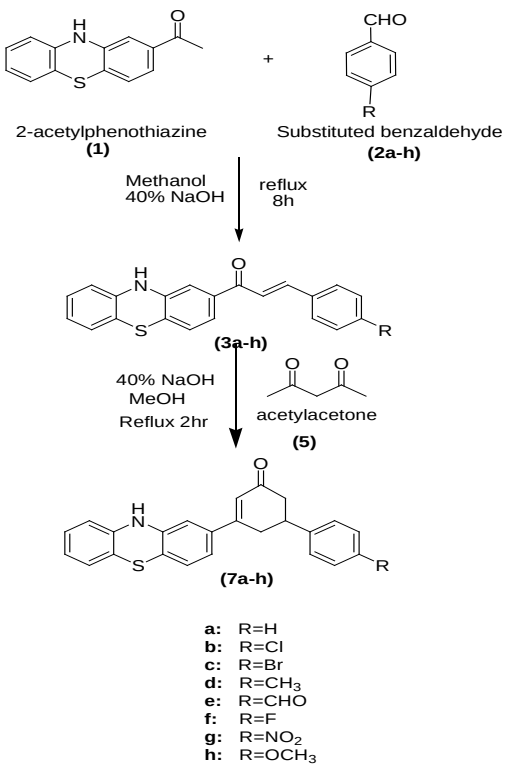


(A)



(B)

Fig. 1: Binding of 7b with 2V7A Protein (A) Ligand seated in the pocket of protein (B) Interaction and H-Bonding of ligand with protein



Scheme 1: **Synthesis route of Cyclohexane derivatives of Phenothiazine preparation from**

Synthesis of cyclohexane derivative of chalcone

The chalcones 3a-c was prepared by Claisen-Schmidt condensation (Scheme 1) of equimolar quantities of phenothiazine methyl ketone 1 with substituted aryl aldehydes 3a-c in the presence of alcoholic alkali. The analytical data, reaction conditions and the yield of the product 3a-c were optimized.

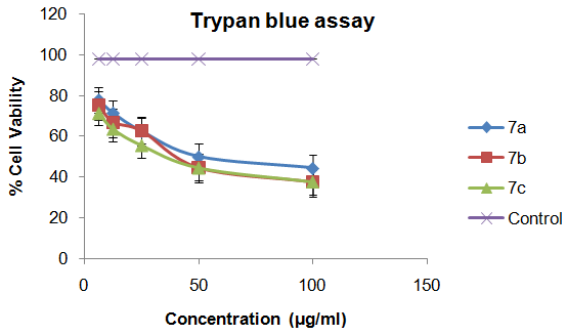


Fig. 2: Viability of K562 cell against 3a-c In Trypan Blue assay Sample size n=3. Values expressed as mean±SEM

Characterization of Cyclohexenones 7a-h

In the UV spectra, all the compounds 7a-h gives rise to the characteristic peak for chalcones at 412 nm and 290 nm respectively for band 1and 2. In the IR spectrum of 7a-h it revealed a strong, sharp band at 1632 cm-1 representing carbonyl group, 1600 cm-1 for C=C and at 3332 cm-1 for the NH stretching band. The addition of ethyl acetoacetate to chalcones 3a-h, leads to the generation of a chiral center in the 7a-h, In ^{1}H-NMR spectrum of compound 7a the pair of methylene protons H-4 and H-6 of cyclohexenone moiety were observed as multiplets at δ 2.83 and 3.37 respectively. The CH (H-5) proton of Cyclohexenone ring resonated as a multiplet at δ 2.62. H-2 carbon appears as singlet at δ 6.42. The protons present in the phenothiazine ring are explained as follows, H-1', 3', 4', 6', 8' appeared as the multiplet between δ 7.01-6.99. H-7' appeared as doublet at δ 6.70 with *J* value 8Hz. H-9' appeared as doublet at δ 6.64 with *J* value 8Hz. H-2'', 6'' appeared as doublet between δ 7.22-7.19 with *J*=8Hz. H-3'', 5'' appeared as multiplet between δ 6.84-6.81. The NH group appeared as a singlet at δ 8.57. The OCH_3 group substituted in the aromatic ring of 7a appeared as singlet at δ 3.81. In ^{13}C-NMR spectrum, C=O group present in the cyclohexenone ring appears at δ 199.30. C-2 appears at δ 124.49; C-3 appears at δ 157.00. Carbons C-4 at δ 36.41, C-5 at 40.21, C-6 at 44.28. All the carbons present in aromatic ring appears between δ 111.58 and 158.67. The 2D NMR spectral data also supports the proposed structures for the compounds. In ^{13}CNMR spectrum, the cyclohexanones showed 25 signals. In the DEPT-135 spectra of the compound, 5a showed only 16 signals. All the quaternary carbon signals at δ 137.66, 141.95, 124.49, 141.05, 120.45, 157.00, 40.21, 141.00, 199.30 disappeared. Further, it exhibits 13 CH signals, 2 (CH_2) methylene signals and one methyl signal.

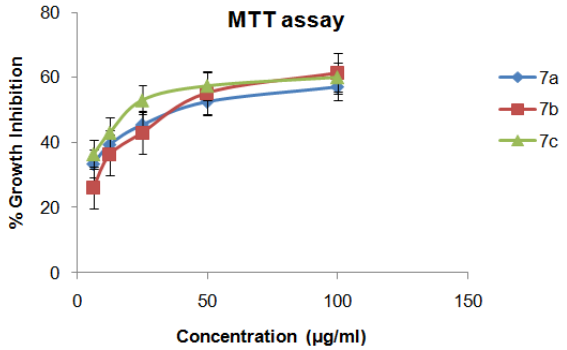


Fig. 3: MTT assay result against K562 cell line Sample size n=3. Values expressed as mean±SEM

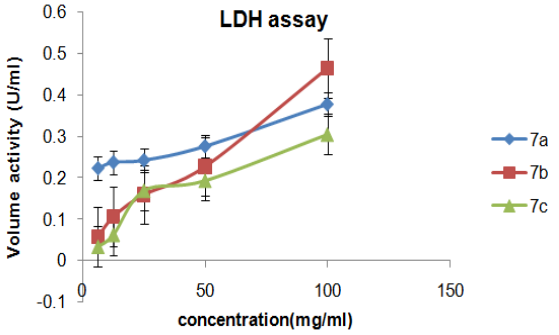


Fig. 4: Lactate dehydrogenase leakage assay Sample size n=3. Values expressed as mean±SEM

Table 3: IC_{50} Values In trypan Blue assay and MTT assay

S. No	Compound	IC_{50}(µg/ml)	
		Trypan blue assay	MTT assay
1	7a	60.02±0.37	41.01±0.43
2	7b	42.3±0.22	38.98±0.18
3	7c	36.06±0.32	36. 75±.0.26

Values are expressed as mean±SD (standard deviation), sample size n=3

CONCLUSION

Compounds 7a-h was docked with tyrosine kinase inhibitor and their scores determined. All the compounds exhibited good scores showing the effective interaction of this group of ligands with the target. Compounds 7a-c are found to be having excellent scores. Lipinski's rule of five has been applied on these compounds to determine the drug likeliness of these compounds. All the compounds have drug like properties. The compounds 7a-c was tested for antiproliferative effect against K562 cell lines by MTT assay LDH assay and Trypan blue assay. All the compounds 7a-h behaves as 3(rd) generation inhibitors for the treatment of patients with Chronic myelogenous leukemia (CML). These can act as a template for the further development and optimization studies.

ACKNOWLEDGMENT

We thank the Department of Science and Technology for the financial assistance (DST-SERB Ref: Sanction No. SR/S1/OC-28/2011). SAIF-STIC, Cochin for NMR spectral studies.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Feig M, Onufriev A, Lee MS, Im W, Case DA, Brooks CL. Performance comparison of generalized born and Poisson methods in the calculation of electrostatic solvation energies for protein structures. J Comb Chem 2004;25:265–84.

2. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, *et al*. Glide: a new approach for rapid, accurate docking and scoring. Method and assessment of docking accuracy. J Med Chem 2004;47:1739–49.
3. Goldman BB, Wipke WT. QSD quadratic shape descriptors. 2. Molecular docking using quadratic shape descriptors (QSDock). Proteins 2000;38:79–94.
4. Jorgensen WL. Rusting of the lock and key model for protein-ligand binding. Science 1991;254:954.
5. Kahraman A, Morris RJ, Laskowski RA, Thornton JM. Shape variation in protein binding pockets and their ligands. J Mol Biol 2007;368:283–301.
6. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discovery 2004;3:935–49.
7. Klebe G, Mietzner T. A fast and efficient method to generate biologically relevant conformations". J Comput Aided Mol Des 1994;8:583–606.
8. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr Opin Struct Biol 1996;6:402–6.
9. Longenecker KL, Stamper GF, Hajduk PJ, Fry EH, Jakob CG, Harlan JE, *et al*. Structure of MurF from streptococcus pneumoniae co-crystallized with a small molecule inhibitor exhibits interdomain closure. Protein Sci 2005;14:3039–47.
10. Meng EC, Shoichet BK, Kuntz ID. Automated docking with grid-based energy evaluation. J Comp Chem 2004;13:505–24.
11. Modungo M, Casale E, Soncini C, Rosettani P, Colombo R, Lupi R, *et al*. Crystal Structure of the T315I Abl Mutant in Complex with the Aurora Kinases Inhibitor PHA-739358. Cancer Res 2007;67:7987.
12. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, *et al*. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comp Chem 1998;19:1639–62.
13. Shoichet BK, Kuntz ID, Bodian DL. Molecular docking using shape descriptors. J Comp Chem 2004;13:380–97.
14. Sousa SF, Ribeiro AJ, Coimbra JTS, Neves RPP, Martins SA, Moorthy HNS, *et al*. Protein-ligand docking in the new millennium-A retrospective of 10 y in the field. Curr Med Chem 2013;20:2296–14.
15. Wang Q, Pang YP. Romesberg, Floyd. ed. Preference of small molecules for local minimum conformations when binding to proteins. PLoS One 2007;2:e820.
16. Wei BQ, Weaver LH, Ferrari AM, Matthews BW, Shoichet BK. Testing a flexible-receptor docking algorithm in a model binding site. J Mol Biol 2004;337:1161–82.
17. Zengming Z, Yu Li, Biaoyang Lin. Michael Schroeder and Bingding Huang, Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction. Bioinform 2011;27:2083-8.
18. An X, Tiwari A, Sun Y, Ding P, Ashby Jr C, Chen Z. BCR-ABL tyrosine kinase inhibitors in the treatment of Philadelphia chromosome-positive chronic myeloid leukemia: a review. Leuk Res 2010;34:1255–68.
19. Bixby D, Talpaz M. Mechanisms of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia and recent therapeutic strategies to overcome resistance. Hematology 2009;1:461-76.
20. Manley PW, Cowan-Jacob SW, Buchdunger E, Fabbro D, Fendrich G, Furet P, *et al*. Imatinib: a selective tyrosine kinase inhibitor. Eur J Cancer 2002:S19-27.
21. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 2002;1:117-23.
22. Druker BJ, Lydon NB. Lessons learned from the development of an ABL tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 2000;105:3–7.
23. Buchanan SG. Protein structure: discovering selective protein kinase inhibitors. Targets 2003;2:101-8.
24. Eck M, Manley P. The interplay of structural information and functional studies in kinase drug design: insights from BCR-ABL. Curr Opin Cell Biol 2009;21:288–95.
25. Mandal S, Moudgil M, Mandal S. Rational drug design. Eur J Pharm 2009;625:90–100.
26. Asaki T, Sugiyama Y, Hamamoto T, Higashioka M, Umehara M, Naito H, *et al*. Design and synthesis of 3-substituted benzamide derivatives as BCR-ABL kinase inhibitors. Bioorgn Med Chem Lett 2006;16:1421–5.
27. Manley P, Cowan-Jacob S, Mestan J. Advances in the structural biology, design and clinical development of BCR-ABL kinase inhibitors for the treatment of chronic myeloid leukaemia. Biochim Biophys Acta 1754;1–2:3–13.
28. Manley P, Stiefl N, Cowan-Jacob S, Kaufman S, Mestan J, Wartmann M, *et al*. "Structural resemblances and comparisons of the relative pharmacological properties of imatinib and nilotinib". Bioorg Med Chem 2010;18:6977–86.
29. Stein B, Smith BD. Treatment options for patients with chronic myeloid leukemia who are resistant to or unable to tolerate imatinib. Clin Ther 2010;32:804-20.
30. Gorre M, Mohammed M, Ellwood K, Hsu N, Paquette RP, Rao PN, *et al*. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876-80.
31. Thomas J, Wang L, Clark R, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. Blood 2004;104:3739–45.
32. Jabbour E, Cortes J, Kantarjian H. Nilotinib for the treatment of chronic myeloid leukemia: an evidence-based review. Core Evidence 2009;4:207-13.
33. Olivieri A, Manzione L. "Dasatinib: a new step in molecular target therapy". Annal oncol 2007;18 Suppl 6:42–6.
34. Breccia M, Alimena G. "Nilotinib: a second-generation tyrosine kinase inhibitor for chronic myeloid leukemia". Leuk Res 2010;34:129–34.
35. http://www.rscb.org/pdb for protein structure data base. [Last accessed on 11 oct 2015].
36. Meta Pocket 2.0 Finder program. Available from: http://metpocket.eml.org. [Last accessed on 11 oct 2015].
37. Saranya AV, Ravi S. Synthesis of 5-phenyl-3-(10H-phenothiazinyl)-Δ^2-cyclohexen-1-ones by conventional and microwave-assisted methods and their antifungal activity. Res Chem Int 2014;40:3085-93.
38. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
39. Shahabuddin MS, Nambiar M, Choudhary B, Advirao GM, Raghavan SC. A novel DNA intercalator, butylamino-pyrimido[4',5':4,5]selenolo(2,3-b)quinoline, induces cell cycle arrest and apoptosis in leukemic cells. Invest New Drugs 2009;28:35-48.
40. Korzeniewski C, Callewaert DM. An enzyme-release assay for natural cytotoxicity. J Immunol Methods 1983;64:313–20.