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Comparison of Some 3-(Substituted-Benzylidene)-1, 3-Dihydro-Indolin Derivatives as Ligands of Tyrosine Kinase Based on Binding Mode Studies and Biological Assay

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A series of 3-(substituted-benylidene)-1, 3-dihydro- indolin-2-one, 3-(substituted-benylidene)-1, 3-dihydro- indolin-2-thione and 2, 2'-dithiobis 3-(substituted-benylidene)-1, 3-dihydro-indole derivatives was investigated as inhibitor of $p60^{c\text{-}Src}$ tyrosine kinase by performing receptor docking studies and inhibitory activity toward tyrosine phosphorylation. Some compounds were shown to be docked at the site, where the selective inhibitor **PP1** [1-*tert*-Butyl-3-p-tolyl-1H-pyrazolo[3,4-*d*]pyrimidine-4-yl-amine] was embedded at the enzyme active site. Evaluation of all compounds for the interactions with the parameters of lowest binding energy levels, capability of hydrogen bond formations and superimposibility on enzyme active site by docking studies, it can be assumed that 3-(substituted- benzylidene)-1, 3-dihydro-indolin-2-one and thione derivatives have better interaction with enzyme active site then 2, 2'-dithiobis 3-(substituted-benzylidene)-1, 3-dihydro indole derivatives. The test results for the inhibitory activity against tyrosine kinase by Elisa method revealed that 3-(substituted-benylidene)-1, 3-dihydro- indolin-2-thione derivatives have more activity then 3-(substituted-benylidene)-1, 3-dihydro- indolin-2-one derivatives.

Key words: Protein tyrosine kinase, $p60^{c\text{-}Src}$, N-Substituted indole derivatives, Dock 4.0

INTRODUCTION

In recent years, designing of new compounds with the inhibitory activity of angiogenesis is considered that this would be a promising approach to cancer chemotherapy (Cortes-funes, 2002). Tumor angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products (Folkman, 1985). It was reported that angiogenesis can induce the formation of cancerous tumors and the inhibition of angiogenesis can also be resulted to inhibit grooving and spreading of tumors (Folkman, 1972). The importance of protein tyrosine kinases (PTKs) in signal transduction and proliferative disorders associated with a variety of human cancers makes agents attractive therapeutic targets and pharmacological probes, which modulate the activity of PTKs (Terrence, 1992; Traxler, 1995; Khols *et al.*, 1997; Fabbro *et al.*, 2002).

Tyrosine kinase inhibitors (TKIs) are an important group of compound for the development of small molecule therapies against angiogenesis (Hamby and Showalter, 1999). TKIs have the capability to directly block growth factor signaling in the course of angiogenesis (Roussidis and Karamanos, 2002; Levistki and Grazit, 1995). Targeting the TKIs could lead to inhibition of tumor growth (Mohammadi *et al.*, 1997; Sun *et al.*, 1999, 2000).

The normal cellular homologue (c-src) of the Rous sarcoma virus oncogene (v-src), the first molecularly defined proto-oncogene, encodes the $p60^{c\text{-}Src}$ PTK (Protein Tyrosine Kinase). The $p60^{c\text{-}Src}$ (2ptk) belongs to non-receptor-linked membrane-anchored PTKs (src-family PTKs) (Noonberg and Benz, 2000). This protein is also involved in oncogenic signal transduction by the receptor tyrosine kinases EGFR/HER1, HER2 and PDGFR (Hamby and Schowalter, 1999). It has been implicated in the development of leukemia, breast, and colon cancer. For these reasons, c-

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Src has been suggested as an important anticancer target (Hanke *et al.*, 1996). The inhibition of $p60^{c\text{-}Src}$ tyrosine kinase could be beneficial for cancer therapy, such as inhibition of uncontrolled tumor cell growth, inhibition of metastasis, inhibition of tumor angiogenesis *via* reducing VEGF levels with low toxicity (Widler *et al.*, 2001). In addition, the inhibitors of $p60^{c\text{-}Src}$ tyrosine kinase particularly have been identified as potential therapeutics for osteoporosis (Thompson *et al.*, 2000). In the last decades, some 3-(substitutedbenzylidene)-1, 3-dihydro indolin-2-one derivatives were reported as potent and selective inhibitor against different Receptor Tyrosine Kinase (RTKs), which have been involved in tumor growth, metastasis and angiogenesis. Among them, SU5416 and SU6668 (Fig. 1) have been utilized as specific inhibitor of VEGF-R (Flk-1/KDR and Flt-1) in *in vitro* studies (Fong *et al.*, 1998, 1999; Sun *et al.*, 2000). These receptors are known to be involved in angiogenesis, and direct evidence from studies show that SU5416 and SU6668 inhibit tumor-dependent angiogenesis *in vivo*. SU5416 has advanced into clinical trials as an antiangiogenic agent (Fong *et al.*, 1998). Among the studies several indole derivatives, 2, 2'-dithiobis (1-methyl-N-phenyl-1H-indole-3-carboxamide)'s were also found as tyrosine kinase inhibitors (Rewcastle *et al.*, 1994). These derivatives showed moderate inhibition against $pp60^{v\text{-}Src}$ and EGFR tyrosine kinase. Since it was demonstrated that some indole derivatives have strong tyrosine kinase inhibitory activity, this fact has prompted us to design new 3-(substituted-benylidene)-1, 3-dihydro-indolin-2-one, 3-(substituted-benylidene)-1, 3-dihydro-indolin-2-thione and 2, 2'-dithiobis 3-(substituted-benylidene)-1, 3-dihydro-indole derivatives. As part of our research project, it has been studied how molecules may be interacting at the target enzyme $p60^{c\text{-}Src}$ tyrosine kinase by performing Dock 4.0 program (Kuntz, 1998). To understanding of the molecular basis of such interaction should help in the design of more active inhibitors. We also investigated the inhibition of enzyme in *in vitro* studies, in the attempt to broaden our understanding the role of enzyme binding capability and activity relationships of compounds.

METHODS

Docking of Inhibitors

The DOCK 4.0 program (Ewing and Kuntz, 1997), was utilized for the study of binding mode of synthesized indole derivatives against protein tyrosine kinase enzyme $p60^{c\text{-}Src}$. Molecular docking is often used in virtual screening methods, whereby large virtual libraries of compounds are reduced in size to a manageable subset, which if successful, includes molecules with high binding affinities to a target receptor (Lyne, 2002). The potential for a docking algoritm to be used as a virtual screening tool is based on both speed and accuracy. DOCK 4.0 generates spheres in the active site pocket around which spheres inhibitors rotate. Since the rotation takes place only at the center of spheres, the entire calculation time is shorter than the other docking system, while the docking precision is not as superior as it should be. Spheres that fill surface indentations are calculated with the program sphgen. Each sphere touches the surface at two points and is centered along the surface normal at one the points. Only one sphere per surface atom, the largest that does not intersect the surface, is generally retained; groups of overlapping spheres are referred to as clusters. The cluster containing the greatest number of spheres tends to occupy the largest indentation of the surface, typically the active site of an enzyme. The user selects one or more clusters for docking. To evaluating molecular complexes, contact score, electrostatic interaction energy and molecular mechanics interaction energy are calculated. The contact grid is automatically constructed to enclose the input atoms, which may form part or all of the receptor. The electrostatic grid encloses a cubic volume, includes the entire receptor molecule. DOCK 4.0 produces the grid for contact and electrostatic scoring (Ewing *et al.*, 2001). The program produces the values for computing force field score. These scores, or molecular mechanics interaction energies, are calculated as a sum of van der waals and electrostatic components (Meng *et al.*, 1992).

The DOCK 4.0 program explores possible orientations of a molecule within a macromolecular active site by superimposing atoms onto pre-computer site points (Taylor *et al.*, 2002). The docking process consists of sampling the coordinate space of the binding site and scoring each possible ligand pose, which is taken as the predicted binding mode for the compound.

Docking energy score of DOCK 4.0 is obtained by using Equation 1 (Akaho *et al.*, 1999). It is a summation of Van der Waals interaction energy and electrostatic energy both taking place between the ligand and the enzyme.

$$\sum_{j=1}^{rec}\left[\frac{A_{ij}}{r_{ij}^{12}}-\frac{B_{ij}}{r_{ij}^{6}}+332\frac{q_i q_j}{Dr_{ij}}\right]$$

r_{ij}: the distance between atoms i and j
q_i, q_j: the point energy charge on atoms i and j
A_{ij}, B_{ij}: van der waals repulsion and attraction parameters
D: the dielectric function
332: factor that converts the electrostatic energy into kilocalories per mole
i: the atom of ligand
j: the atom of enzyme

RMSD is computed and expressed in Å, and it is a

structural comparison of two molecules in terms of distance. Assume that the structure is defined in terms of the cartesian coordinates of the atoms and represented by an *n*x3 coordinate matrix, where *n* is the number of atoms in the molecule. Structure translation can be done by adding a translation vector to each row of the coordinate matrix, and structure rotation *via* multiplying the coordinate matrix by a rotation matrix. *X* and *Y* are the coordinate matrices of two structures after they are translated so that their centers of geometry coincide (Schulz-Gasch and Stahl, 2003).

The docking experiments as well as receptor and ligand preparations were performed on SGI Indigo Extreme (R4400) workstation. Insight II software (MSI) was used for drawing compounds. **PP1** (Fig. 1) and inhibitors have been docked manually into the active site. To attach hydrogen atoms, molecules are converted to the Sybyl (Tripos) mol2 files. The charge was assigned on the drawn compound, which was optimized by Discover. Empirical partial atomic charges were taken from the CVFF force field with the assistance of Insight II software. The enzyme 2ptk ($p60^{c\text{-}Src}$) were taken out from PDB (Protein Data Bank) sum (URL: http://www.biochem.ucl.ac.uk/bsm/pdbsum/), were placed into the Insight II where docking with inhibitors were conducted. The tyrosine kinase $p60^{c\text{-}Src}$ used for docking was chicken SRC tyrosine kinase. The three dimensional structures were obtained by X-ray diffraction analysis. The amino acid residues representing the active site of tyrosine kinase $p60^{c\text{-}Src}$ where the pocket is created were considered at Lys 295, Glu 310. The secondary structure of enzymes in the PDB sum database was shown in Fig. 2. As far as the geometry of the protein is concerned, TK $p60^{c\text{-}Src}$ consists of 5 sheets, 15 helices, 54 β turns, 8 γ turns, and 11 hairpins.

DOCK 4.0 (Sun *et al.*, 1998), filled the pocket with spheres, moved an inhibitor to the center of each sphere, and rotated it to score the docking energy. This procedure of transforming and rotating the inhibitor was repeated. To rank each potential inhibitor, a pre-calculated contact-scoring grid, based on distance between potential inhibitor atoms and target area atoms, and a force-field scoring grid, based on molecular mechanics interaction energies consisting of Van der Walls and electrostatic components were generated. The resulting output file for each screening, based on distance or force field grids, contains the highest scoring compounds ranked in order of their scores.

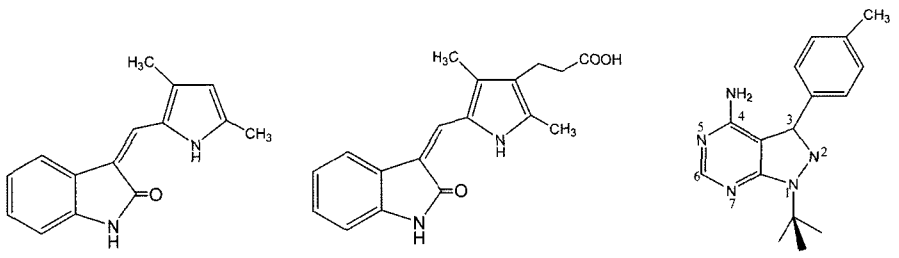
After docking of our proposed compounds against $p60^{c\text{-}Src}$ tyrosine kinase, the top 25 docking results, which are arranged in order with the one with lowest energy were examined. The hydrogen bonds between compounds and enzyme were evaluated by using Insight II software. The compound with the highest number of hydrogen bond with the lowest binding energy was noted for evaluating inhibition capacity and selectivity for the enzyme active site. It was considered that compound shows the lowest binding energy with the greatest number of hydrogen bonds, will be docked more firmly and inhibit the enzyme more strongly than the other compounds.

Tyrosine Kinase assay

Takara Universal Tyrosine Assay Kit (Takara-bio co.) was used to test the synthesized compounds. This assay kit determines the inhibition activities of inhibitors against substrate of tyrosine kinase (Taylor *et al.*, 1999). The main purpose of this assay is to block the phosphorylation of the substrate with inhibitors, which are competitive with substrate. The details of methods were reported in our previous publication (Olgen *et al.*, 2005). The specific $p60^{c\text{-}Src}$ protein kinase inhibitor **PP1** (IC_{50}= 170 mM) was used as an effective standard to evaluate in vitro biological result (Zhu *et al.*, 1999).

Synthesis

The synthesis of 3-(substituted-benylidene)-1, 3-dihydro-indolin-2-one and 3-(substituted-benylidene)-1, 3-dihydro-indolin-2-thione derivatives have been reported in our previous publication (Olgen *et al.*, 2005). Synthesis of oxoindole was achived by a Wolff-Kishner like reduction of isatin with hydrazine hydrate (Sun *et al.*, 1998). The oxoindole was changed to thioindole by using P_2S_5 and Na_2CO_3 in THF (Rewcastle *et al.*, 1994). The resulted compounds were synthesized by condensation of oxoindole



SU 5416 SU 6668 PP1

Fig. 1. SU 5416 and **SU 6668** as spesific inhibitor of VEGF-R (Flk-1/KDR ve Flt-1) and **PP1** as spesific $p60^{c\text{-}Src}$ inhibitors

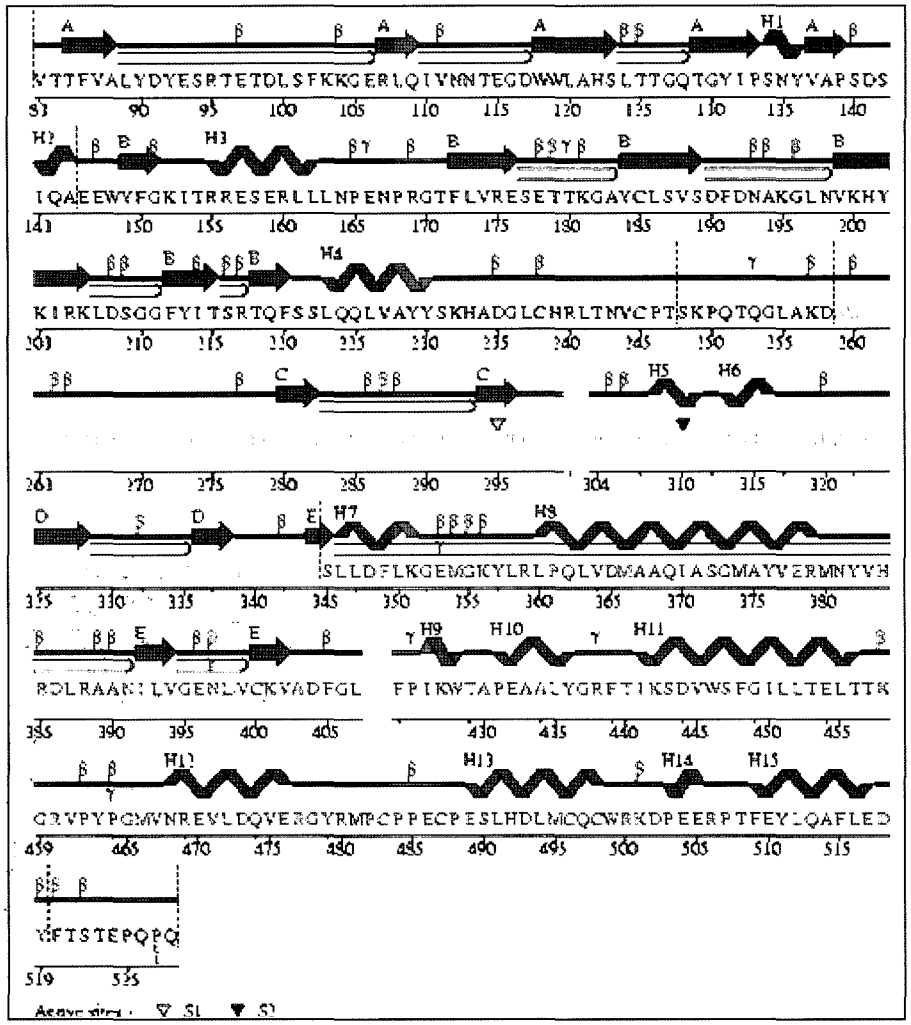
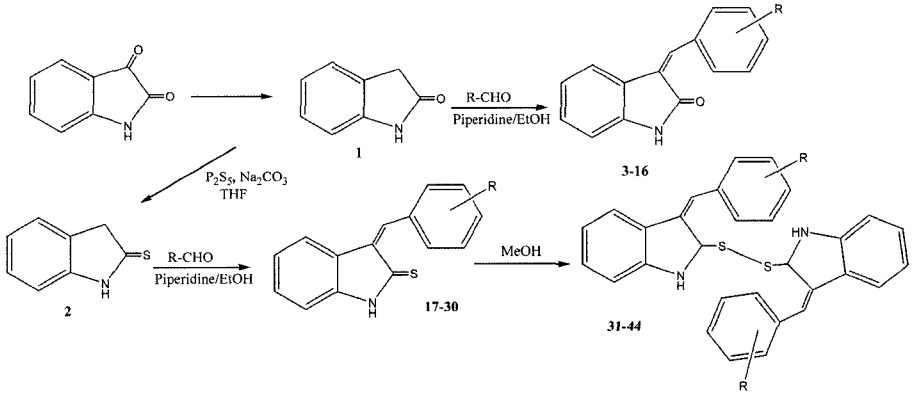


Fig. 2. Secondary structure of PTK $p60^{c\text{-}Src}$ taken from protein data bank. S1- and S2-represent the active site



Scheme 1. Synthesis of 3-(substituted-benylidene)-1, 3-dihydro-indolin derivatives

and thioindole with substituted aryl aldehydes in the presence of the bases (Coda *et al.*, 1984; Braud *et al.*, 2003) (Scheme 1). 2, 2'-Dithiobis 3-(substituted-benylidene)-1, 3-dihydro-indole derivatives could be synthesized by stirring the 3-(substituted-benylidene)-1, 3-dihydro- indolin-2-thione derivatives in MeOH at the room temperature for several days. Indeed, the synthetic difficulties, week binding capability of dithiobis compounds by molecular docking studies, and also moderate activity results of thioindole and oxoindole derivatives, prompted us to give up synthesis

Table I. H bonds between 3-(substituted-benzylidene)-1, 3-dihydro indolin derivatives and active site amino acid Lys 295 for enzyme $p60^{c\text{-}Src}$

Comp. No	Energy (kcal/mol)	Against $p60^{c\text{-}Src}$ H bond (distance Ao)	RMSD (root mean square deviation)
8	08. -18.91	O-1 with N-4 of Lys 295 (2.74)	10.17
20	09. -15.98	O-4 with N-4 of Lys 295 (2.98)	11.35
	10. -15.89	O-4 with N-4 of Lys 295 (2.84)	11.36
29	10. -9.69	S-1 with N-4 of Lys 295 (3.29)	9.78
	11. -9.06	S-1 with N-4 of Lys 295 (3.17)	9.64
30	09. -5.62	S-1 with N-4 of Lys 295 (3.64)	9.92
	10. -5.49	S-1 with N-4 of Lys 295 (3.26)	9.68
35	01. -22.57	S-5 with N-4 of Lys 295 (3.47)	14.38
	02. -22.55	S-5 with N-4 of Lys 295 (3.47)	14.37
	03. -22.54	S-5 with N-4 of Lys 295 (3.47)	14.36
	04. -22.32	S-5 with N-4 of Lys 295 (3.47)	14.76
	05. -22.32	S-5 with N-4 of Lys 295 (3.47)	14.76
	06. -20.86	S-5 with N-4 of Lys 295 (3.47)	14.40
	07. -20.20	S-5 with N-4 of Lys 295 (3.47)	14.38
	08. -20.16	S-5 with N-4 of Lys 295 (3.47)	14.38
	09. -20.11	S-5 with N-4 of Lys 295 (3.47)	14.73
	10. -20.09	S-5 with N-4 of Lys 295 (3.47)	14.75
38	01. -3.53	S-3 with N-4 of Lys 295 (3.62)	12.81
	02. -3.38	S-3 with N-4 of Lys 295 (3.62)	12.81
	03. -3.29	S-3 with N-4 of Lys 295 (3.62)	12.81
	04. -3.15	S-3 with N-4 of Lys 295 (3.62)	12.81
39	01. -16.90	S-3 with N-4 of Lys 295 (3.63)	12.98
43	01. -22.73	S-3 with N-4 of Lys 295 (3.18) S-4 with N-4 of Lys 295 (3.14) O-6 with N-4 of Lys 295 (2.94)	12.53
	02. -19.85	S-3 with N-4 of Lys 295 (3.18)	13.03
	04. -18.99	O-2 with N-4 of Lys 295 (2.98)	13.10
	05. -17.80	O-2 with N-4 of Lys 295 (2.98)	13.20
	06. -16.05	O-2 with N-4 of Lys 295 (2.98)	15.05
	07. -15.42	O-2 with N-4 of Lys 295 (2.98)	13.93
	08. -12.01	S-3 with N-4 of Lys 295 (3.18) S-4 with N-4 of Lys 295 (3.14)	12.53
	10. -8.39	O-2 with N-4 of Lys 295 (2.98)	13.11
	11. -8.29	O-2 with N-4 of Lys 295 (2.98)	15.33
44	02. -9.58	S-4 with N-4 of Lys 295 (3.67) N-6 with N-4 of Lys 295 (2.18)	13.18
	04. -5.15	S-4 with N-4 of Lys 295 (3.42)	12.37

Table II. H bonds between 3-(substituted-benzylidene)-1, 3-dihydro indolin derivatives and other amino acids neighbor at the active site amino acid Lys 295 for enzyme $p60^{c\text{-}Src}$

Comp. No	Energy (kcal/mol)	Against $p60^{c\text{-}Src}$ H bond (distance A°)	RMSD (root mean square deviation)
3	12. -14.21	N-3 with O-4 of Asp 404 (2.87)	11.18
6	01. -13.78	N-3 with O-4 of Asp 404 (2.59) O-4 with O-5 of Asp 404 (2.64)	11.13
7	01. -22.71	N-4 with O-4 of Asp 404 (2.69)	11.71
10	03. -19.36	O-3 with O-4 of Asp 404 (2.85)	10.28
13	06. -9.63	O-4 with O-1 of Thr 338 (2.68)	10.41
16	01. -23.36	N-3 with O-4 of Asp 404 (2.43)	9.73
21	13. -2.57	N-3 with O-2 of Met 341 (2.48) N-3 with O-2 of Gly 344 (2.59) N-3 with O-2 of Val 394 (2.41) O-5 with N-4 of Lys 343 (2.67)	17.76
24	03. -18.68	O-3 with O-4 of Asp 404 (2.95)	10.63
29	01. -23.40	O-3 with O-5 of Asp 404 (2.92)	9.81
30	02. -23.41	N-3 with O-5 of Asp 404 (2.35)	9.82
33	03. -29.56	N-4 with O-2 of Leu 273 (2.26)	18.80
35	15. -7.88	N-7 with O-2 of Val 323 (2.11) N-8 with O-4 of Gln 324 (2.16) O-9 with N-5 of Gln 324 (2.27) O-10 with N-5 of Gln 324 (2.70)	12.43
38	14. -0.46	N-1 with N-3 of Leu 325 (2.47) O-2 with N-3 of Lys 315 (0.85) O-2 with N-3 of Lys 316 (1.96) O-2 with O-2 of Lys 315 (1.89)	11.35
41	03. -28.37	O-2 with N-2 of Ser 345 (2.97) O-9 with O-4 of Tyr 340 (1.47) O-10 with O-4 of Tyr 340 (1.79) N-8 with O-4 of Tyr 340 (1.80) N-8 with S-4 of Met 283 (3.00)	19.03
42	06. -5.05	N-1 with O-2 of Val 323 (2.08)	11.73
43	03. -19.40	O-2 with O-5 of Asp 404 (2.75) O-2 with N-5 of Lys 295 (2.98) S-4 with N-2 of Ser 245 (3.32)	15.34
44	04. -5.15	S-4 with N-4 of Lys 295 (3.42) N-8 with O-5 of Asp 404 (2.64)	12.37

of 2, 2'-dithiobis 3-(substituted-benylidene)-1, 3-dihydro-indole derivatives.

RESULTS AND DISCUSSION

In this study, the docking results of synthesized 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-one, 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-thione and 2, 2'-dithiobis 3-(substituted-benzylidene)-1, 3-dihydro-indole derivatives for $p60^{c\text{-}Src}$ tyrosine kinase were evaluated and are shown in Table I and II. Atoms responsible for H (hydrogen) bonds are shown in Fig. 3 and 4. Docking experiments report a similar binding mode for **PP1** and the set of studied compounds. The hydrogen binding capability of compounds to enzyme active site with their lowest binding energy, 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-one and 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-thione derivatives were shown H bonds with the amino acid Asp 404 (Fig. 5). As seen from this figure more numbers of 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-one derivatives have H bonds than 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-thione derivatives.

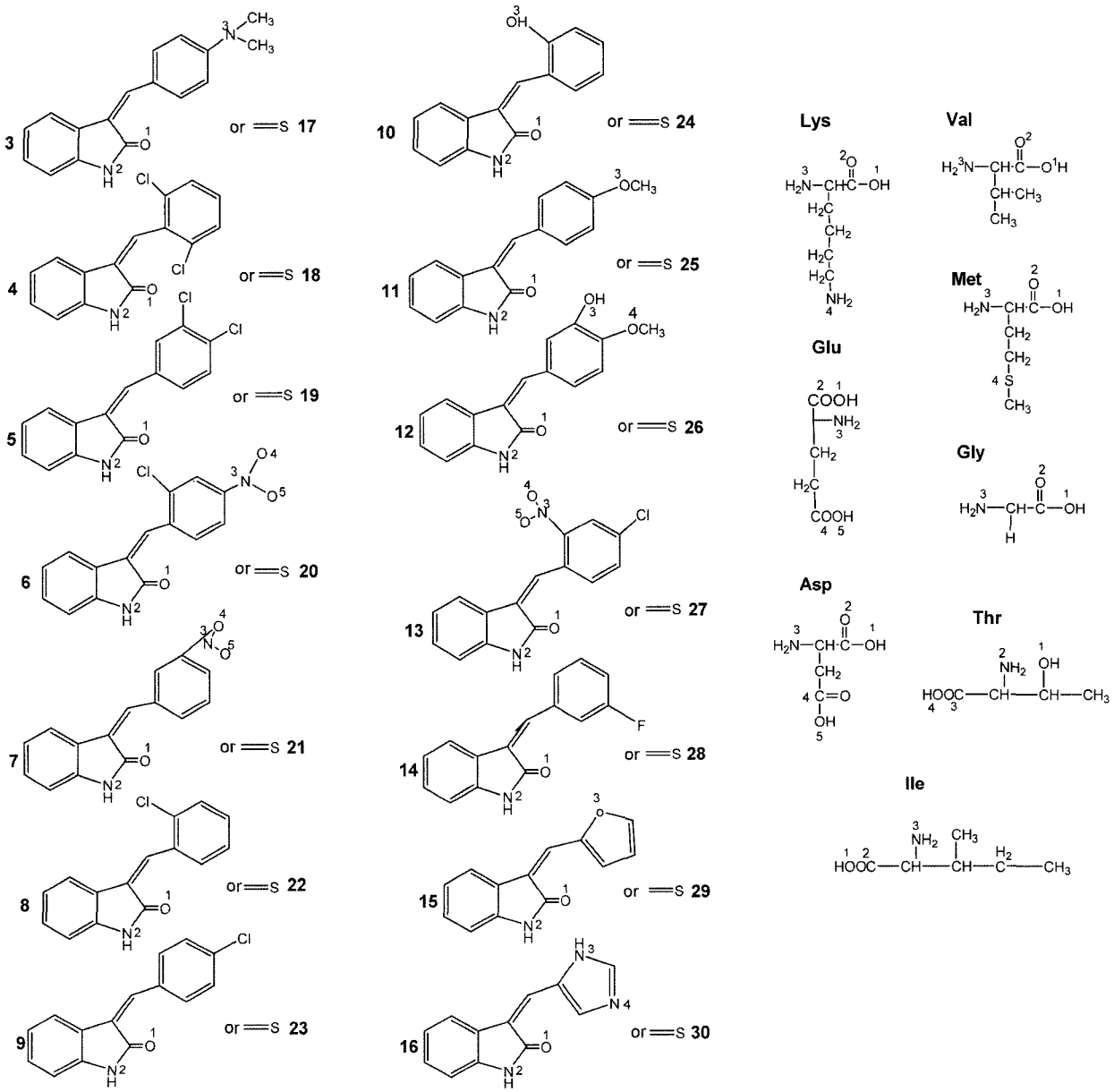


Fig. 3. Atom responsible of compounds which is involved in hydrogen bonding for 3-(substituted-benzylidene)-1, 3-dihydro indolin-2-one and 2-thione derivatives: superscripts indicate the atom responsible for hydrogen bonding with amino acids. Amino acids (Lys, Val, Met, Glu, Gly, Asp, Thr, Ile) represent the H bonds with compounds.

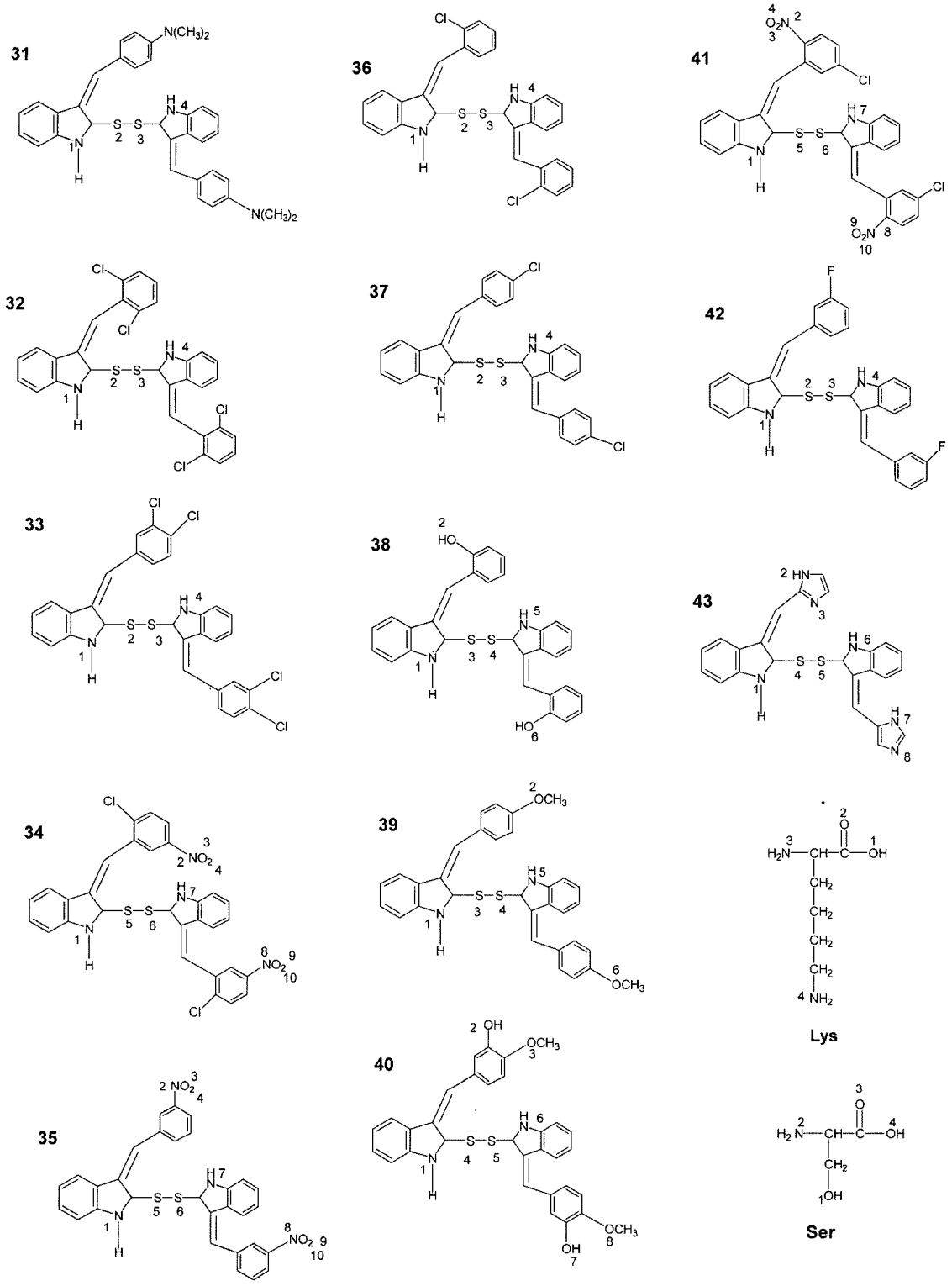


Fig. 4. Atom responsible of compounds which is involved in hydrogen bonding 2, 2'-dithiobis 3-(substituted-benylidene)-1, 3-dihydro-indole derivatives: superscripts indicate the atom responsible for hydrogen bonding with amino acids. Amino acids (Lys, Ser) represent the H bonds with compounds.

Both groups of compounds adopt a similar binding mode (with amino acid Asp 404) into the active site of the enzyme. Based on the data obtained from docking studies, compounds **8**, **20**, **29**, **30**, **35**, **38**, **39**, **43**, and **44** had

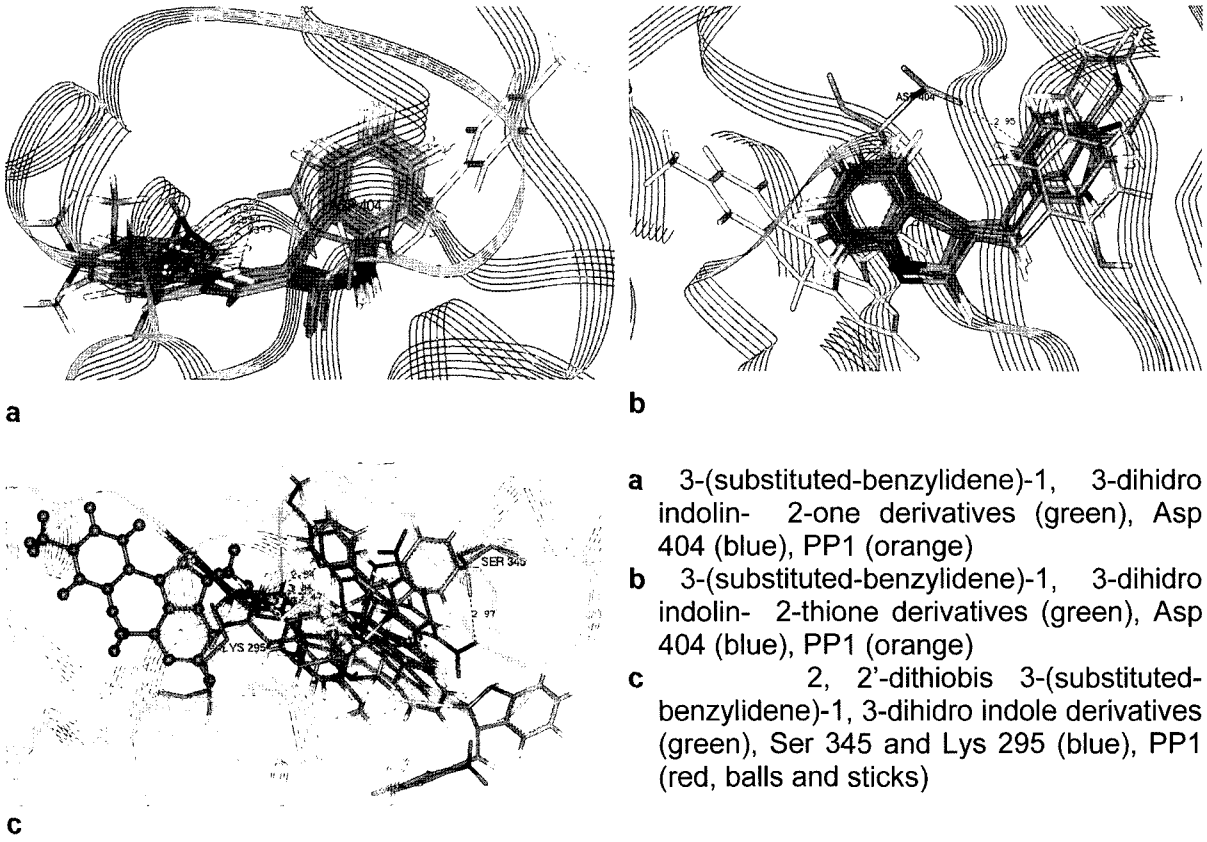


Fig. 5. Comparison of docking sites of 3-(substituted-benzylidene)-1, 3-dihidro indolin derivatives with the lowest energy level against $p60^{c\text{-}Src}$. Compounds were shown in Figure represent only lowest energy bindings.

hydrogen bonding ability with active site amino acid Lys 295 (Table I). In addition, compound **43** showed three H bonds with the active site amino acid Lys 295 at the lowest energy level. Evaluation of all compounds for hydrogen bonding ability at the possible lowest energy level into the active site, compounds **3**, **6**, **7**, **10**, **16**, **24**, **29**, and **30** had hydrogen bonds with Asp 404; the other compounds **13**, **21**, **33**, **35**, **38**, **41**, and **42** had hydrogen bonds with amino acids neighbor to active site amino acids Lys 295 (Table II). Interestingly, compounds **43** and **44** had hydrogen bonds with both amino acids Asp 404 and lys 295. Among all compounds, 2, 2'-dithiobis 3-(substituted-benzylidene)-1, 3-dihydro-indole derivatives have more H bonds with the active site amino acid Lys 295 than the others (Fig. 6). When we examine the hydrogen bonding capability of oxo and thio derivatives, it was found that only thio derivatives **20**, **29**, **30** and oxo derivative **8** have render satisfactory interaction with active site amino acid Lys 295. Since more numbers of thio compounds have hydrogen bonds with active site amino acids, it can be concluded that thio derivatives have more binding potency then oxo derivatives. In general, compounds which have hydrogen bonds with active site amino acids, did not show any significant activity for the inhibition of $p60^{c\text{-}Src}$ tyrosine kinase. In the docking literatures, if compounds have hydrogen bonding capability with active site amino acid, it can be assumed that these compounds would be docked more firmly and inhibit the enzyme more strongly then the other derivatives (Kuntz, 1998, Ewing and Kuntz, 1997). Among the active compounds, only compound **8** 3-(o-chloro benzylidene)-1, 3-dihydro indolin-2-one had hydrogen bond with active site amino acid Lys 295. On the other hand, the most active compounds **17**, **18**, **26** did not show any hydrogen bond with active site amino acid Lys 295. These findings suggest that there is no paralel result between the hydrogen binding capability and activity potency of our compounds.

The docking energy levels and RMSD (root mean square deviation) value of compounds are also shown in Table I, and II. Docking energy levels of all compounds showed well low negative values, which are considered the fact that these energy values are low enough for compounds to be docked into the active site. The RMSD value tells us how far the docked compound is situated from the ideal dock compound (the ligand), and it also tells us the small RMSD value representing the super-imposability of the two compounds. Taking into consideration of RMSD values at their lowest energy levels, all

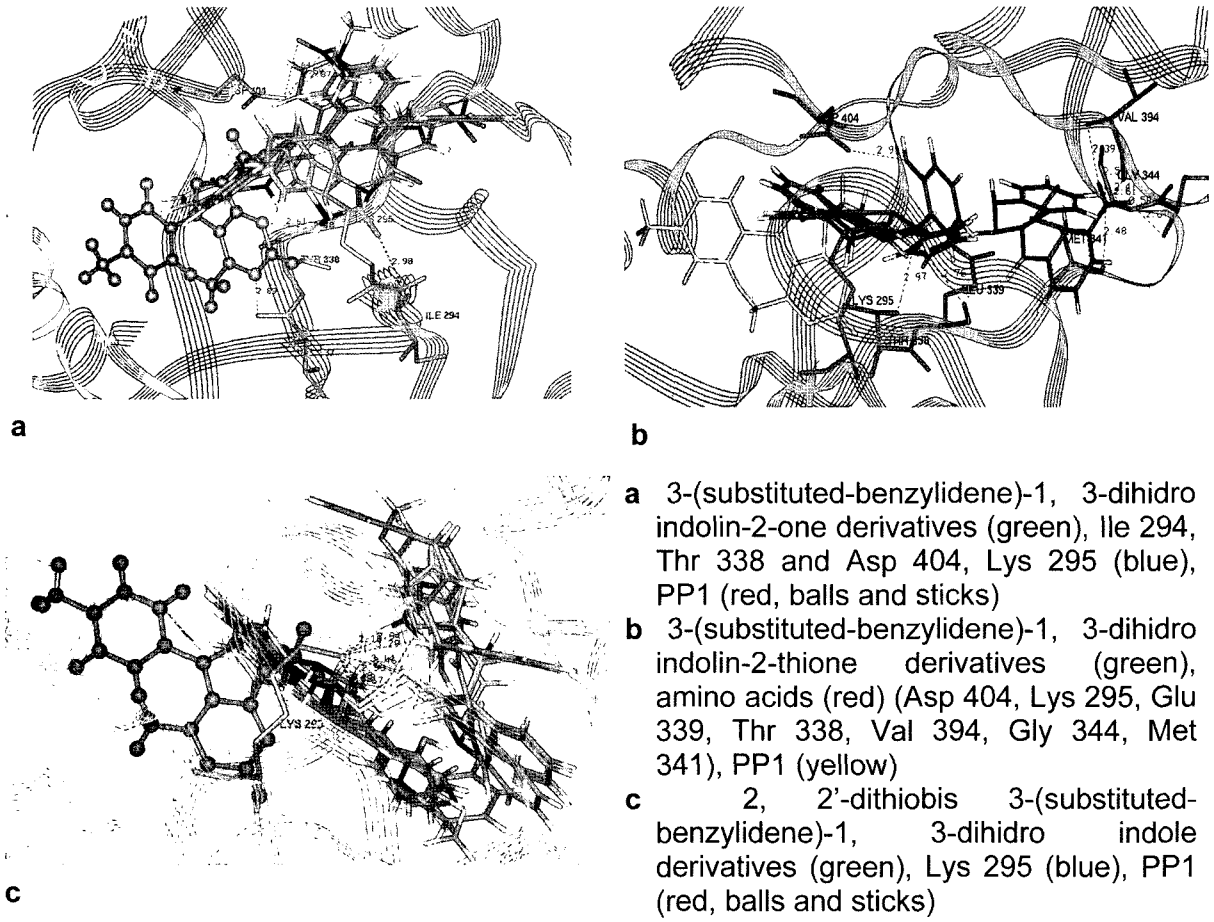
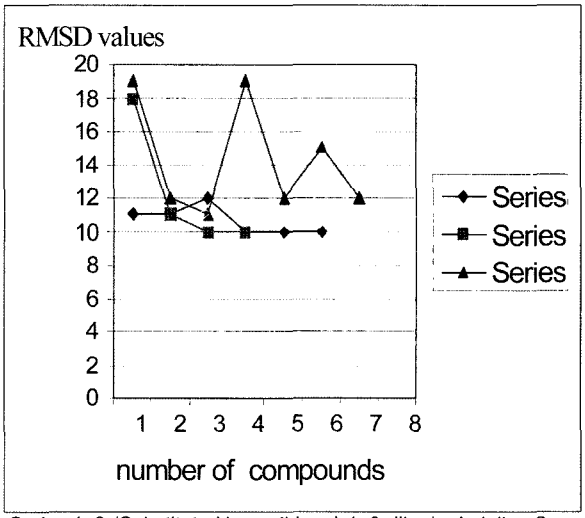


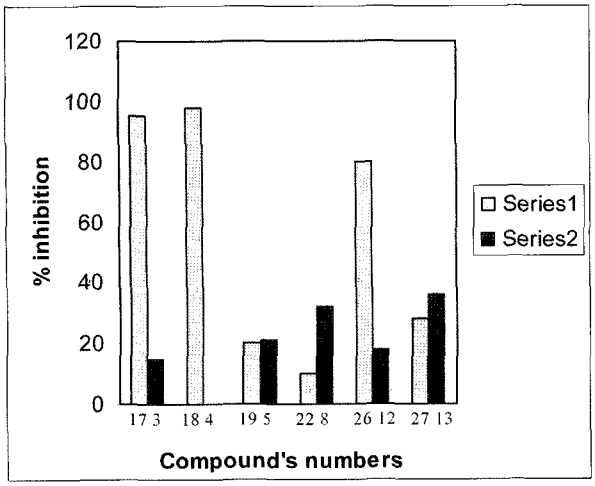
Fig. 6. Comparison of docking sites of 3-(substituted-benzylidene)-1, 3-dihidro indolin derivatives for H bonding capability against $p60^{c\text{-}Src}$. Compounds show possible number of hydrogen bonds in figure.

compounds in Fig. 7 demonstrated that 3-(substituted-benzylidene)-1, 3-dihydro-indolin-2-one derivatives show better superimposability then the other derivatives. X, axis represents, how many compounds of each series had H bonds. Y, axis represents the RMSD values of compounds, which had H bonds with active site amino acids. These compounds have ability to get closer to the active site of the enzyme comparing the other derivatives and lower RMSD of them can lead to the increased binding capacity of compounds (Table I and II). Although more numbers of 2, 2'-dithiobis 3-(substituted-benzylidene)-1, 3-dihydro-indole derivatives had H bonds at their lowest energy level, their RMSD values were found more fluctuated (Fig. 7) and they did not show better superimposabilty then the other compounds (Fig. 5). In order to docking evaluation, both the lower energy levels and the small RMSD values of compounds demonstrate the fact that all compounds are enough closer to the active site of the enzyme and exert the better binding capacity of the inhibitor. As far as the lowest energy is concern, the lowest energy does not necessarily give us a stable docking, if the docking energy



Series 1: 3-(Substituted benzylidene)-1, 3-dihydro indoline-2-ones
Series 2: 3-(Substituted benzylidene)-1, 3-dihydro indoline-2-thiones
Series 3: 2, 2'-dithiobis 3-(substituted benzylidene)-1, 3-dihydro indoles

Fig. 7. Comparison the RMSD values of compounds which have H bonds with active site at the lowest energy level



Series 1: 3-(Substituted benzylidene)-1, 3-dihydro indoline-2-thiones
Series 2: 3-(Substituted benzylidene)-1, 3-dihydro indoline-2-ones

Fig. 8. Comparison the % inhibition of active compounds at 100 μM concentrations. Only active compounds and their congeners were compared in this figure.

is more or less scattered evenly so that the docked compound cannot be stable as it should be. On the other hand, if docked compound demonstrate a hydrogen bond on where docking orientation is created to make compound stably situated even though the total docking energy is not low as otherwise. In some cases, a good correlation between the potency and the number of hydrogen bonds is observed, while other cases not. It is our understanding that our current study belongs to the latter case. Hydrophobic interaction between an inhibitor and an enzyme may also an important factor to determine the interaction capability of inhibition against the enzyme. To analyze docking solutions in terms of structure-activity relationship, both binding energies and % inhibitor of enzyme were evaluated for each compound. Nevertheless compounds, which show the lowest binding energy do not score them as the most active molecules. This contradiction could be explained by the role of water affecting the binding of the inhibitors to the enzyme. The compound with the highest number of hydrogen bonds, with the lowest binding energy, and with the smallest RMSD is generally said to be a reasonable candidate for the inhibition of the enzyme. Considering all these factors, docking results of compounds suggested us that none of them have more advantages than the others (Fig. 5 and 6).

Both thio and oxo congeners of 3-(substituted-benzylidene)-1, 3-dihydro-indolin derivatives were evaluated for their inhibitory activity toward tyrosine phosphorylation for *the p60$^{c\text{-}Src}$ tyrosine kinase. The results were expressed* as a percentage of inhibition shown in Fig. 8 and IC_{50} values were determined by graphic analysis and reported in our previous publication (Olgen *et al.*, 2005). The maximum inhibition attained among the tested compound was IC_{50}= 21.20 μM of compound **18**. The other active compounds **5**, **8**, **12**, **13**, **17**, **19**, **26**, and **27** have IC_{50} inhibition range among 21-305 μM. Fig. 8 shows comparison the % inhibitions of most active compounds and their congeners. When we examine the structure activity relationships of most effective compounds **17**, **18**, and **26** with others, this activity results showed that thio compounds more potent than the others. Due to the structure-activity relationship between indole derivatives and p60$^{c\text{-}Src}$ tyrosine kinase, we have suggested that the replacement of oxo with a thio group play a more important role in the enhancement of activity. The same substitutions of different congeners also have different impacts on the activity. As predicted by activity results, only 3', 4'-dichloro congeners **5**, **19** produced equal activity. However, there are not any clear relationships, between the activity profiles of identical congeners. The potency of this chemical series may depend, to a large extent, on the three dimensional structure of R substituents. Both docking and activity results will be used the address to design and synthesis of new potent tyrosine kinase inhibitors.

In overall evaluation of docking and activity test results show that there is not any correlation between them in this study. Nevertheles, both activity and docking studies on indole esters showed a parallel result in our previous study (Olgen *et al.*, 2003). For this reason it is still beleived that docking results might explain the correlation of biological activity and enzyme binding capability of compounds in some cases.

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