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# Synthesis, Biological and Docking Studies of Thiadiazole Amide Derivatives Containing Anthranilic Acid

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## Abstract

Cancer has been become threatening the health and life of humans. Therefore, attempt to discovery more effective anticancer agents continue. Thiadiazole derivatives have been shown remarkable effects among chemotherapeutic agents. In this study, hybrid molecules containing thiadiazole amide derivatives -aromatic amines (anthranilic acid and 2, 5 dichloro aniline) were synthesized in multi-step reactions including N-amidation and S-alkylation of thiadiazole. Synthesized compounds were assayed by MTT test against three cell lines. Molecular docking was performed on Src tyrosine kinases. Compounds **5a**, **5b** and **8** rendered remarkable cytotoxic properties on HeLa with $IC_{50}$‹ 60 µM. Compound **5b** showed significant cytotoxic effect on both cancer cell lines compared to other tested derivatives. Compound **5a** with ΔG = -8.05kcal/mol was the best compound among docked compounds.The results revealed that the quiddity of the amide substitution has a definitive role in the cytotoxic activity.

**Keywords:** Anthranilic acid; Anti cancer; Thiadiazole; Molecular Docking.

## Introduction

Cancer is a growing disease in the human population. Millions of people in the world are affected by this disease. Despite major advances in cancer treatment, including surgery, chemotherapy, and radiation, there is a need to discover more effective and selective anticancer agents with better bioavailability and fewer side effects especially for metastatic and resistant cancers (1-6). The azoles as heterocyclic compounds are very well known in the field of anticancer research. These compounds displayed interesting in-vitro and in-vivo antitumor activity (1, 2, 5-10). Small molecules containing diverse azoles in particular thiadiazole were prepared as cytotoxic agents. The extensive activity of thiadiazole derivatives has definited them as pharmacologically remarkable scaffolds (11-13). Substituted thiadiazole derivatives, including amide (3), schiff base (1), s-alkylate (3, 4), phthalimide (14), and hybrid analogues of thiadiazole have been shown anticancer activity in literature surveys (2, 15). Some anticancer mechanisms have been assigned for thiadiazole derivatives, including apoptotic, angiogenesis (1), potent binary inhibitors of ABL and src tyrosine kinases (3, 4) and anti tubulin (5). Thiadiazole derivatives shown in Figure 1 (A) can inhibit both Src and ABL thyrosine kinases (dual inhibitors) or only one of the them, depending on the substitution on the benzamido moiety (3, 4, 16). In a cell-based screening study performed by Zhao et al, a hit compound, Figure 1(B), was found which exhibited moderate inhibition against some the human cancer

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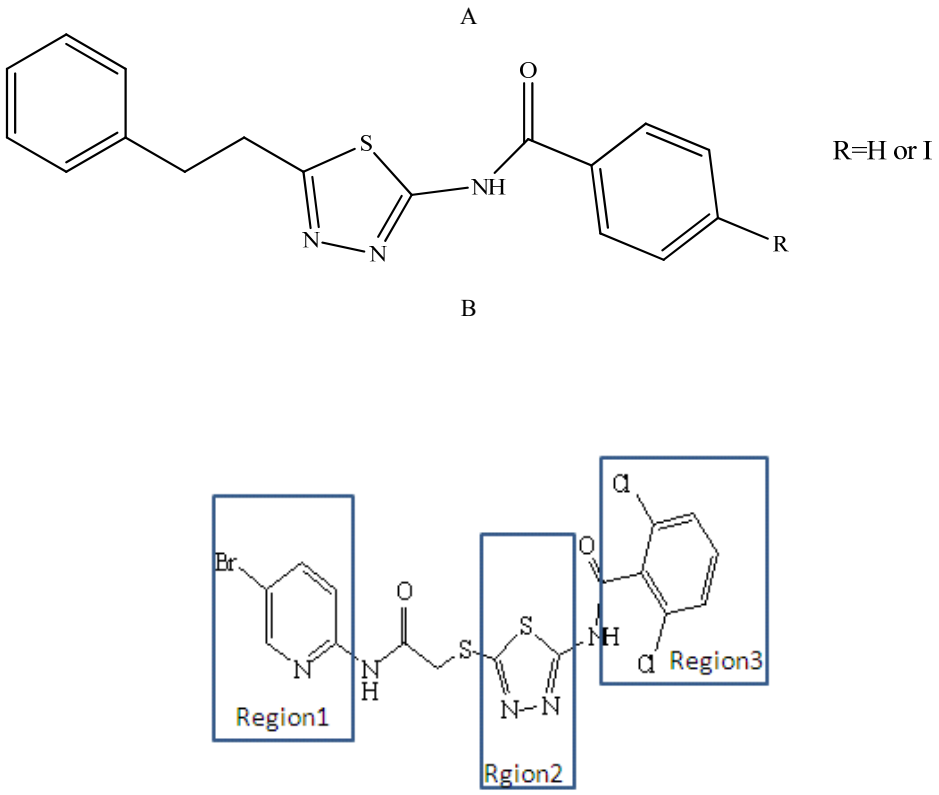



**Figure 1.** Thiadiazole derivatives as inhibitors Src (or/and) ABL (A), Important regions of hit compound for structure activity relationship evaluation (B)

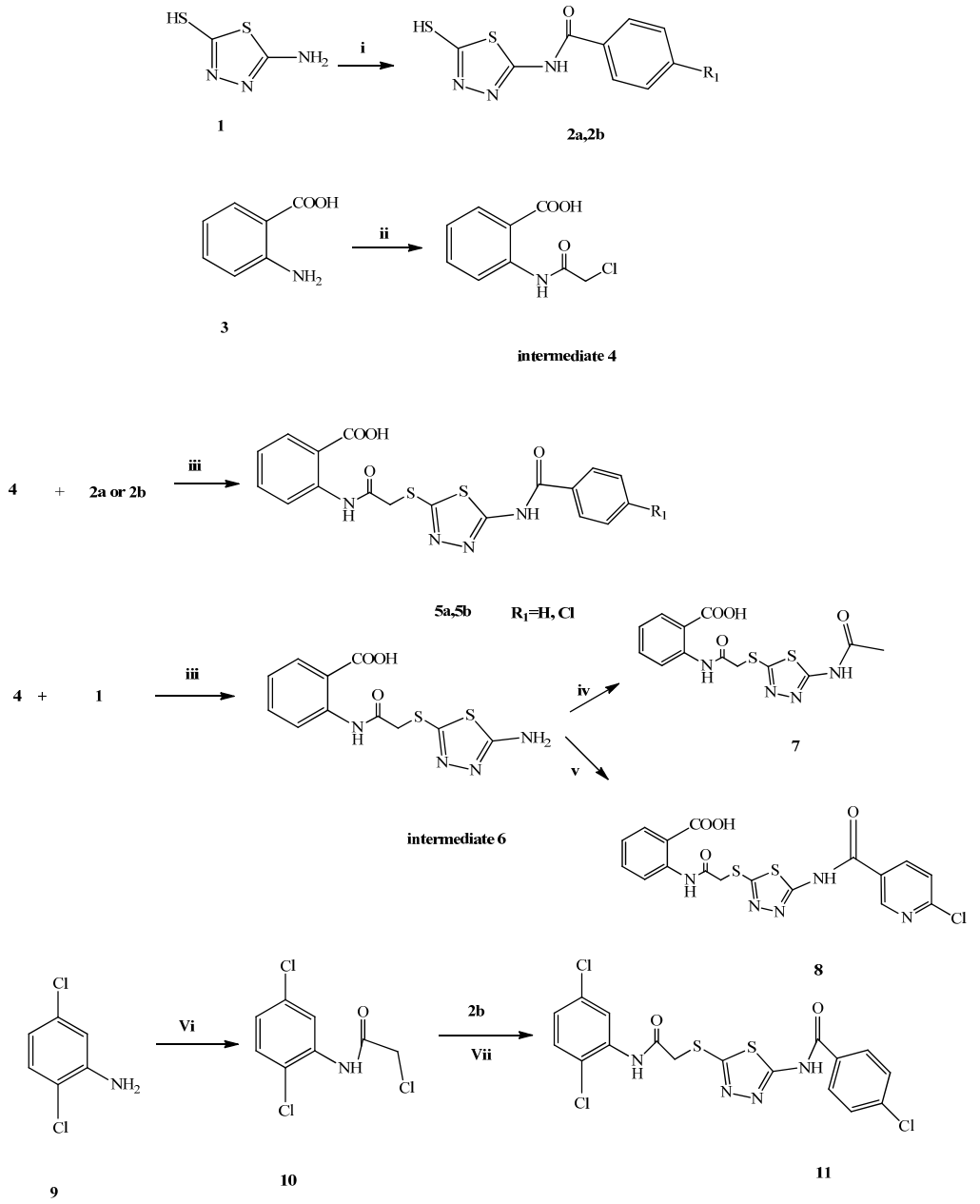
cells in the MTT assay (17). On the other hand, among the wide range of compounds known as anti-cancer agents, anthranilic acid based structures have been attracted much interest in recent years. Experimental and clinical assays show that some of these compounds have prominent anti-cancer activity in particular by inhibiting tyrosine kinase (18-20). Some of the anthranilic acid-based diamide derivatives incorporating aryl-isoxazoline moiety were constructed by shi et al as anticancer agents (21). Based on these considerations, and modifications applied in structures in Figure 1, we prepared novel hybrid molecules via S-alkylation of thiadiazole with aromatic amines (anthranilic acid and 2, 5 dichloro aniline) and N-amidation of thiadiazole core in order to determine the effect and importance of amide and alkyl components. Cytotoxicity of final compounds was assayed against three cell lines. The synthesized compounds were subjected against kinase domain of Src in complex with dasatinib (PDB code: 3G5D) to explore the binding pattern.

## Materials and Methods

Chemical materials and solvents were obtained from Samchun (Korea) and Merck (Germany) companies. The reactions were controlled by silica gel 60 $F_{254}$ plates (Germany). Open capillary tubes and electro thermal IA 9200 apparatus (England) were used for taking melting points and are uncorrected. Infrared (IR, KBr discs) was recorded with a WQF-510 Fourier-transform (FT)-IR spectrophotometer (China). Proton nuclear magnetic resonance spectrometer (HNMR) were obtained by (Bruker 400 MHz, Germany) spectrometer. Mass spectra were run on Agilent Technologies 5975C mass spectrometer (USA).

### *Compounds 2a, 2b*

According to Scheme 1, (benzoyl chloride or 4-chlorobenzoyle chloride) (0.08 mol) was added drop wise to a solution of **1** (0.04mol) and pyridine (catalytic amount) in dry tetrahyrofuran (THF) (20mL). The solution was stirred at room temperature (rt) for 2 h to form solid product (8). The obtained material was




**Scheme 1.** (i) benzoyl chloride derivatives, THF, Pyridine, rt, 2h: (ii) chloroacetyl chloride; DMF rt, 2h: (iii) THF, $K_2CO_3$, reflux: (iv) glacial acetic acid, reflux, 72h:(v) 6-Chloronicotinoyl chloride, DMF, rt, 48h: (vi) glacial acetic acid/ sodium acetate ,Chloroacetyl chloride, rt, 3h: (vii)THF, $K_2CO_3$, **2b**, reflux ,72h.

filtered, washed with water, and recrystallized from methanol to yield compounds **2a**, **2b**.

***Synthesis of intermediate 4***

Chloroacetyl chloride (0.0 8mol) was added drop wise to a solution of anthranilic acid **3** (0.04mol) in dimethyl formamide (DMF) (30 mL) and stirred at room temperature for 2 h. The mixture was added into water (22, 23). The filtration and washing with water were done for collection of white solid **4** (Scheme 1).

***Synthesis of final compounds (5a, 5b)***

To a solution of (0.03 mol ) **2a** or **2b** in THF (20mL), was added anhydrous potassium carbonate (0.03mol), and mixture was stirred at room temperature for 5 min , then the solution of intermediate **4** (0.03mol) in THF was added and refluxed for 48h (24). The precipitate was filtered off and the organic solution was

**Table 1.** The $IC_{50}$ (μM) of compounds on MCF7, HeLa and Huvec cell lines.

| | $IC_{50}$ (μM) | | |
|---|---|---|---|
| **Tested Compounds** | MCF-7 | HeLa | HUVEC |
| **5a** | 92±3 | 31±1 | 72±2 |
| **5b** | 50±3 | 59±2 | 37±2 |
| **6** | >100 | >100 | 92±4 |
| **7** | >100 | >100 | >100 |
| **8** | 93±4 | 49±2 | 45±3 |
| **11** | >100 | 92±2 | >100 |
| **Doxorubicin** | 17.4±5.2 | 1.45±0.15 | 678 (nM) |

purified by chromatography on silicagel to give **5a** and obtained solid washed with water and recrystallized from THF to give **5b** (Scheme 1)**.**

***Intermediate 6***

To a solution of **1** (0.04 mol) in THF (30 mL) were added potassium carbonate and the solution of intermediate **4** *(*0.04 mol) in THF (24)*,* mixture was refluxed for 4 h. The formed solid was filtered, after washing with water, crystallized from THF to give **6** (Scheme1)**.**

***Synthesis of 2-(2-(5-acetamido-thiadiazol-2-ylthio) acetamido) benzoic acid (7)***

The mixture of intermediate **6** (0.03 mol) in glacial acetic acid (20mL) was heated up to 50°C to dissolve completely. Then refluxed for 72h to complete reaction. It was poured in ice water. The separated material was filtered, washed with cold water to give compound **7** (Scheme 1).

***Synthesis of 2-(2-(5-(2-chloronicotinamido)-thiadiazol-2-ylthio) acetamido) benzoic acid (8)***

To intermediate **6** solution (0.03 mol) in DMF (200 mL), 6-chloronicotinoyl chloride (0.04 mol) was added (8). The solution was stirred for 48 h at room temperature, and then was added into ice-water; obtained white solid was crystallized from methanol to give **8** (Scheme 1).

***Synthesis of 4-Chloro-N-(5-(2-(2, 5-dichlorophenylamino)-2-oxoethylthio)- thiadiazole-2-ylbenzamide (11)***

Final compound **11** was prepared in a two-step process. At the first, 2, 5 dichloroaniline (0.01 mol) was dissolve in a mixture of saturated solution of sodium acétate and glacial acetic acid (50mL/50ML), chloroacetyl chloride (0.02mol) was slowly added and stirred in room température for 3h (24), then poured in ice water. Obtained product was collected, washed with cold water to give **10** (Scheme1)**.**

In the second step, compound **2b** (0.02 mol) was dissolved in THF (20 mL), then potassium carbonate (0.02 mol) and the first step product **10** (0.02 mol) were added to this mixture (24) and refluxed for 72h to complete the reaction. The product was collected and purified on silica gel to yield **11** (Scheme 1)**.**

***MTT assay***

Cell lines including HeLa (cervical), MCF-7 (breast) and Huvec (human umbilical vein endothelial cell), were used for cytotoxicity effects évaluation of synthesized compounds (**5a**, **5b**, **6**, **7**, **8** and**11**). Grown cell lines in supplemented Roswell Park Memorial Institute (RPMI) 1640 was spilled in plates at the concentration of ($5 \times 10^4$ cells/mL) and incubated for 24 h to allow celles attachement. Then, the celles were treated with concentrations (1, 10,100 μM) of the synthesized compounds. Doxorubicin and dimethyl sulfoxide (DMSO, 1%) were considered as the positive and negative controls, respectively. These plates were incubated in a humidified incubator at 37 °C for 48 h. After that, 20 μL of MTT dye 3- (dimethylthiazol-2-yl)-diphenyl tetrazolium bromide solution (5 mg/mL) was added to each well and incubated for another 3 h. Dissolving the obtained formazan crystals was done with DMSO (150 μL per well) and absorbance was recorded at 570 nm using an ELISA plate reader. The expérimentés were performed in triplicate. Analysis of variance (ANOVA) and Tukey test was used to obtain the differences between groups with negative control. Cell viability was calculated using the following equation (7,8, 25). Cytotoxic activity was represented in Table 1 and Figure 2. Significant differences were observed compare to the negative control against cell lines for final compounds.

Cell viability was calculated

$$Cellsurvival\ (\%) = \frac{\text{MA of treated wells} - \text{MA of blank}}{\text{MA of negative control} - \text{MA of blank}} \times 100$$

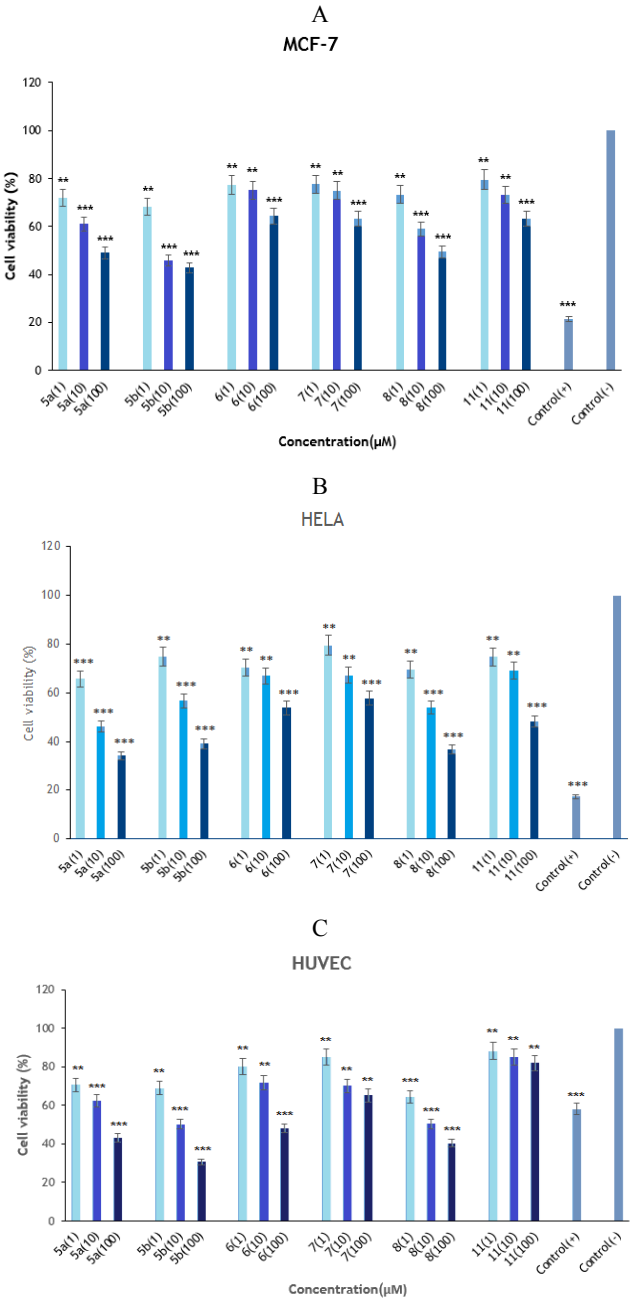



**Figure 2.** Cytotoxic effect of compounds **5a**, **5b**, **6-8** and **11** on MCF-7 (A), HELA (B) and HUVEC cells (C) in different concentrations (1, 10, and 100 μM). Data are presented as mean ± SD, n = 3. * $P$ <0.1 ** $P$ < 0.01, *** $P$ < 0.001 Show significant differences in comparison with negative control group. Doxorubicin was used as positive control.

Where, MA is mean absorbance.

***Docking study***

The target compounds were built and optimized using hyperchem program 7.0 software (Version 7.0) and saved as PDB files. These files were subjected to Auto Dock Tools and processed then saved as PDBQT files. The kinase domain crystallographic structure of cSrc in complex with dasatinib (3G5D) with resolution 2.2 A was obtained from the Protein Data Bank. The enzyme preparation process began with the removal of co-crystal ligand and water molecules of structure. Polar hydrogens and Kollman charges were inserted by using AutoDockTools 1.5.6 (ADT). Generated PDBQT file of

the protein was saved. A grid box with 60 × 60 × 60 dimensions and 0.375 Å spacing was used. The center of the grid box was assigned (0.66, -5.45, and 31.26 Å). Routine procedure and default parameters of molecular docking AutoDock 4.2 software and implemented empirical free energy function (8) were used. Estimated free binding energy values (kcal/mol), and the interactions with key amino acid residues at the active site of enzymes are expressed in Table 2 and Figure 3.

## Results and Discussion

***2-(2-(5-Benzamido-thiadiazol-2-ylthio) acetamido) benzoic acid (5a)***

Yield:59 %, white, m.p.212-214 °C, IR ($\nu_{max}$ $cm^{-1}$), 3592, 3396 (OH), 3263 (NH), 2929 (C-H, Aliphatic), 1734, 1668, (C=O), $^1$HNMR: ($CDCl_3$): δ 12.1 (1H, broad, COOH), 8.21(1H, d, *J*=8Hz, H-Ar), 8.19-8.16 (3H, m, H-Ar), 7.82 (1H, t, *J*=8Hz, H-Ar), 7.65 -7.56 (6H, m, H-Ar and NH-amids),4.45 (2H, s, $CH_2$); MS ( m/z, %): 414 ($M^+$), for $C_{18}H_{14}N_4O_4S_2$, M.W. 414g/mol .

***2-(2-(5-(4-Chlorobenzamido)- -thiadiazol-2-ylthio) acetamido) benzoic acid (5b)***

Yield: 78%, white, m.p. 248-250°C, IR ($\nu_{max}$ $cm^{-1}$), 3667 (OH), 3308 (NH), 1775, 1668 (C=O). $^1$HNMR: (DMSO- $d_6$): δ 13.2 (1H, broad, COOH), 8.15-8.10 (3H, m, H-Ar), 7.94 (1H, t, *J*=8Hz, H-Ar), 7.67-7.61 (5H, m, H-Ar and NH-amide), 4.54(2H, s, $CH_2$).

***2-(2-(5-Amino--thiadiazol-2-ylthio) acetamido) benzoic acid (6)***

Yield:80 %, white, mp.183-185°C, IR ($\nu_{max}$ $cm^{-1}$), 3300 (OH), 3157 (NH), 1666 (C=O) $^1$HNMR: (DMSO-$d_6$): δ 13.59 (1H, broad, COOH), 8.45 (1H, d, *J*=8Hz, H-anthranilic), 7.97 (1H, d , H-anthranilic acid), 7.41(1H, t, *J*=8Hz , H-anthranilic), 7.28 (2H, s, $NH_2$), 7.05 (1H, t, *J*=8Hz , H-anthranilic), 4.01 (2H, s, $CH_2$).

***2-(2-(5-Acetamido--thiadiazol-2-ylthio) acetamido) benzoic acid (7)***

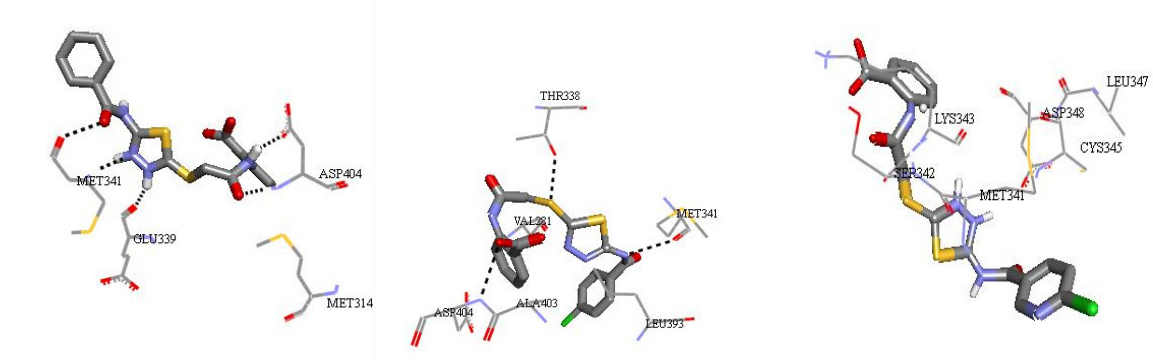
Yield: 46%, yellow, m.p. 240-242°C, IR ($\nu_{max}$ $cm^{-1}$), 3442 (OH), 3157 (NH), 3047 (C-H, Ar), 2924 (C-H, Aliphatic), 1734, 1685 , (C=O), $^1$HNMR: (DMSO- $d_6$): δ 13.67 (1H, broad, COOH), 12.6 (1H,s, NH), 11.75 (1H, s, NH), 8.50 (1H, d, *J*=8Hz, H-anthranilic), 7.97 (1H, d , H-anthranilic acid), 7.60 (1H, t, *J*=8Hz , H-anthranilic), 7.18 (1H, t, *J*=8Hz , H-anthranilic), 4.26 (2H, s, $CH_2$), 2.16 (3H, s, $CH_3$).

***2-(2-(5-(2-Chloronicotinamido)- thiadiazol-2-ylthio) acetamido) benzoic acid (8)***

Yield: 39 %, gray, m.p 262-264 °C, IR ($\nu_{max}$ $cm^{-1}$), 3541(OH), 3323 (NH) , 3100 (C-H, Ar), 2908 (C-H, Aliphatic), 1723, 1664 (C=O), $^1$HNMR: (DMSO- $d_6$): δ 11.82 (1H, broad, COOH), 9.04 (1H, s, H-pyridine),

**Table 2.** Energy interactions and hydrogen bonds for compounds docked into kinase domain crystallographic structure of cSrc

| No. | $\Delta G_{bind}$ (Kcal/mol) | Hydrogen bond (Distance, Å) |
|---|---|---|
| **5a** | -8.05 | Asp 404 (1.7Å) (1.84 Å), Met 341 (1.08 Å),Glu 339 (1.08 Å) |
| **5b** | -7.29 | Asp 404 (1.9 A), Met 341 (2.01 Å), Thr 338(1.08 Å) |
| **6** | -5.92 | - |
| **7** | -6.37 | Lys 343 (1.79 A) ,Cys 345 (2.03) |
| **8** | -6.04 | - |
| **11** | -7.33 | Cys 345 (2.1 A), Asp 348 (1.97) |




A **B** C

**Figure 3.** Conformation of compounds (A) **5a**, (B) **5b**, and (C) **8** in the binding site of cSrc kinase.

8.52 (1H, d, *J*=8Hz, H-Ar), 8.45 (1H,d, *J*= 8HZ, H-pyridine),7.98 (1H, d, *J*=8Hz, H-Ar), 7.74 (1H, d, *J*= 8HZ, H-pyridine),7.61 (1H, t, *J*=8Hz, H-Ar), 7.19 (1H, t, *J*=8Hz, H-Ar), 4.31(2H, s, $CH_2$); MS ( m/z, %): 449 ($M^+$), for $C_{17}H_{12}ClN_5O_4S_2$, M.W. 449g/mol .

***4-Chloro-N-(5-(2-(2,5-dichlorophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)benzamide (11)*** Yield:64%, yellow , m.p.281-283°C, IR ($\nu_{max}$ $cm^{-1}$), 3221 (NH) , 2965 (C-H, Aliphatic), 1666, 1658 (C=O) $^1$HNMR: (DMSO-$d_6$): δ10.23 (1H, s, NH)**,** 10.05 (1H, s, NH), 8.23 (2H, d, *J*=8Hz, H-Ar), 7.76 (1H, d, *J*=8Hz, H-Ar), 7.73-7.71 (2H, m, H-Ar), 7.57 (2H, d, *J*=8Hz, H-Ar), 4.33 (2H, s, $CH_2$).

Substituted 1, 3, 4-thiadiazole, including amide (3), s-alkylate (3, 4) and hybrid analogues have been shown anticancer activity in literature surveys. Some of the anthranilic acid-based diamide derivatives were constructed as potential anticancer (21). Derivatives of thiadiazole containing both amide (3) and s-alkylate moiety showed prominent cytotoxic effects (3, 4, 17).Based on these considerations, structural modications were started by preserving region 2 and changing regions 1 and 3 of hit compound shown in Figure 1 (B). 5-Amino-thiadiazole-2-thiol as starting material was treated with corresponding acyl chlorides to obtain amid derivatives. The resultants were reacted with intermediate **4** prepared according to the previously reported method (22) to afford the relevant final products **5a** and **5b**. 5-Amino-thiadiazole-2-thiol was reacted with intermediate **4** to yield the intermediate **6**. The reaction of amine group of intermediate **6** with glacial acetic acid or 6-chloronicotinoyl chloride afforded the corresponding target compounds**7** and **8** respectively. S-alkylation reaction of intermediate **10** with amid derivative of thiadiazole **2b** produced final compound **11**. Compounds were confirmed by IR, $^1$HNMR spectral. The IR spectrum of the final compounds containing anthranilic acid showed absorption bands around 3667 – 3300 $cm^{-1}$ for (COOH) and 3323-3157$cm^{-1}$ for NH. Absorption peaks C=O for acid was seen in the range 1775-1723 $cm^{-1}$ and C=O for amide was identified in the range 1664-1685 $cm^{-1}$. The$^1$ H-NMR results showed that all the aryl protons presented at approximately 9.04–7.05ppm, and the characteristic -S-methylene group were observed at 4.54-4.01 ppm. The compounds were subsequently tested against MCF7, HeLa and Huvec cell lines. According to Table 1, compounds **5a**, **5b** and **8** rendered remarkable cytotoxic effects against HeLa cell line with $IC_{50}$‹ 60 µM. Compounds **5a** and **8** showed higher cytotoxicity against HeLa cell line and lower cytotoxicity against MCF-7. Chlorine atom at para position of the phenyl ring in compound **5b** increased activity against MCF-7 cell line. Compound **6** with amine group on thiadiazole ring had no activity against tested cell lines. This suggested that the presence of an amide region on thiadiazole scaffold is essential for cytotoxic activity. Radi et al and Mohammadi-Farani were individually reported cytotoxic activities of thiadiazole amide derivatives as potent ABL tyrosine kinase inhibitors (16, 26).

The difference between our work and Radi's study is in s-alkylate moiety of thiadiazole. Radi et al showed that less active compounds were characterized by the presence of an ortho-chloro substituent on the benzamide moiety (16).

Comparison of $IC_{50}$ values in case of compounds **7**, **5a**, **5b** and **8** showed that presence heterocyclic or phenyl substitutions at amide region can increase activities against HeLa cell line. In another variation, replacement of anthranilic acid with 2, 5 dichloroaniline in compound **5b** led to decreased activity in the compound**11**. Comparing the cytotoxicity results of intermediate 6 with other derivatives, showed that the hybridization of anthranilic acid with thiadiazole is not sufficient for cytotoxic effects, and the presence of amid moiety in thiadiazole is also necessary.

All compounds expect **8** and **5b** showed $IC_{50}$>50 on Huvec cells. Due to the cytotoxic effects on normal cell lines, target therapy should be considered. Docking studies have been performed to identify possible interactions of derivatives with kinase domain of Src. Appraised free binding energy values (kcal/mol), and the interactions with key amino acid residues at the active site of enzymes are declarated in Table 2 and Figure 3. The highest dock score belongs to compound 5**a** which Asp 404 , Met341 , and Glu 339 residues have been detected for hydrogen bind constitution with nitrogen atoms of the 1,3,4-thiadiazole ring and amide regions . Asp 404, Met341 residues are also responsible formation hydrogen binding in compound **5b**. Although the hybridized anthranilamide derivative participated in the hydrogen bind interactions, but the hydrogen bind interactions of the thiadiazole amide part with the receptor are also necessary.

In similar study conducted by Mohammadi-Farani et al Asp 404 was detected as important residue in hydrogen binding interactions of thiadiazole derivatives and Src tyrosine kinase (26). Rady et al detected hydrogen binds formation between Met 318 and nitrogen atoms, amide regions of the 1, 3, 4-thiadiazole ring (16). The thiadiazole derivatives synthesized by Radi *et al* showed an interaction in the active site of ABL tyrosine kinase, similar to imatinib. Regardless of the structure of imatinib and prepared

derivatives, thiadiazole derivatives mimic the pharmacophoric portion of imatinib in the receptor site (26).

### *Conclusion*

Thiadiazole amide derivatives containing anthranilic acid was synthesized and their cytotoxic properties were evaluated by MTT assay. Compound **5b** with para chlophenyl moiety has the best cytotoxic effects against both cell lines. Compound **5a** without any electron withdrawing and donating moiety, indicated the highest anticancer activity toward HeLa cell lines ($IC_{50}$= 31μM). 2,5 Dichloroaniline moiety as applied in compound **5b** instead of anthranilic acid caused an decrease in the cytotoxic potency against both cancer cell lines. It can be concluded that both nature and the presence of amides and the presence of antranilic acid contribute to the cytotoxic activity.

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