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Synthesis, Docking and Cytotoxicity Evaluation of *N*-(5-(Benzyl-Thio)-1,3,4-Thiadiazol-2-yl)-2-(3-Methoxyphenyl)Acetamide Derivatives as Tyrosine Kinase Inhibitors With Potential Anticancer Activity

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A B S T R A C T

In the recent years, targeted therapy of the neoplastic diseases is a current strategy used by oncologists. Hence, design and discovery of novel targeted anticancer therapeutics is an interesting topic in the current research of medicinal chemistry. A new series of 1,3,4-thiadiazole derivatives were prepared and their anticancer activity was assessed against PC3, SKNMC and HT29 cell lines by application of the MTT assay. Compound **3e** with *para* positioning of the methoxy moiety demonstrated the highest inhibitory potency against PC3 (IC_{50} = 22.19 ± 2.1 µM**)** and SKNMC (IC_{50} = 5.41 ± 0.35 µM**)** cell lines in this series. This compound rendered a superior cytotoxic activity than imatinib. Compound **3f** with *ortho* positioning of the fluorine displayed the most cytotoxic activity against HT29 cell line compared to other tested derivatives (IC_{50} = 12.57 ± 0.6 µM). Molecular docking studies on Abl as well as Src tyrosine kinases were also performed and potential hydrogen bindings were observed for ligand-receptor interaction.

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Introduction

Tyrosine kinases are enzymes that catalyze the transfer of the phosphate group from adenosine triphosphate (ATP) to target proteins [1-4]. Protein tyrosine kinases (PTKs) have pivotal role in cellular processes like proliferation, differentiation, death, motility, etc in multicellular organisms. Receptor tyrosine kinases (RTKs) and nonreceptor tyrosine kinases (NRTKs) are the two main categories of PTKs. various forms of neoplastic conditions and also benign proliferative diseases can be presented due to point mutations or overexpression of these enzymes [5]. Standard cytotoxic agents for treating cancer were developed based on their effectiveness to kill rapidly dividing cells, not on their ability to selectively kill cancer cells and spare normal tissue. Much of contemporary cancer research is aimed at identifying specific molecular features of cancers to directly target tumor cells with the hope of reducing or eliminating unwanted side effects. Targeted therapy for the treatment of cancer can be divided into two main categories: monoclonal antibodies and small molecules[6] .This targeted approach, predominantly via inhibition of tyrosine kinase activity, has markedly improved the management of cancers including chronic myeloid leukemia (CML), breast cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC), and colon carcinoma. Inhibitors of tyrosine kinases are of two classes: monoclonal antibodies (mAbs), typically targeting growth factor receptor tyrosine kinases, and small molecules, referred to as TKIs (tyrosine kinase inhibitors), targeting both receptor and non-receptor tyrosine kinases (**Fig. 1**). The goal of targeted therapy is to improve anti-tumor activity with fewer toxic side-effects [7] .Resistance is a major problem because it can develop at any time and lead to disease progression. Hence, new inhibitors are required to deal with this problem[8] .In recent years, numerous small molecules with potential activity for tyrosine kinase inhibition have been reported (**Fig. 2**) [9-12] .In the present study we concentrated on the development of some tyrosine kinase inhibitors containing 1,3,4-thidiazole in the main pharmacophore.

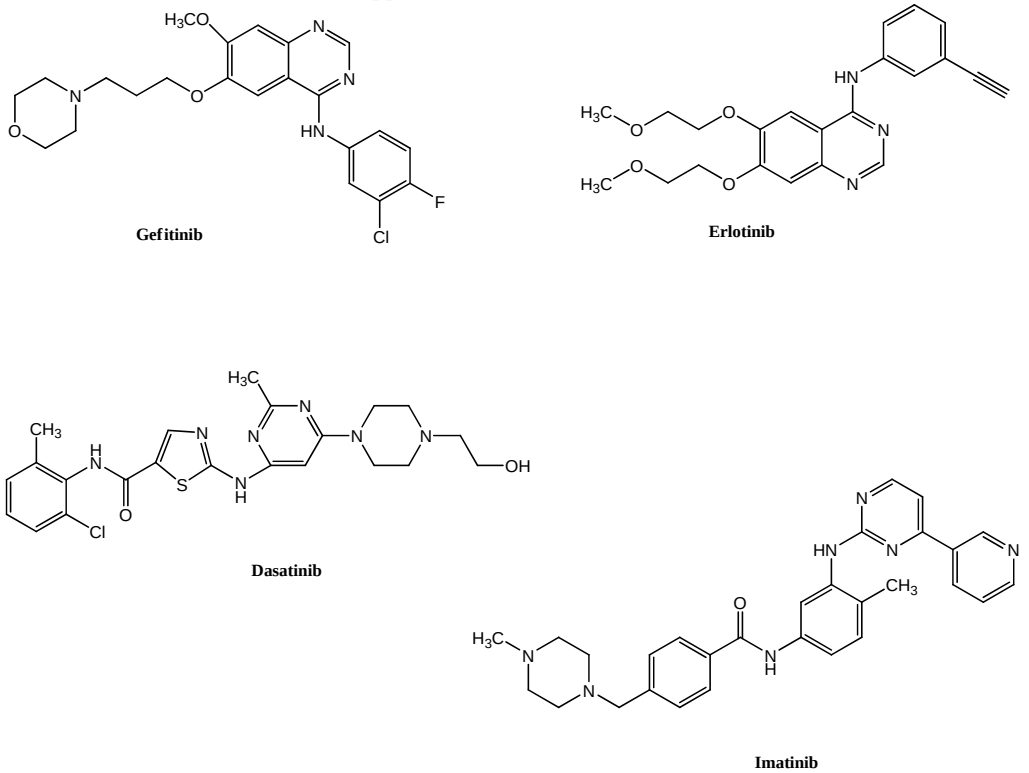


Fig. 1. Structures of four tyrosine kinase inhibitors (gefitinib,erlotinib, dasatinib and imatinib) as anticancer agents in the clinic for targeted therapy.

A comprehensive investigation of different pharmacophores and chemical classes containing 1,3,4-thiadiazole ring have been carried out during recent years. Many of these derivatives possess

interesting and potential biological effects such as antimicrobial, antitubercular, antiviral, antiinflammatory, anticonvulsant, antihypertensive, antioxidant, antifungal and anticancer activity and now there are in the market as common used drugs such as cefalothin and acetazolamide[13-16].

Diverse chemical structures containing 1,3,4-Thiadiazole nucleus have been reported with potential anticancer activity. The 1,3,4-thiadiazole ring in anticancer agents performs its role in pharmacophroes of apoptosis inducers and caspase activators, tyrosine kinase inhibitors, carbonic anhydrase inhibitors and etc[17-19] .Hence, various mechanisms could be imagined for anticancer chemical structures that contain the 1,3,4-thiadiazole ring. Recently, we reported some 1,3,4-thiadiazole derivatives with potential anticancer activity (**Fig. 3**) [20, 21].Therefore, in order to develop and continue our investigation, a new series of 1,3,4-thiadiazole derivatives were designed, synthesized and their cytotoxic effects were assessed *in vitro*.

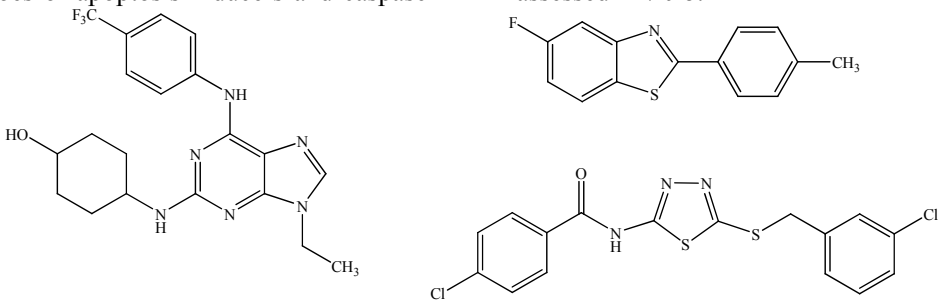
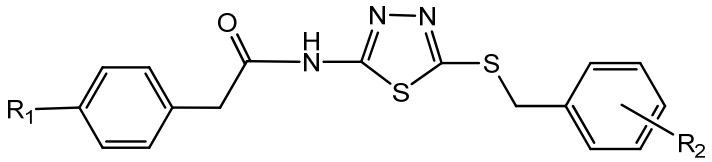


Fig. 2. Structure of three small molecules as potential tyrosine kinase inhibitors.



R_1: -OCH_3, -CF_3

R_2: F, Cl, NO_2, -OCH_3

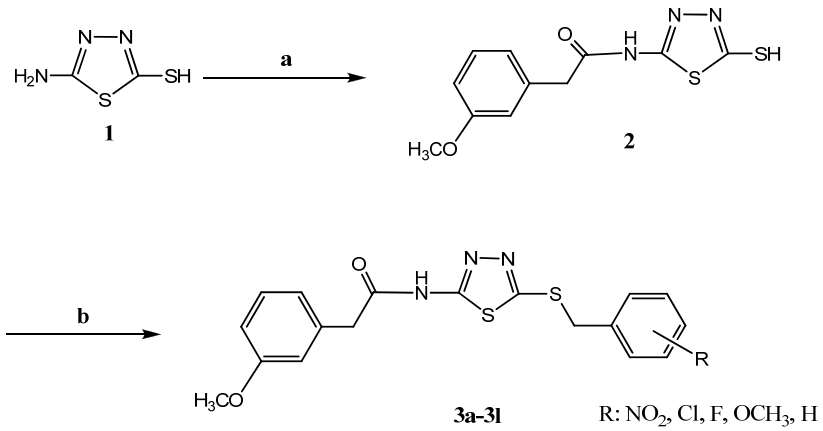
Fig. 3. Structure of 1,3,4-thiadiazole based derivatives as potential anticancer agents hat previously reported.

Material and Methods

Chemistry

The preparation of all compounds was done according to the **scheme 1**. All chemical substances, reagents and solvents were purchased from Merck and Sigma-Aldrich companies. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} plates were applied for analytical TLC. Column chromatography was performed on Merck silica gel (70-230 mesh) to purify the intermediate and final compounds. ^{1}H-NMR spectra were recorded using a Bruker 250 spectrometer, and chemical shifts were expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). Melting points were determined by electrothermal melting point analyzer and were uncorrected. The

mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV.



Scheme 1. Synthesis of compounds **3a-3l**. Reagnets and conditions: a) *m*-methoxyphenylacetic acid, EDC, HOBt, CH_3CN, rt, 24 h, b) Benzyl chloride derivatives, EtOH, KOH, reflux, 20-30 h.

Synthesis of N-(5-Mercapto-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (2)

In a flat bottom flask 3 g (0.018 mol) of 4-methoxyphenylacetic acid, 3.37 g (0.018 mol) of *N*-ethyl-*N*-dimethylaminopropyl- carbodiimide (EDC) and 2.4 g (0.018 mol) of hydroxybenzotriazole (HOBt) were added to 50 ml of acetonitrile as solvent. The reaction mixture was stirred at room temperature for 30 minutes and then 2.4 (o.o18 mol) of 5-amino-1,3,4-thiadiazole-2-thiol (**1**) was added to the reaction medium and stirring was continued for 24 hours. The reaction completion was confirmed by thin layer chromatography (TLC). The acetonitrile was evaporated under reduced pressure and 100 ml of ethyl acetate and water (1:1) were added. The aqueous phase was separated and the organic phase was washed two times by alkaline solution of sodium bicarbonate 5%, diluted sulfuric acid (2%), and saturated sodium chloride solution (Brine). Anhydrous sodium sulfate was used for drying the organic phase. Then, the sodium sulfate was filtered and the ethyl acetate was evaporated using rotary evaporator apparatus. The obtained yellow powder was washed by diethyl ether and purified by column chromatography (Ethyl acetate/Petroleum ether 3:2). The spectroscopic data was compared with previous synthesized and reported information [22, 23].

General procedure for the synthesis of compounds 3a-3l

In a flat bottom flask 200 mg (0.71 mmol) of compound **2**, 39 mg (0.71 mmol) potassium hydroxide, and 20 ml absolute ethanol were mixed. The prepared mixture was heated and stirred for 5 minutes and then the equimolar amount of appropriate benzyl chloride derivative was added to the reaction medium. The reaction mixture was refluxed for 20-30 h. The completion of the reactions was approved by TLC. After completion, cool water was added to the reaction medium and the reaction flask was put in a water/ice container to perform extra cooling and also to enhance the yield of precipitation. The related precipitate filtered and washed by cool water. Column chromatography was done by EtOAc/petrol- eum ether if needed.[24]

2-(3-Methoxyphenyl)-N-(5-(2-nitrobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (3a)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.71 (s, 3H, -OCH_3), 3.75 (s, 2H, -CH_2CO-), 4.72 (s, 2H, -S-CH_2-), 6.81 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.21 (t, 1H, H_5-3-Methoxyphenyl), 7.42 (t, 1H, H_4-2-Nitrophenyl), 7.69 (m, 2H, 2-Nitrophenyl), 8.02 (d, 1H, *J* = 7.5 Hz, H_6-3-Methoxyphenyl), 8.13 (d, 1H, *J* = 7.5 Hz, H_3-2-Nitrophenyl), 12.85 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3429, 3329, 3155, 3093, 3035, 2924, 2850, 1701, 1597, 1566, 1527, 1442, 1342, 1307, 1261, 1168,

1091, 1037, 941, 871, 790, 740. MS (*m/z*, %): 416 (M^+, 10), 268 (10), 148 (100), 121 (60), 91 (20), 77 (30), 63 (10).

2-(3-Methoxyphenyl)-N-(5-(3-nitrobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (3b)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2CO-), 4.60 (s, 2H, -S-CH_2-), 6.80 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.85 (s, 1H, H_2-3-Methoxyphenyl), 7.20 (t, 1H, H_4-3-Methoxyphenyl), 7.40 (t, 1H, 3-Nitrophenyl), 7.95 (d, 1H, H_6-3-Methoxyphenyl), 8.26 (d, 1H, H_4-3-Nitrophenyl), 8.38 (s, 1H, H_2-3-Nitrophenyl), 12.95 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3433, 3151, 3051, 2927, 2854, 1693, 1608, 1531, 1442, 1350, 1319, 1261, 1238, 1153, 1091, 1049, 831, 810, 748, 729. MS (*m/z*, %): 416 (M^+, 35), 268 (10), 148 (100), 121 (45), 91 (25), 77 (30), 63 (10).

2-(3-Methoxyphenyl)-N-(5-(4-nitrobenzylthio)-1,3,4-thiadiazol-2-yl)acetamide (3c)

^{1}H NMR (DMSO-d_6 , 250 MHz) δ:3.77(s, 2H, -CH_2CO-), 3.79 (s, 3H, -OCH_3), 4.57 (s, 2H, -S-CH_2-), 6.82 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.91 (s, 1H, H_2-3-Methoxyphenyl), 7.23 (t, 1H, H_5-3-Methoxyphenyl), 7.99 (d, 1H, H_6-3-Methoxyphenyl), 7.64 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-Nitrophenyl), 8.15 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-Nitrophenyl), 12.80 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3433, 3332, 3159, 3047, 2920, 2850, 1689, 1600, 1566, 1519, 1346, 1261, 1149, 1091, 1049, 964, 875, 852, 740. MS (*m/z*, %): 416 (M^+, 15), 268 (10), 193 (10), 148 (100), 121 (90), 106 (10), 91 (30), 77 (30), 63 (10).

N-(5-(3-methoxybenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3d)

^{1}H NMR (DMSO-d_6, 250 MHz) δ:3.69 (s, 3H, -OCH_3), 3.71 (s, 3H, -OCH_3), 3.73 (s, 2H, -CH_2CO-), 4.41 (s, 2H, -S-CH_2-), 6.86 (m, 5H, Aromatic), 7.21 (m, 2H, Aromatic). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3429, 3329, 3151, 3035, 2997, 2927, 2850, 1689, 1608, 1562, 1512, 1492, 1458, 1438, 1350, 1300, 1249, 1172, 1053, 821, 763, 690. MS (*m/z*, %): 401 (M^+, 40), 368 (15), 148 (15), 121 (100), 91 (35).

N-(5-(4-Methoxybenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3e)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.69 (s, 3H, -OCH_3), 3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2-CO-), 4.39 (s, 2H, -S-CH_2-), 6.85 (m, 4H, Aromatic), 7.27 (d, 4H, Aromatic), 12.80 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$:3421,3329, 3159, 3039, 3008, 2927, 2846, 1705, 1600, 1566, 1492, 1462, 1435, 1354, 1303, 1269, 1176, 1091, 1045, 968, 871, 840, 790, 759, 729, 690.MS (*m/z*, %): 401 (M^+, 40), 368 (10), 148 (10), 121 (100), 91 (15).

N-(5-(2-Fluorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3f)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.71 (s, 3H, -OCH_3), 3.75 (s, 2H, -CH_2CO-), 4.45 (s, 2H, -S-CH_2-), 6.80 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.12-7.50 (m, 5 H, Aromatic), 8.01 (d, 1H, H_6-3-Methoxyphenyl), 12.80 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3448, 3329, 3147, 3039, 2924, 2850, 1693, 1597, 1558, 1492, 1454, 1408, 1342, 1288, 1261, 1230, 1172, 1087, 1037, 960, 871, 821, 763. MS (*m/z*, %): 389 (M^+, 55), 376 (40), 241 (15), 166 (45), 148 (50), 121 (45), 109 (100), 91 (20), 83 (10).

N-(5-(3-Fluorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3g)

^{1}H NMR (DMSO-d_6, 250 Hz) δ: 3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2CO-), 4.46 (s, 2H, -S-CH_2-), 6.81 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.15 (t, 1H, H_4-3-Methoxyphenyl), 7.19-7.55 (m, 4H, 3-Fluorophenyl), 8.01 (d, 1H, H_6-3-Methoxyphenyl), 12.90 (brs, NH). IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3329, 3259, 3147, 3005, 2927, 2850, 1693, 1616, 1585, 1554, 1489, 1450, 1408, 1350, 1292, 1265, 1153, 1095, 1053, 937, 871, 786, 748. MS (*m/z*, %): 389 (M^+, 35), 376 (30), 241 (30), 166 (15), 148 (70), 121 (55), 109 (100), 91 (25), 83 (10).

N-(5-(4-Fluorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3h)

^{1}H NMR (DMSO-d_6, 250 MHz) δ:3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2CO-), 4.44 (s, 2H, -S-CH_2-), 6.81 (d, 1H, *J* = 7.5 Hz, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.17 (m, 3H, Aromatic), 7.40 (m, 2H, Aromatic), 7.99 (d, 1H, *J* = 10 Hz, H_6-3-

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Methoxyphenyl), 12.95 (brs, NH). IR (KBr, cm^{-1}) $\bar{U}$: 3325, 3151, 3047, 2927, 2850, 1693, 1604, 1566, 1508, 1442, 1354, 1296, 1265, 1230, 1153, 1091, 1053, 956, 929, 864, 833, 794, 744. MS (*m/z*, %): 389 (M^+, 70), 376 (50), 241 (20), 208 (10), 166 (30), 148 (60), 121 (80), 109 (100), 91 (25), 83 (15).

N-(5-(2-Chlorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3i)

^{1}H NMR (DMSO-d_6, 250 MHz) δ:3.71 (s, 3H, -OCH_3), 3.75 (s, 2H, -CH_2-CO-), 4.52 (s, 2H, -S-CH_2-), 6.83 (d, 1H, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.22 (d, 1H, J = 7.5 Hz, H_6-3-Methoxyphenyl), 7.26 (m, 3H, Aromatic), 7.38-7.55 (m, 4H, Aromatic), 7.99 (d, 1H, J = 7.5 Hz, H_3-2-Chlorophenyl), 12.90 (brs, NH). IR (KBr, cm^{-1}) $\bar{U}$: 3428, 3271, 3143, 3055, 2997, 2916, 2854, 1697, 1597, 1554, 1492, 1469, 1435, 1354, 1265, 1168, 1091, 1053, 964, 924, 867, 763, 744, 686. MS (*m/z*, %): 407 (M^++2, 15), 405 (M^+, 40), 257 (45), 224 (15), 182 (15), 148 (70), 125 (100), 123 (90), 91 (30).

N-(5-(3-Chlorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3j)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.78 (s, 3H, -OCH_3), 3.94 (s, 2H, -CH_2CO-), 4.41 (s, 2H, -S-CH_2-), 7.21-7.43 (m, 7H, Aromatic), 7.98 (d, 1H, J = 7.5 Hz, H_4-3-Chlorophenyl), 11.95 (brs, NH). IR (KBr, cm^{-1}) $\bar{U}$: 3429, 3325, 3151, 3062, 2927, 2850, 1693, 1624, 1577, 1477, 1435, 1357, 1303, 1242, 1153, 1095, 1080, 1053, 933, 883, 786, 767, 744, 686. MS (*m/z*, %): 407 (M^++2, 20), 405 (M^+, 55), 257 (25), 182 (35), 148 (55), 125 (100), 123 (75), 91 (40).

N-(5-(4-Chlorobenzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3k)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2CO-), 4.44 (s, 2H, -S-CH_2-), 6.82 (d, 1H, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.22 (t, 1H, H_5-3-Methoxyphenyl), 7.39 (m, 4H, 4-Chlorophenyl), 8.01 (d, 1H, 3-Methoxyphenyl), 11.80 (brs, NH). IR (KBr, cm^{-1}) $\bar{U}$: 3441, 3329, 3151, 3051, 2927, 2850, 1693, 1558, 1489, 1442, 1404, 1350, 1319, 1300, 1265, 1238, 1153, 1087, 1053, 929, 856, 813, 763, 744. MS (*m/z*, %): 407 (M^++2, 30), 405 (M^+, 55), 257 (20), 224 (10), 182 (25), 148 (90), 125 (100), 123 (90), 91 (30).

N-(5-(Benzylthio)-1,3,4-thiadiazol-2-yl)-2-(3-methoxyphenyl)acetamide (3l)

^{1}H NMR (DMSO-d_6, 250 MHz) δ: 3.71 (s, 3H, -OCH_3), 3.74 (s, 2H, -CH_2CO-), 4.44 (s, 2H, -S-CH_2-), 6.80 (s, 1H, J = 7.5 Hz, H_4-3-Methoxyphenyl), 6.86 (s, 1H, H_2-3-Methoxyphenyl), 7.18-7.49 (m, 5H, Aromatic), 8.01 (d, 1H, H_6-3-Methoxyphenyl), 12.80 (brs, NH). IR (KBr, cm^{-1}) $\bar{U}$: 3325, 3159, 3032, 3008, 2931, 2912, 2846, 1732, 1693, 1624, 1597, 1570, 1492, 1454, 1404, 1346, 1296, 1265, 1172, 1087, 1037, 960, 871, 837, 794, 756, 698.

Cytotoxicity evaluation

Diverse derivatives of 1,3,4-thiadiazole (compounds **3a-3l**) were tested for cytotoxic activity at 0.1-250 µg/mL concentration in three human cancer cell lines of PC3 cell (prostate cancer), SKNMC (neurobalstoma), and HT29 (colorectal cancer). Cells from different cell lines were seeded in 96-well plates at the density of 8000-10,000 viable cells per well and incubated for 48 h to allow cell attachment. The cells were then incubated for another 48-96 h (depending on cell cycle of each cell line) with various concentrations of compounds **3a-3l**. Cells were then washed in PBS, and 20 µL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution (5 mg/mL) were added to each well. An additional 4 h of incubation at 37°C were done, and then the medium was discarded. Dimethyl sulfoxide (60 µL) was added to each well, and the solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020; Austria) at a test wavelength of 550 nm against a standard reference solution at 690 nm. The amount of produced purple formazan is proportional to the number of viable cells [22, 24].

Docking

ArgusLab 4.0 software was applied to perform molecular docking studies [25, 26].All intended ligands were constructed in arguslab workspace and all ligands **3a-3l** were energy minimized by AM1 as semiemperical method. The pdb file of Abl tyrosine kinase in complex with imatinib (pdb code: 3K5V) and Src tyrosine kinase in complex with dasatinib (pdb code: 3G5D) were downloaded from brookhaven protein databank [27] .The geometry optimization of structure of each enzyme was performed using universal force field (UFF) as a

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molecular mechanic method. The docking process was done for all ligands in the workspace of Argus Lab 4 software after defining the related groups for each ligand and also for related proteins. The binding location of imatinib as well as dasatinib were defined as binding site for finding the best pose and conformation for all ligands. Binding mode and related interactions of all ligands were explored in ArgusLab software (**Fig.4**).

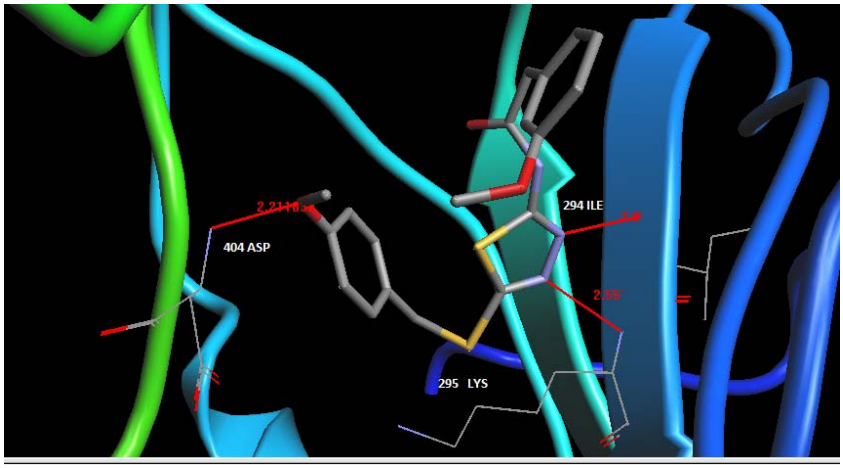

Fig. 4. Interaction of compound **3e** (4-OCH_3) into the active site of Src tyrosine kinase (pdb code: 3G5D). Three hydrogen bind interactions were observed with Asp 404, Lys 295 and Ile 294.

Results and Discussion

Chemistry

A new series of 1,3,4-thiadiazole based anticancer derivatives were synthesized (**Table 1**) and their cytotoxic activity was assessed using MTT protocol *in vitro* (**Table 2**). Amidation of the 3-methoxyphenylacetic acid was carried out with 5-amino-1,3,4-thiadiazole-2-thiol by utilization of the *N*-ethyl-*N*-dimethylaminopropylcarbodiimide (EDC) as coupling reagent to achieve the intermediate thiol derivative (**2**). In fact, EDC facilitates the coupling process of the acidic group with amine group of the 5-amino-1,3,4-thiadiazole-2-thiol (**Scheme 1**). Hydroxybenzotriazole (HOBt) was also applied as additive agent to prevent the formation of the *N*-acylureas as side products of this reaction. For affording the final derivatives **3a-3l**, compound **2** was treated by potassium hydroxide (KOH) to form a thiolate anion and then appropriate benzyl chloride derivative was added to the reaction medium for benzylation. Spectroscopic data consisting of ^{1}HNMR, IR and MS of the intermediate as well as final derivatives were prepared and provided. Physicochemical properties and other characteristics of the prepared compounds were listed in **Table 1**. Closed formulas, molecular weight (MW) and melting points (mp) were calculated for all compounds. Yield of synthesis was also calculated and presented as percentage (%).

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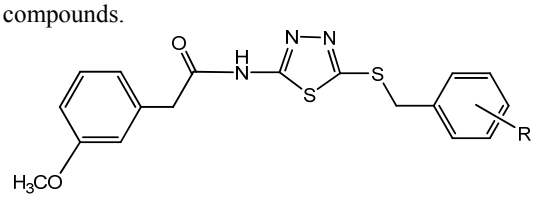
Table 1. Properties of synthesized compounds.



Compounds	R	Closed Formula	MW (g/mol)	mp (°C)	Yield (%)
2	-	$C_{11}H_{11}N_3O_2S_2$	281	98	71
3b	2-NO_2	$C_{18}H_{16}N_4O_4S_2$	416	94	52
3c	3-NO_2	$C_{18}H_{16}N_4O_4S_2$	416	123	66
3d	4-NO_2	$C_{18}H_{16}N_4O_4S_2$	416	146	56
3e	3-OCH_3	$C_{19}H_{19}N_3O_3S_2$	401	153	23
3f	4-OCH_3	$C_{19}H_{19}N_3O_3S_2$	401	115	26
3g	2-F	$C_{18}H_{16}N_3O_2FS_2$	389	122	33
3h	3-F	$C_{18}H_{16}N_3O_2FS_2$	389	90	27
3i	4-F	$C_{18}H_{16}N_3O_2FS_2$	389	66	21
3j	2-Cl	$C_{18}H_{16}N_3O_2ClS_2$	405	85	45
3k	3-Cl	$C_{18}H_{16}N_3O_2ClS_2$	405	63	49
3l	4-Cl	$C_{18}H_{16}N_3O_2ClS_2$	405	95	53
3a	H	$C_{18}H_{17}N_3O_2S_2$	371	111	24

Structure Activity Relationship (SAR)

Cytotoxicity of the final derivatives **3a-3l** was measured by MTT assay and obtained results were provided as IC_{50} ± SD (μM) in **Table 2**. PC3 (Prostate carcinoma), SKNMC (Neuroblastoma),and HT29 (Colorectal cancer) were selected as representative cell lines. Inhibitory potency of the tested compounds was compared with imatinib as reference drug. Various electron withdrawing (F, Cl, NO_2) and also electron donating substituents (-OCH_3) were introduced on the phenyl residue to investigate the role and impact of the electronic and steric effects of the moiety on this part of the molecule. Compound **3e** with *para* positioning of the methoxy moiety demonstrated the highest inhibitory potency against PC3 (IC_{50} = 22.19 ± 2.1 μM**)** and SKNMC (IC_{50} = 5.41 ± 0.35 μM**)** cell lines in this series. This compound rendered a superior cytotoxic activity than imatinib. According to this evidence, it is likely that electron donating property of the methoxy substituent is so effective for interaction with related receptor. Nitro containing derivatives (**3a, 3c**) at position *ortho* and *para* were also very effective towards SKNMC cell line and exerted better cytotoxic potency in comparison with imatinib (18.57 ± 2.7 μM). Capability of the free radical generation by nitro moiety maybe the probable cytotoxic mechanism for this group. Compound **3f** with *ortho* positioning of the fluorine displayed the most cytotoxic activity against HT29 cell line compared to other tested derivatives (IC_{50} = 12.57 ± 0.6 μM). Only this derivative was more active than imatinib (18.1 ± 2.6 μM) towards HT29 cell line. Movement of the fluorine atom to the other possible positions of the phenyl residue reduced the anticancer activity remarkably.

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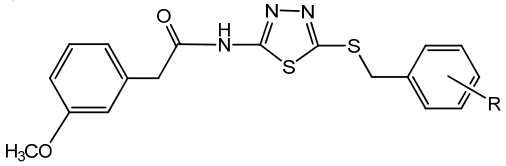
Table 2. Cytotoxicity results (IC_{50}±SD, µM) of compounds **3a-3l** against PC3 (prostate carcinoma), HT29 (Colorectal cancer) and SKNMC (Neuroblastoma) cell lines.



Compounds	R	PC3	SKNMC	HT29
3a	**2-NO_2**	96.47 ± 5.2	**10.29 ± 1.1**	>200
3b	**3-NO_2**	126.5 ± 3.9	85.49 ± 6.3	99.14 ± 5.9
3c	**4-NO_2**	24.01 ± 1.3	**6.26 ± 0.7**	100.40 ± 7.3
3d	**3-OCH_3**	67.38 ± 3.4	52.74 ± 4.2	56.53 ± 2.6
3e	**4-OCH_3**	**22.19 ± 2.1**	**5.41 ± 0.35**	62.24 ± 3.4
3f	**2-F**	38.30 ± 3	89.28 ± 9.3	**12.57 ± 0.6**
3g	**3-F**	38.96 ± 2.5	81.38 ± 6.7	87.39 ± 5.5
3h	**4-F**	66.03 ± 5.1	63.88 ± 5.9	63.07 ± 2.1
3i	**2-Cl**	63.72 ± 2.4	24.99 ± 4.3	23.79 ± 0.8
3j	**3-Cl**	39.08 ± 3.1	44.95 ± 3.7	24.42 ± 1.6
3k	**4-Cl**	28.30 ± 3	40.43 ± 6	55.62 ± 3.6
3l	**H**	48.55 ± 4.1	51.65 ± 4.9	40.02 ± 1.2
Imatinib	-	25.17 ± 3.3	18.57 ± 2.7	18.1 ± 2.6

Docking

Molecular docking of synthesized molecules was carried out by Arguslab software to explore the probable binding mode and interaction of the intended ligands. In **Figure 3**, interaction of compound **3e** (4-OCH_3) into the active site of Src tyrosine kinase enzyme has been shown. Three potential hydrogen binding interactions have been detected for this ligand. Asp 404, Lys 295, and Ile 294 are the responsible amino acids for formation of hydrogen binds. Oxygen of the methoxy moiety of the benzyl residue has participated in an hydrogen bond interaction with Asp 404. Nitrogen atoms of the 1,3,4-thiadiazole ring has been formed two hydrogen bindings with Lys 295 and Ile 294. These hydrogen bonds maybe the probable reason for potent cytotoxic activity of the *p*-methoxy derivative.

Conclusion

According to the recent reports, a new series of 1,3,4-thiadiazole derivatives as tyrosine kinase inhibitors were prepared and their anticancer activity was evaluated by MTT assay. Compound **3e** with *para*methoxy substituent exhibited a significant cytotoxic effect against PC3 and SKNMC cell lines. Compounds **3a** (*o*-nitro) and **3c** (*p*-nitro) also displayed a remarkable activity towards SKNMC cell line. Compound **3f** (*m*-fluorine) was more active than imatinib against HT29 cell line. Molecular docking of the intended ligands was also done to explore the probable binding mode and interactions with tyrosine kinase receptors (Abl and Src). Based on the obtained results, synthesized 1,3,4-thiadiazole derivatives could be introduced as potential anticancer agents via inhibitory activity in tyrosine kinase pathways. However, further experimental assay such as *in vitro* inhibitory study against tyrosine kinases as well as *in vivo* investigation are necessary to prove and complete these findings.

Conflict of interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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