

**Abstract:** C-Src is involved in a number of signaling pathways that ultimately lead to angiogenesis. Recently derived data leads to deduction that the most important consequence of increased c-Src activity is promotion of an aggressive phenotype in multiple human tumors. Based on that information, inhibition of c-Src kinase is essential and might be considered a treatment of cancer. There are currently a number of drug molecules targeting c-Src which are in clinical trials. These include Bosutinib which has been shown to significantly reduce the renal growth in cystic *bpk* mouse models. Drug design can be accomplished with the aid of a number of computer software programs. These help in mapping the active site of the target molecule, which will then be used as a template for possible drug molecules. Minimum energy structures of these molecules can be obtained from programs like Spartan. However, experimental work to establish actual binding constants, solubility and toxicity of chosen drug molecules will have to be carried out.

C-Src kinase is a non receptor tyrosine kinase, encoded by the c-Src (cellular-Src) gene (Src pronounced "sarc", which is short for sarcoma, a cancer type which derives from changed connective tissue cells). C-Src protein acts as a signal transduction inhibitor that is a critical component of multiple signaling pathways that control cell growth, proliferation, invasion, and apoptosis [1, 2]. One of the signaling pathways that c-Src is involved in is the VEGF (Vascular Endothelial Growth Factor) pathway, shown below:

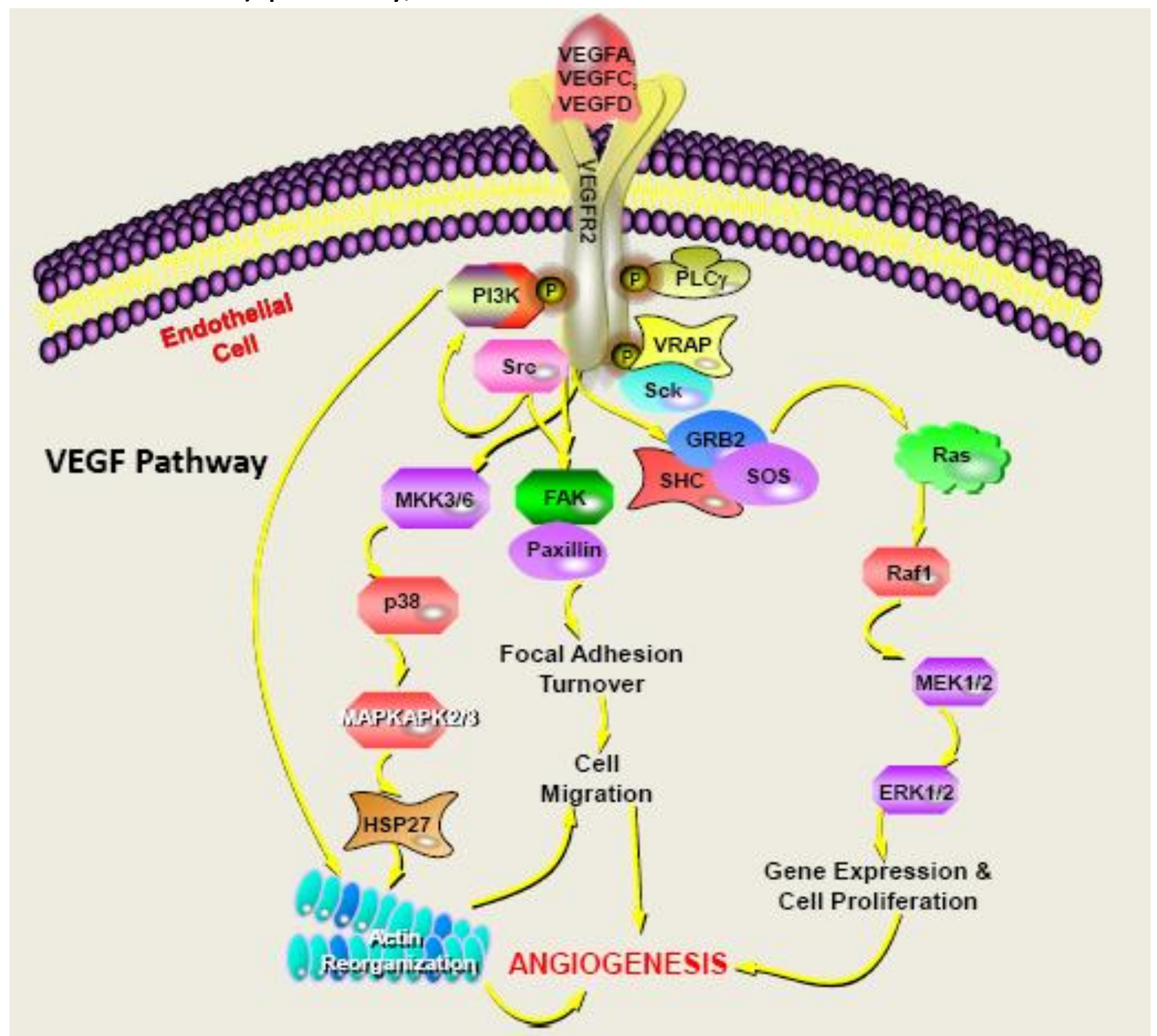


Figure 1: Involvement of c-Src in the VEGF pathway. Source: SABiosciences, ProteinLounge.com

Phosphorylation of proteins by kinases is important in signal transduction and regulation of other cellular activities such as cell division. In some cases, the protein kinase will mutate so the kinase is always active, causing the cell to grow uncontrollably [3]. Therefore, inhibition of c-Src can be used as a way of regulating cell growth, ultimately resulting in cancer treatment. Imatinib and Bosutinib are some examples of drug compounds that inhibit the autophosphorylation of c-Src, resulting in inhibition of cell growth and apoptosis [4].

As illustrated below, in the inactive form of c-Src, Tyr527 is phosphorylated and binds to the SH2 domain, Tyr416 is dephosphorylated, and the SH3 domain is engaged with the SH2 kinase linker. C-Src is activated by dephosphorylation of Tyr527 which leads to an "open" conformation, allowing autophosphorylation of Tyr416 and interaction of c-Src with substrates.

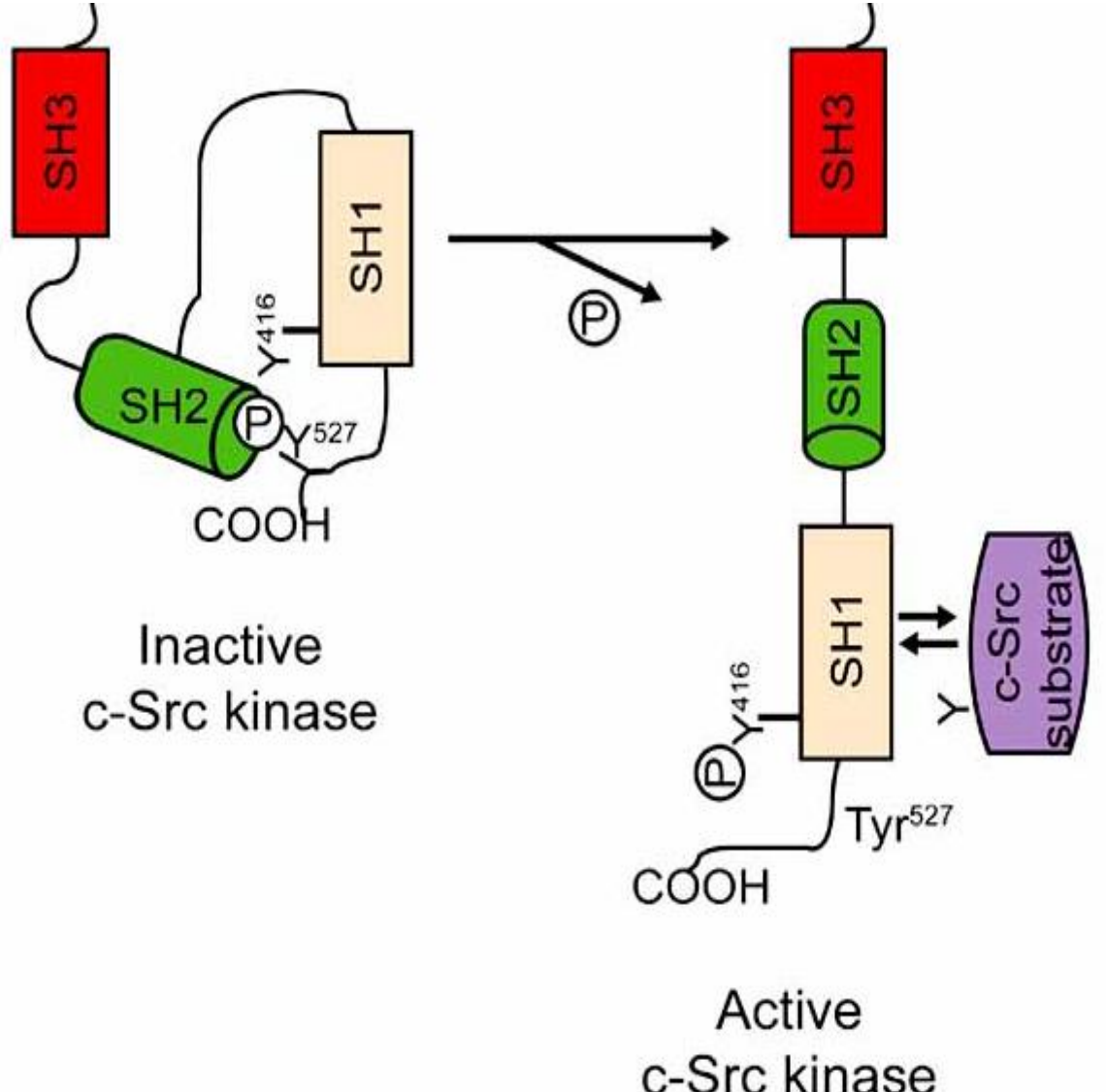


Table 1: Structures of Src inhibitors presently in clinical trials (From Ref 10)

Compound	Structure	Pharmaceutical Company
Saracatinib AZD0530		AstraZeneca
Dasatinib BMS-354825 Sprycel		Bristol Myers Squibb
Bosutinib SKI-606		Wyeth
KX01 KX2-391		Kinex

The inhibition of c-Src has been shown to result in considerable reduction of renal growth in cystic *bpk* mice. The *bpk* mouse model is one of several PKD (Polycystic Kidney Disease) mouse models genetically modified to have a desired phenotype. In this case *bpk* means BALB/c polycystic kidneys, which means that BALB/c, a laboratory-bred strain of house mouse, was transformed to get the polycystic kidney disease phenotype [5]. Figure 3 below illustrates some of the experimental results.

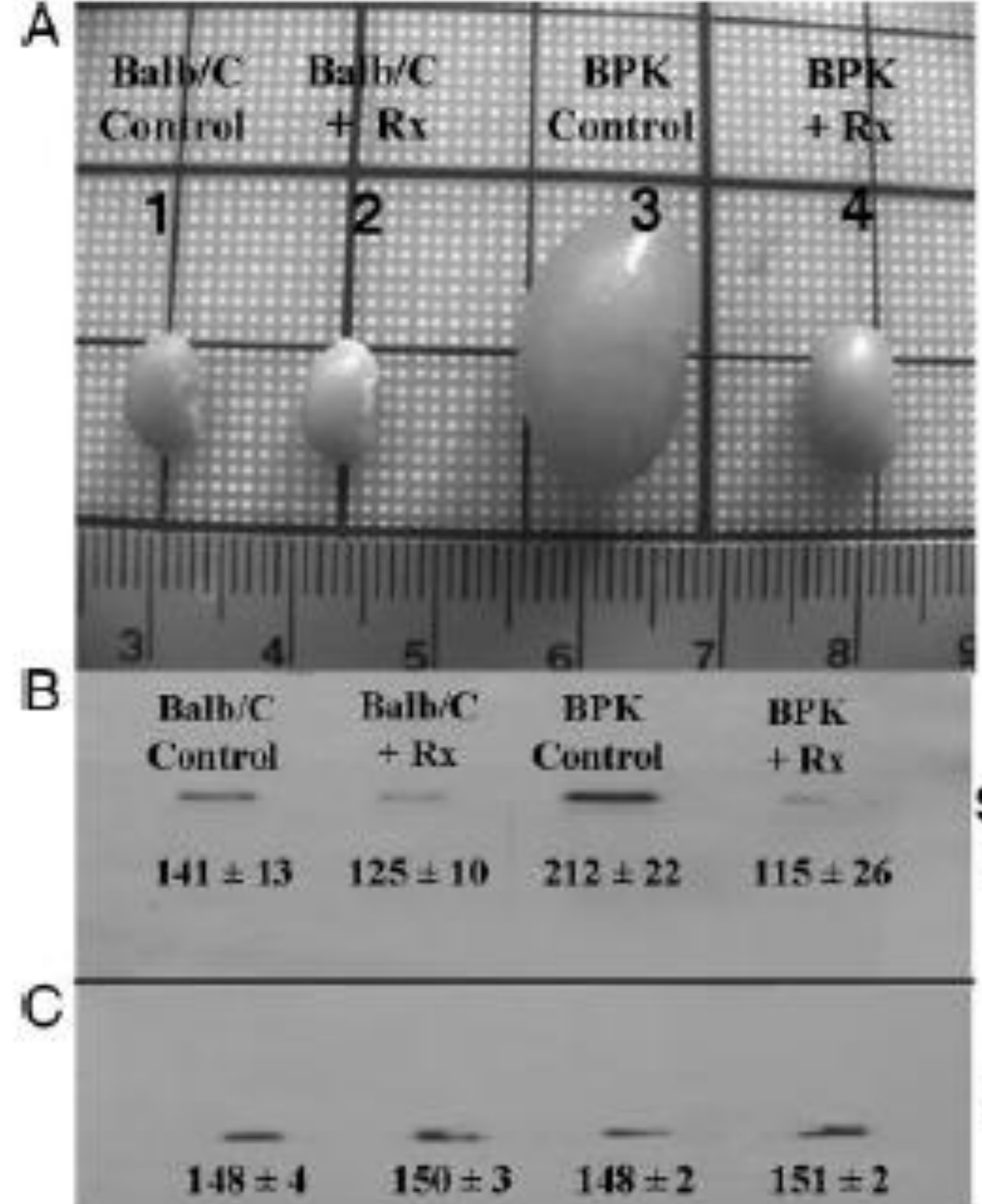


Figure 3: Effect of c-Src on renal size. (From Ref 6)

Evaluation of c-Src as a drug target for different compounds can be performed in mice as was done for the above experiment. *Bpk* (cystic) and BALB/c pups received SKI-606 (Bosutinib) at 30 mg/kg per day by i.p., starting at PN7 (postnatal day 7). Animals were treated from PN7 to PN20 (14 doses). The kidney and liver tissues were routinely harvested at PN21 and the heart, spleen, pancreas, stomach, and thymus were periodically harvested at PN21 to evaluate possible toxicity of vehicle or SKI-606.

The active site of c-Src has different residues leading to different types of interactions with ligands. These interactions include H bonds, pi - cationic interactions and hydrophobic interactions. These interactions are kept in mind when designing potential drug molecules to target c-Src.

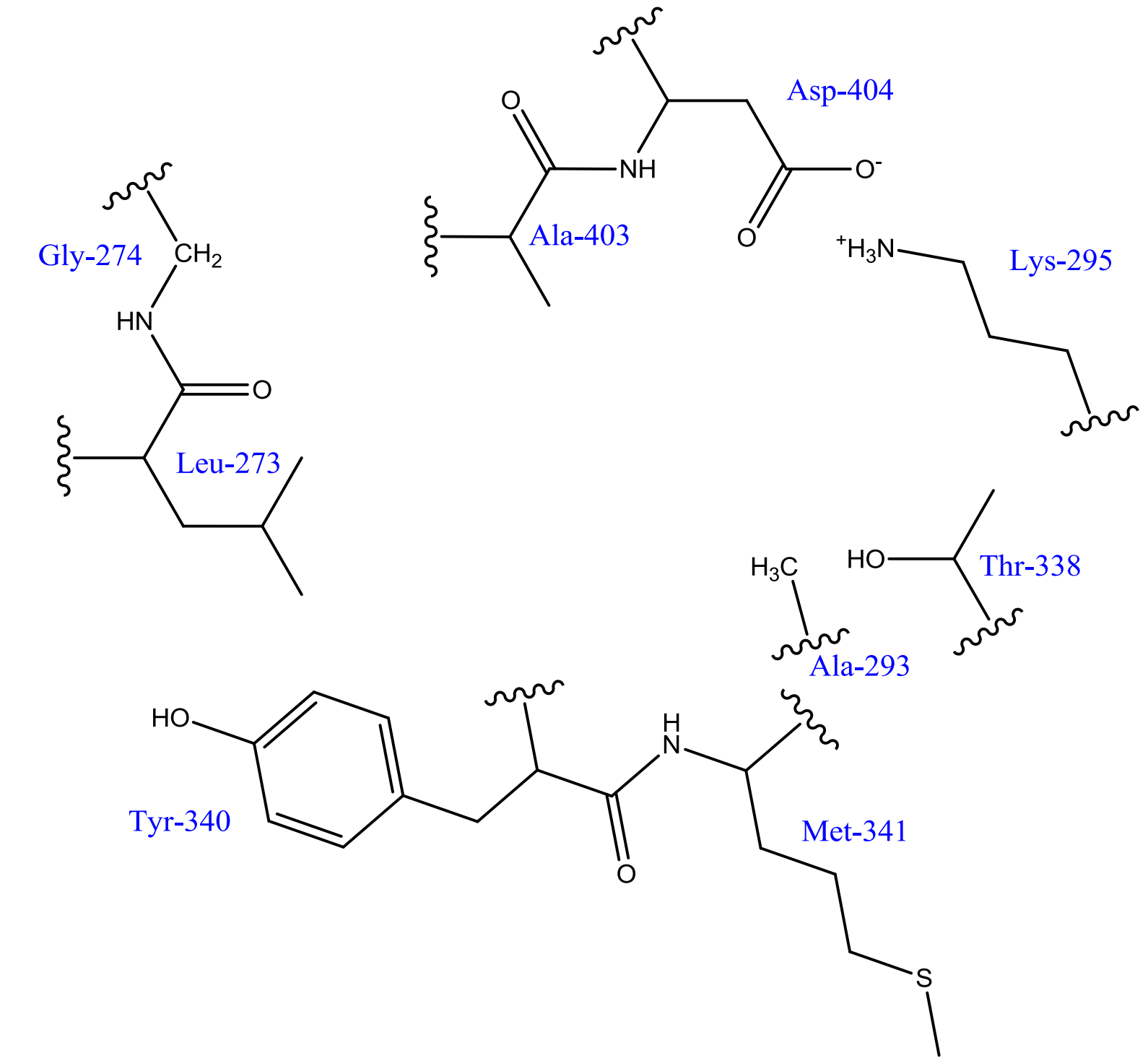


Figure 4: 2D active site of c-Src showing the amino acid residues.

Potential drug molecules were designed using the known inhibitor AZD0530 as a starting point. Computer generations of the ligands were created using Spartan software. Minimum energy 3D structures were determined using the Hartree-Fock method and 3-21G as the basis set. The 3D ligands were docked in the active site of c-Src using Discovery Studio Visualizer software. Improvements and optimizations of the ligand structures were done based on how the ligands were fitting into the active site.

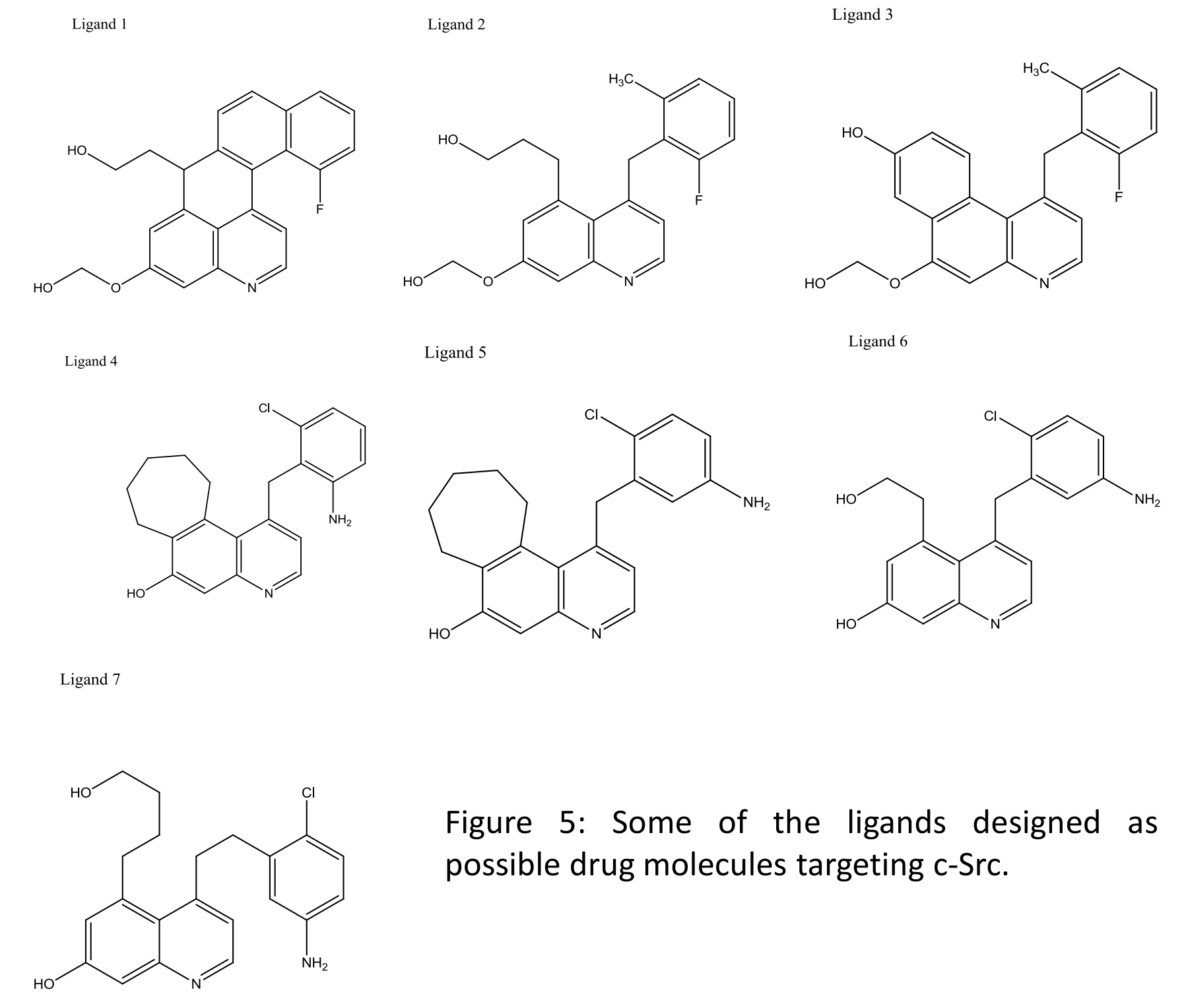


Figure 5: Some of the ligands designed as possible drug molecules targeting c-Src.

The interactions of Ligand 7 in the active site of c-Src are shown in the 3D view below with Ligand 7 docked in the active site.

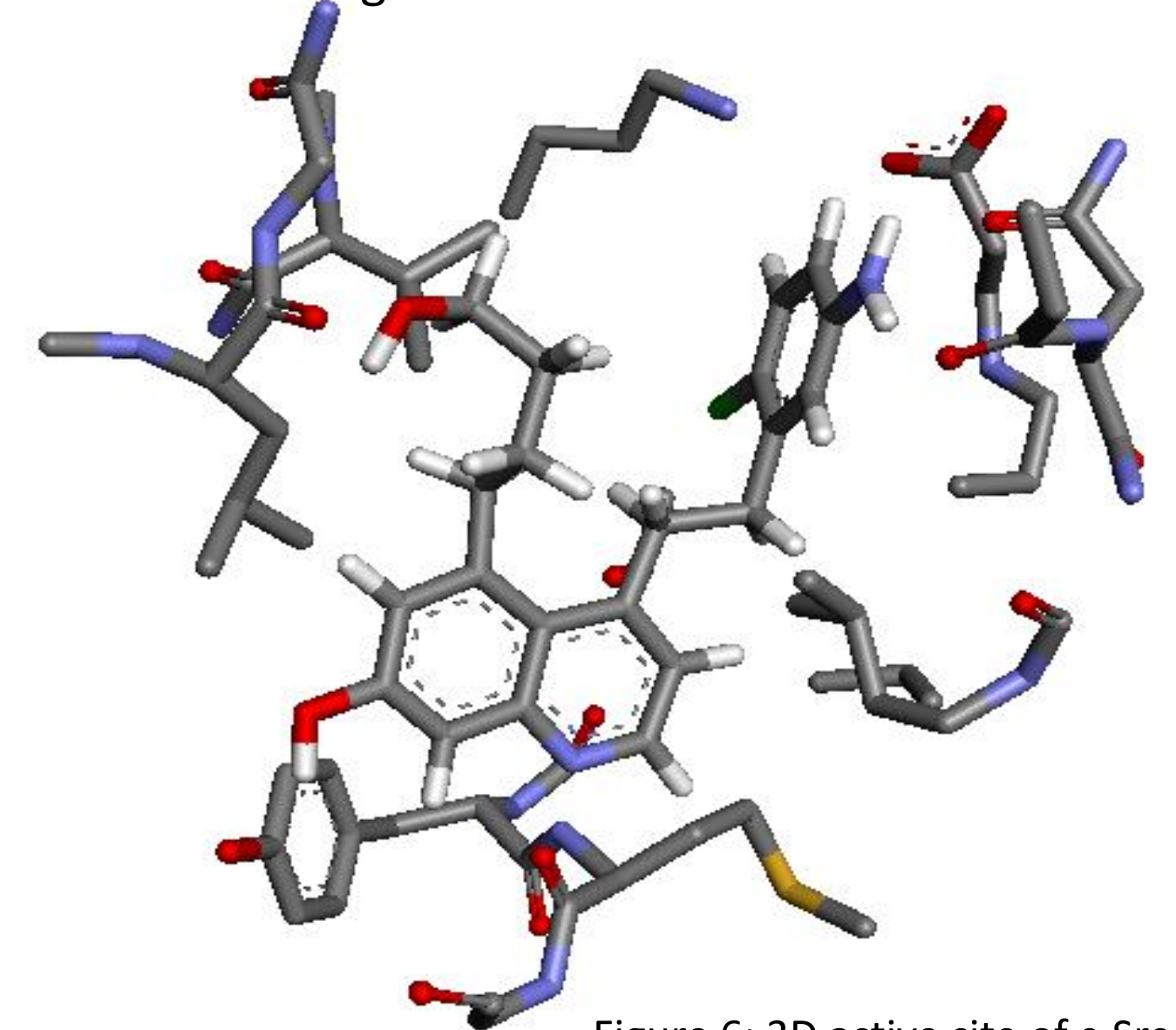


Figure 6: 3D active site of c-Src

The results on the size of kidneys in mice clearly show the effectiveness of targeting c-Src in cancer treatment. Various software packages are available now that can be used in designing ligands that can be used as potential drugs. Insertion of these ligands in the protein active site and visualization of possible interactions with amino acid residues using software packages such as Discovery Studio Visualizer are very useful in optimizing the structures of the possible drug molecules. However, experimental work will still have to be done to determine such parameters like binding constants and toxicity of those molecules that would have been selected.

There are already a number of drug molecules targeting c-Src. However, in an effort to widen the drug bank, it will be interesting to determine the binding affinities of some of the 7 ligands above. Toxicity experiments with the above ligands is also an important step in widening potential drug molecules targeting c-Src.

[1]Russello SV, Shore SK. SRC in human carcinogenesis. *Front Biosci.* (2004), 9, 139-144. [2] Hennequin LF, Allen J, Breed J, Curwen J, Fennell M, Green TP, Lambert-van der Brempt C, Morgentern R, Norman RA, Olivier A, Otterben L, Ple PA, Warin N, Costello, G. N-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2H-pyran-4-yloxy)quinazolin-4-amine, a novel, highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor. *J. Med. Chem.* (2006) 49 (22), 6465-6488. [3] Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT. pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. *Proc. Natl. Acad. Sci. U.S.A.* (June 1992) 89 (11): 5192-6. [4] Ye L, Santarpia L, Gagel RF. The evolving field of tyrosine kinase inhibitors in the treatment of endocrine tumors. *Endocr Rev.* (2010) 31 (4): 578-99. [5] Nauta J, Ozawa Y, Sweeney W, Rutledge J, Avner E. Renal and biliary abnormalities in a new murine model of autosomal recessive polycystic kidney disease. *Pediatr Nephrol* (1993) 7, 163-172. [6] Sweeney WE, Jr, von Vigier RO, Frost P, Avner ED. Src inhibition ameliorates polycystic kidney disease. *J Am Soc Nephrol* (2008) 19: 1331-1341. [7] Yeatman TJ. A renaissance for SRC. *Nat Rev Cancer* (2004) 4, 470-480. [8] Jove R, Hanafusa H. Cell transformation by the viral src oncogene. *Ann Rev Cell Biol* (1987) 3, 289-297. [9]Dhanasekaran N, Premkumar Reddy E. Signaling by dual specificity kinases. *Oncogene.* (1998) 17, 1447-55. [10] Boschelli DH, Boschelli F. Bosutinib *Drugs Fut.* (2007) 32 (6): 481. [11] Recchia I, Rucci N, Festuccia C, Bologna M, Mackay AR, Migliaccio S, Longo M, Sosa M, Fabbro D, Teti A. Pyrrolopyrimidine c-Src inhibitors reduce growth, adhesion, motility and invasion of prostate cancer cells in vitro. *Eur J Cancer.* (2003) 39 (13): 1927-35. [12] Boschelli, F, Arndt K, Gambacorti-Passerini C. Bosutinib: a review of preclinical studies in chronic myelogenous leukaemia. *Eur J Cancer.* (2010) 46: 1781-1789.