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## Synthesis of novel 1,3,4-thiadiazole analogues with expected anticancer activity

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### ABSTRACT

Synthesis of substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles **2a,b-6**, substituted 1,3,4-thiadiazolo[3,2-*a*]pyrimidines **7-9** and 1,3-disubstituted thioureas **10a-e** was reported. Structures of all synthesized compounds were elucidated using IR, NMR and mass spectroscopy. All the prepared derivatives were evaluated for their cytotoxic activity against tumor cell line A549 (Non-Small Cell Lung Cancer Cell Line) using Sulfo-Rodamine B (SRB) standard method. Most of the tested compounds exhibited potent cytotoxicity especially compounds **4**, **5**, **8** and **10b-d** ( $IC_{50}$  2.58-6.47  $\mu$ M). In order to find a molecular target for newly synthesized compounds, docking study was performed to explore the possible binding mode of these compounds with the binding site of fibroblast stromelysin-1 enzyme, which is involved in several pathological conditions including cancer.

**Keywords:** Imidazo[2,1-*b*]-1,3,4-thiadiazole, 1,3,4-Thiadiazolo[3,2-*a*]pyrimidine, Thiourea, Anticancer, A549 cell line.

### INTRODUCTION

Cancer is a leading cause of death worldwide. Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year [1]. Based on type and stage of cancer, treatments include surgery, radiation therapy, chemotherapy and targeted therapies [2]. Management of cancer still represents a major challenge in medicine despite of significant progress achieved in anticancer therapy. Therefore, the development of novel effective anticancer drugs and strategies is eagerly being pursued.

1,3,4-Thiadiazole derivatives possessed a wide range of therapeutic activities like antimicrobial [3], antifungal [4], antimycobacterial [5], antileishmanial [6], analgesic, antiinflammatory [7] antidepressant [8], antipsychotic [9] and anticonvulsant [9, 10]. 1,3,4-Thiadiazole derivatives exhibited interesting *in vitro* [11-13] and *in vivo* [14-17] antitumor activities. Different mechanisms of action were attributed to antitumor activity of 1,3,4-thiadiazole ring such as inhibited DNA and RNA syntheses specifically without appreciably affecting protein synthesis [18], inhibition of carbonic anhydrase [19], phosphodiesterase-7 (PDE7) [20], histone deacetylase [21] or as adenosine A3 receptor antagonists [22].

2-Amino-1,3,4-thiadiazole (ATDA, NSC4728) **I** (Fig. 1) and structurally related compounds had antitumor and uricogenic activity that can be prevented or reversed by nicotinamide [23-27]. In addition, phenyl-1,3,4-thiadiazole derivatives were found to have anticancer activity against different human cell lines [28, 29]. Substitution of 1,3,4-thiadiazole ring with both amino and phenyl groups resulted in compounds with promising anticancer activity against several cell lines [30, 31]. 2-(4-Fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT), **IIa**

(Fig. 1), is one of the most promising derivatives [32]. The newly synthesized derivative 4ClABT **IIIb** (Fig. 1), in addition to FABT **IIa** were not toxic to normal cells [32, 33]. Moreover, imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives **III**, **IV** (Fig. 1), were reported to possess antitumor activity against different human cell lines [34-36].

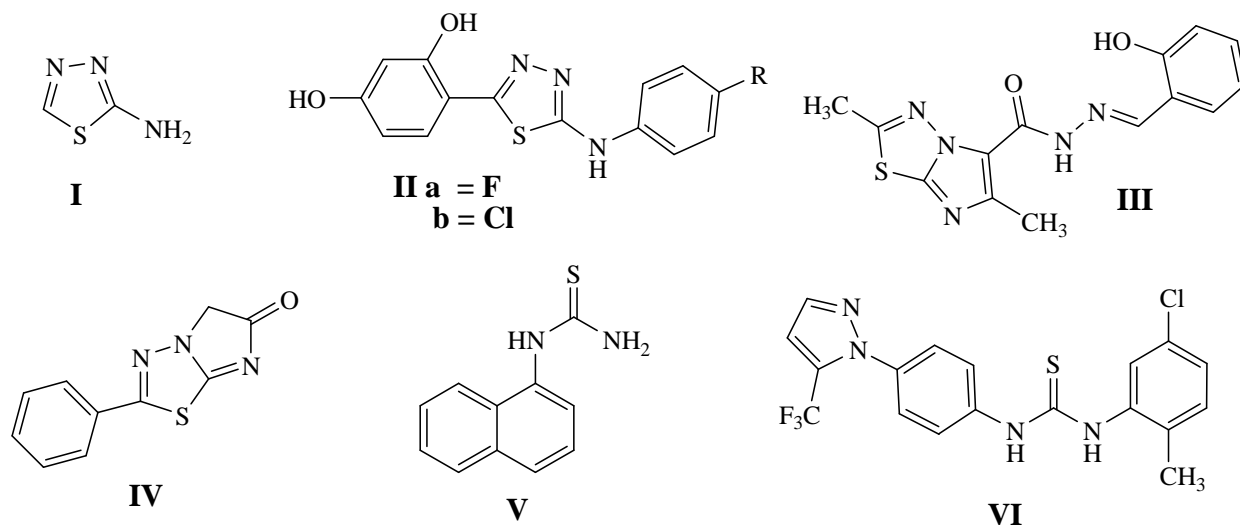


Fig. 1. Literature antitumor active compounds

Furthermore, use of thiourea derivatives was proved in treatment of cancer. It was observed that treatment of tumor-immunized rats with  $\alpha$ -naphthylthiourea (ANTU) **V** (Fig. 1) (although ANTU had no cytotoxic or immunosuppressive action) caused apparent "breakdown" of tumor immunity in 50% of rats due to a possible mechanism for the ANTU-induced decrease in innate resistance to growth of tumor in the lungs [37]. Also thiourea derivative (AW00178) **VI** (Fig. 1) was able to sensitize TRAIL-resistant human lung cancer H1299 cells to TRAIL-mediated apoptosis [38].

In view of the above mentioned facts, the 1,3,4-thiadiazole scaffold is selected as a building block for the design and synthesis of new potent antitumor agents. The present work describes the synthesis of condensed heterocyclic substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles **2a,b-6**, substituted-1,3,4-thiadiazolo[3,2-*a*]pyrimidines **7-9** and a structure hybrid comprised of thiourea and 1,3,4-thiadiazole as 1,3-disubstituted thioureas **10a-e**. All the newly synthesized compounds were evaluated for the antitumor properties of the prepared compounds against human tumor cell line A549 "Non-small cell lung cancer cell line". In addition, attempt to elucidate a molecular target for activity was achieved via molecular docking of the prepared compounds in the active site of fibroblast stromelysin-1 enzyme using Molecular Operating Environment (MOE).

## MATERIALS AND METHODS

### 2.1. Chemistry

Melting points were determined by open capillary tube method using Electrothermal 9100 melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. Microanalyses were carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded as potassium bromide discs on Shimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer.  $^1\text{H}$  spectra were run at 300 MHz and  $^{13}\text{C}$  spectra were run at 75.46 MHz in dimethylsulphoxide ( $\text{DMSO}-d_6$ ). Chemical Shifts are quoted in  $\delta$  as parts per million (*ppm*) downfield from tetramethylsilane (TMS) as internal standard and *J* values are reported in Hz. Mass spectra were performed as EI at 70eV on Hewlett Packard Varian (Varian, Palo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX. TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents was chloroform/methanol 9.5:0.5 and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

**2.1.1.** The starting compound 5-(4-Bromophenyl)-1,3,4-thiadiazol-2-amine **1** was prepared according to reported procedure [39].

**2.1.2. General procedure for the synthesis of 2,6-Bis(4-substituted phenyl)imidazo[2,1-b]-1,3,4-thiadiazole (2a,b) (Scheme 1)**

A mixture of the amine compound **1** (0.51 g, 0.002 mol) and the appropriate phenacylbromide derivative (0.002 mol) in dioxane (5 ml) was heated under reflux for 5 h. After cooling, a saturated solution of sodium acetate (5 ml) was added. The formed precipitate was filtered, washed with water and dried.

**2.1.2.1. 2-(4-Bromophenyl)-6-(4-chlorophenyl)imidazo[2,1-b]-1,3,4-thiadiazole (2a)**

Yield 60%, mp 120-123 °C (DMF/water). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3062 (CH Ar), 1650, 1631, 1589, 1539 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 7.10 (s, 1H, CH imidazole), 7.56 (d, 2H, *J* = 8.4 Hz, H-3',5' Ar), 7.63 (d, 2H, *J* = 8.1 Hz, H-2',6' Ar), 7.81 (d, 2H, *J* = 9.0 Hz, H-2,6 Ar), 7.88 (d, 2H, *J* = 8.7 Hz, H-3,5 Ar). MS (*m/z*) %: 390 (M<sup>+</sup>) 0.12%. Anal. Calc. For C<sub>16</sub>H<sub>9</sub>BrClN<sub>3</sub>S (390.68): C, 49.19; H, 2.32; N, 10.76. Found: C, 49.32; H, 2.36; N, 10.91.

**2.1.2.2. 2,6-Bis(4-bromophenyl)imidazo[2,1-b]-1,3,4-thiadiazole (2b)**

Yield 62%, mp 110-112 °C (DMF/water). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3066 (CH Ar), 1658, 1605, 1573 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 7.19 (s, 1H, CH imidazole), 7.60 (d, 2H, *J* = 9.1 Hz, H-2',6' Ar), 7.80 (d, 4H, *J* = 9.0 Hz, H-2,6,3',5' Ar), 7.88 (d, 2H, *J* = 8.7 Hz, H-3,5 Ar). MS (*m/z*) %: 435 (M<sup>+</sup>) 3.96%, 437, (M<sup>+</sup>+2) 4.50%. Anal. Calc. For C<sub>16</sub>H<sub>9</sub>Br<sub>2</sub>N<sub>3</sub>S (435.14): C, 44.16; H, 2.08; N, 9.66. Found: C, 44.22; H, 2.13; N, 9.79.

**2.1.3. 2-(4-Bromophenyl)imidazo[2,1-b]-1,3,4-thiadiazol-6(5H)-one (3) (Scheme 1)**

A mixture of the amine compound **1** (0.51 g, 0.002 mol) and chloroacetyl chloride (0.34 g, 0.003 mol) was refluxed in toluene (10 ml) in the presence of anhydrous potassium carbonate (0.27 g, 0.002 mol) for 8 h. The reaction mixture was then filtered while hot and the filtrate was evaporated to dryness under vacuum. The residual mass was washed with water and dried. Yield 70%, mp 248-250 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3028 (CH Ar), 2920, 2850 (CH aliphatic), 1712 (C=O), 1643, 1593, 1558 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.88 (s, 2H, CH<sub>2</sub>), 7.61 (d, 2H, *J* = 6.0 Hz, H-2,6 Ar), 7.72 (d, 2H, *J* = 6.6 Hz, H-3,5 Ar). MS (*m/z*) %: 296 (M<sup>+</sup>) 21.15%, 298 (M<sup>+</sup>+2) 19.13%. Anal. Calc. For C<sub>10</sub>H<sub>6</sub>BrN<sub>3</sub>OS (296.14): C, 40.56; H, 2.04; N, 14.19. Found: C, 40.63; H, 2.11; N, 14.27.

**2.1.4. 2-(4-Bromophenyl)imidazo[2,1-b]-1,3,4-thiadiazole-5,6-dione (4) (Scheme 1)**

Oxalyl chloride (0.38 g, 0.003 mol) was added dropwise with stirring to a cooled suspension of the amine compound **1** (0.51 g, 0.002 mol) in toluene (10 ml) then refluxed for 6 h. The solid product separated on cooling was filtered and dried. Yield 80%, mp 224-226 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3097 (CH Ar), 1766, 1685 (2 C=O), 1635, 1589, 1519 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 7.64 (d, 2H, *J* = 8.7 Hz, H-2,6 Ar), 7.90 (d, 2H, *J* = 8.4 Hz, H-3,5 Ar). MS (*m/z*) %: 310 (M<sup>+</sup>) 9.69%. Anal. Calc. For C<sub>10</sub>H<sub>4</sub>BrN<sub>3</sub>O<sub>2</sub>S (310.13): C, 38.73; H, 1.30; N, 13.55. Found: C, 38.77; H, 1.32; N, 13.69.

**2.1.5. Methyl 2-[2-(4-bromophenyl)-6-hydroxyimidazo[2,1-b]-1,3,4-thiadiazol-5-yl]acetate (5) (Scheme 1)**

A mixture of the amine compound **1** (0.51 g, 0.002 mol), maleic anhydride (0.2 g, 0.002 mol) and triethylamine (0.2 g, 0.002 mol) in methanol (20 ml) was refluxed for 6 h. The solution obtained was concentrated and poured onto ice-water. The formed precipitate was filtered, washed with water and dried. Yield 64%, mp 211-213 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3437 (OH), 3016 (CH Ar), 2920, 2850 (CH aliphatic), 1700 (C=O), 1650, 1600, 1523 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.14 (s, 2H, CH<sub>2</sub>), 3.52 (s, 3H, CH<sub>3</sub>), 7.62 (d, 2H, *J* = 7.2 Hz, H-2,6 Ar), 7.77 (d, 2H, *J* = 6.6 Hz, H-3,5 Ar), 11.46 (s, 1H, OH exchang. with D<sub>2</sub>O). MS (*m/z*) %: 368 (M<sup>+</sup>) 5.95%. Anal. Calc. For C<sub>13</sub>H<sub>10</sub>BrN<sub>3</sub>O<sub>3</sub>S (368.21): C, 42.41; H, 2.74; N, 11.41. Found: C, 42.58; H, 2.79; N, 11.49.

**2.1.6. Ethyl 2-(4-bromophenyl)-5-methylimidazo[2,1-b]-1,3,4-thiadiazole-6-carboxylate (6) (Scheme 1)**

To a solution of the amine compound **1** (0.51 g, 0.002 mol) in ethanol (10 ml), ethyl  $\alpha$ -chloroacetoacetate (0.32 g, 0.002 mol) was added. The reaction mixture was heated under reflux for 24 h. The liquid was chilled and treated with 3N ammonium hydroxide to pH 8 then diluted with water (30 ml). The precipitate formed was filtered, washed with water and dried. Yield 82%, mp 182-184 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3063 (CH Ar), 2920, 2850 (CH aliphatic), 1681 (C=O), 1662, 1600, 1554, 1536 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.27 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 2.08 (s, 3H, CH<sub>3</sub>), 4.21 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 7.57 (d, 2H, *J* = 7.2 Hz, H-2,6 Ar), 7.72 (d, 2H, *J* = 6.9 Hz, H-3,5 Ar). <sup>13</sup>C NMR (75.46 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 14.22 (CH<sub>3</sub>), 16.90 (CH<sub>2</sub>CH<sub>3</sub>), 60.06 (CH<sub>2</sub>CH<sub>3</sub>), 122.88 (C-4, C-4 imidazole (d)), 126.44 (C-1,2,6), 131.77 (C-3,5), 133.25 (C2 thiadiazole (b)), 143.22 (C5 imidazole (c)), 161.77 (C5 thiadiazole (a)), 169.00 (C=O). MS (*m/z*) %: 366 (M<sup>+</sup>) 32.42%, 368 (M<sup>+</sup>+2) 33.40%. Anal. Calc. For C<sub>14</sub>H<sub>12</sub>BrN<sub>3</sub>O<sub>2</sub>S (366.23): C, 45.91; H, 3.30; N, 11.47. Found: C, 45.98; H, 3.37; N, 11.56.

**2.1.7. 2-(4-Bromophenyl)-5-imino-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-7-amine or 2-(4-bromophenyl)-7-imino-7H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-amine (7) (Scheme 2)**

A solution of the amine compound **1** (0.51 g, 0.002 mol), malononitrile (0.13 g, 0.002 mol) in ethanol (20 ml) in presence of triethylamine (0.2 ml) was heated under reflux for 10 h. The precipitate formed was filtered and dried.

Yield 60%, mp 210-212 °C (DMF/water). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3336, 3261, 3155 (NH<sub>2</sub>, NH), 3061 (CH Ar), 1660, 1641, 1629, 1610, 1589 (C=N, NH, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.92 (s, 2H, NH<sub>2</sub> exchange. with D<sub>2</sub>O), 7.27 (s, 1H, CH pyrimidine), 7.32 (d, 2H, *J* = 8.1 Hz, H-2,6 Ar), 7.72 (d, 2H, *J* = 7.8 Hz, H-3,5 Ar), 8.38 (s, 1H, NH exchange. with D<sub>2</sub>O). MS (*m/z*) %: 322 (M<sup>+</sup>) 0.01%. Anal. Calc. For C<sub>11</sub>H<sub>8</sub>BrN<sub>5</sub>S (322.18): C, 41.01; H, 2.50; N, 21.74. Found: C, 41.09; H, 2.57; N, 21.82.

### 2.1.8. 2-(4-Bromophenyl)-5-hydroxy-7H-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-7-one or 2-(4-bromophenyl)-7-hydroxy-5H-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (8) (Scheme 2)

A solution of the amine compound **1** (0.51 g, 0.002 mol), diethyl malonate (0.32 g, 0.002 mol) and sodium metal (0.046 g, 0.002 mol) in methanol (30 ml) were refluxed for 10 h. After concentration and cooling, the solution was poured onto cold water. The solids separated was filtered and dried. Yield 63%, mp 185-188 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3388 (OH), 3000 (CH Ar), 1680 (C=O), 1641, 1595, 1583, 1550 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 6.69 (s, 1H, OH exchange. with D<sub>2</sub>O), 7.34 (s, 1H, CH pyrimidine), 7.56 (d, 2H, *J* = 8.7 Hz, H-2,6 Ar), 7.73 (d, 2H, *J* = 8.4 Hz, H-3,5 Ar). MS (*m/z*) %: 324 (M<sup>+</sup>) 2.40%. Anal. Calc. For C<sub>11</sub>H<sub>6</sub>BrN<sub>3</sub>O<sub>2</sub>S (324.15): C, 40.76; H, 1.87; N, 12.96. Found: C, 40.78; H, 1.85; N, 12.99.

### 2.1.9. 2-(4-Bromophenyl)-5-methyl-7H-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-7-one (9) (Scheme 2)

Equimolar amounts of the amine compound **1** (0.51 g, 0.002 mol) and ethyl acetoacetate (0.26 g, 0.002 mol) were heated at 160 °C in oil bath for 12 h. Yield 88%, mp 240-242 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3089 (CH Ar), 2924, 2850 (CH aliphatic), 1680 (C=O), 1620, 1589, 1535 (C=N, C=C). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 2.08 (s, 3H, CH<sub>3</sub>), 7.20 (s, 1H, CH pyrimidine), 7.55-7.80 (m, 4H, ArH). MS (*m/z*) %: 323 (M<sup>+</sup>+1) 84.44%. Anal. Calc. For C<sub>12</sub>H<sub>8</sub>BrN<sub>3</sub>OS (322.18): C, 44.74; H, 2.50; N, 13.04. Found: C, 44.83; H, 2.49; N, 13.16.

### 2.1.10. General procedure for the synthesis of 1-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-3-substituted thiourea (10a-e) (Scheme 3)

A mixture of the amine compound **1** (0.51 g, 0.002 mol) and the appropriate isothiocyanate (0.002 mol) in ethanol (20 ml) was heated under reflux for 24 h. The separated solid was filtered, washed with water and dried.

#### 2.1.10.1. 1-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-3-ethylthiourea (10a)

Yield 68%, mp 186-190 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3410 (2 NH), 3090 (CH Ar), 2920, 2850 (CH aliphatic), 1650, 1630, 1610, 1593 (C=N, NH, C=C), 1280 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.06 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>), 3.44 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 6.45 (s, 1H, NH exchange. with D<sub>2</sub>O), 7.62 (d, 2H, *J* = 8.7 Hz, H-2,6 Ar), 7.81 (d, 2H, *J* = 8.4 Hz, H-3,5 Ar), 11.26 (s, 1H, NH exchange. with D<sub>2</sub>O). <sup>13</sup>C NMR (75.46 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 13.70 (CH<sub>2</sub>CH<sub>3</sub>), 36.73 (CH<sub>2</sub>CH<sub>3</sub>), 122.95 (C-4), 129.07 (C-2,6), 131.52 (C-3,5), 133.61 (C-1), 144.97 (C2 thiadiazole), 155.07 (C5 thiadiazole), 179.31 (C=S). MS (*m/z*) %: 343 (M<sup>+</sup>) 1.54%. Anal. Calc. For C<sub>11</sub>H<sub>11</sub>BrN<sub>4</sub>S<sub>2</sub> (343.27): C, 38.49; H, 3.23; N, 16.32. Found: C, 38.53; H, 3.28; N, 16.39.

#### 2.1.10.2. 3-Allyl-1-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]thiourea (10b)

Yield 63%, mp 205-207 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3437, 3286 (2 NH), 3078 (CH Ar), 2920, 2850 (CH aliphatic), 1619, 1600, 1589, 1523 (C=N, NH, C=C), 1286 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 3.47 (d, 2H, *J* = 18.0 Hz, CH<sub>2</sub>CH=CH<sub>2</sub>), 4.87-4.94 (m, 2H, CH<sub>2</sub>CH=CH<sub>2</sub>), 5.20-5.35 (m, 1H, CH<sub>2</sub>CH=CH<sub>2</sub>), 6.48 (s, 1H, NH exchange. with D<sub>2</sub>O), 7.62 (d, 2H, *J* = 8.7 Hz, H-2,6 Ar), 7.92 (d, 2H, *J* = 8.4 Hz, H-3,5 Ar), 8.21 (s, 1H, NH exchange. with D<sub>2</sub>O). MS (*m/z*) %: 355 (M<sup>+</sup>) 3.10%. Anal. Calc. For C<sub>12</sub>H<sub>11</sub>BrN<sub>4</sub>S<sub>2</sub> (355.28): C, 40.57; H, 3.12; N, 15.77. Found: C, 40.64; H, 3.14; N, 15.92.

#### 2.1.10.3. 1-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-3-butylthiourea (10c)

Yield 60%, mp 167-170 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3437, 3286 (2 NH), 3057 (CH Ar), 2918, 2846 (CH aliphatic), 1621, 1600, 1589, 1523 (C=N, NH, C=C), 1280 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.05 (t, 3H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.15-1.35 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.47 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.35 (s, 1H, NH exchange. with D<sub>2</sub>O), 6.49 (s, 1H, NH exchange. with D<sub>2</sub>O), 7.63 (d, 2H, *J* = 8.7 Hz, H-2,6 Ar), 7.82 (d, 2H, *J* = 9.0 Hz, H-3,5 Ar). MS (*m/z*) %: 369 (M<sup>+</sup>-2) 14.87%. Anal. Calc. For C<sub>13</sub>H<sub>15</sub>BrN<sub>4</sub>S<sub>2</sub> (371.32): C, 42.05; H, 4.07; N, 15.09. Found: C, 42.17; H, 4.05; N, 15.21.

#### 2.1.10.4. 1-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-3-cyclohexylthiourea (10d)

Yield 70%, mp 202-205 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3437, 3286 (2 NH), 3010 (CH Ar), 2926, 2830 (CH aliphatic), 1660, 1645, 1600, 1589, 1523 (C=N, NH, C=C), 1260 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 1.03-1.07 (m, 6H, cyclohexyl H), 1.20-1.30 (m, 4H, cyclohexyl H), 3.20-3.46 (m, 1H cyclohexyl H), 4.35 (s, 1H, NH exchange. with D<sub>2</sub>O), 6.49 (s, 1H, NH exchange. with D<sub>2</sub>O), 7.64 (d, 2H, *J* = 8.1 Hz, H-2,6 Ar), 7.82 (d, 2H, *J* = 8.7 Hz, H-3,5 Ar). MS (*m/z*) %: 397 (M<sup>+</sup>) 14.72%. Anal. Calc. For C<sub>15</sub>H<sub>17</sub>BrN<sub>4</sub>S<sub>2</sub> (397.36): C, 45.34; H, 4.31; N, 14.10. Found: C, 45.42; H, 4.33; N, 14.24.

**2.1.10.5. 1-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-3-phenylthiourea (10e):**

Yield 80%, mp 188-190 °C (ethanol). IR  $\nu_{\max}/\text{cm}^{-1}$ : 3435, 3221 (2 NH), 3072 (CH Ar), 1647, 1616, 1595, 1539 (C=N, NH, C=C), 1280 (C=S).  $^1\text{H NMR}$  (300 MHz, DMSO- $d_6$ )  $\delta$  ppm: 7.14-8.07 (m, 9H, ArH), 8.21 (s, 1H, NH exchang. with D<sub>2</sub>O), 11.03 (s, 1H, NH exchang. with D<sub>2</sub>O). MS ( $m/z$ ) %: 389 ( $M^{+2}$ ) 0.02%. Anal. Calc. For C<sub>15</sub>H<sub>11</sub>BrN<sub>4</sub>S<sub>2</sub> (391.31): C, 46.04; H, 2.83; N, 14.32. Found: C, 46.21; H, 2.91; N, 14.43.

**2.2. Antitumor activity**

All newly synthesized compounds were tested against the tumor cell line A549 (Non-Small Cell Lung Cancer Cell Line) at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University using the Sulfo-Rhodamine B stain (SRB) assay by the method of Skehan et al [40].

**Procedure:**

A549 human lung cancer cells were grown in DMEM, supplemented with 10% heat inactivated FBS, 50 units/ml of penicillin and 50 g/ml of streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. The cells were maintained as "monolayer culture" by serial subculturing.

Cells were plated in 96-multiwell plate (10<sup>4</sup> cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted to the appropriate volume. Different concentrations of the compound under test (0.01, 0.1, 1, 10 and 100  $\mu\text{M}$ ) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO<sub>2</sub>. After 48 h, cells were fixed, washed and stained for 30 min. with 0.4% (w/v) Sulfo-Rhodamine B dissolved in 1% acetic acid. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The IC<sub>50</sub> values were calculated according to the equation for Boltzman sigmoidal concentration-response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

**2.3. Molecular docking****2.3.1. Docking procedure**

Docking studies of all the synthesized compounds were performed by Molecular Operating Environment (MOE) 2008.10 release of Chemical Computing Group, Canada [41]. The program operated under "Window XP" operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. All minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol<sup>-1</sup> Å<sup>-1</sup> with MMFF94 force field and the partial charges were automatically calculated. The score function, dock function (S, Kcal/mol) developed by MOE program was used for the evaluation of the binding affinity of the ligand.

**2.3.1.1. Preparation of the target Fibroblast stromelysin-1**

The X-ray crystal structure of the enzyme with thiadiazole ligand PNU-142372, 2-[3-(5-mercapto-1,3,4-thiadiazol-2-yl)ureido]-*N*-methyl-3-pentafluorophenylpropionamide, (PDB code 1USN) [42] was obtained from the protein data bank in PDB formate. The enzyme was prepared for docking studies. (i) 3D protonation of the amino acid side chain and ligand PNU-142372. (ii) Deleting all water of crystallization away from the active site. (iii) Isolation of the active site, fixation to be dealt with as rigid structure and recognition of the amino acids. (iv) Creation of dummies around the active site. (v) Studying the interactions of the ligand (PNU-142372) with the amino acids of the active site.

**2.3.1.2. Preparation of compounds for docking**

The 3D structures of the synthesized compounds were built using MOE and subjected to the following procedure: (i) 3D protonation of the structures. (ii) Running conformational analysis using systemic search. (iii) Selecting the least energetic conformer. (iv) Applying the same docking protocol used with PNU-142372.

**2.3.1.3. Docking running**

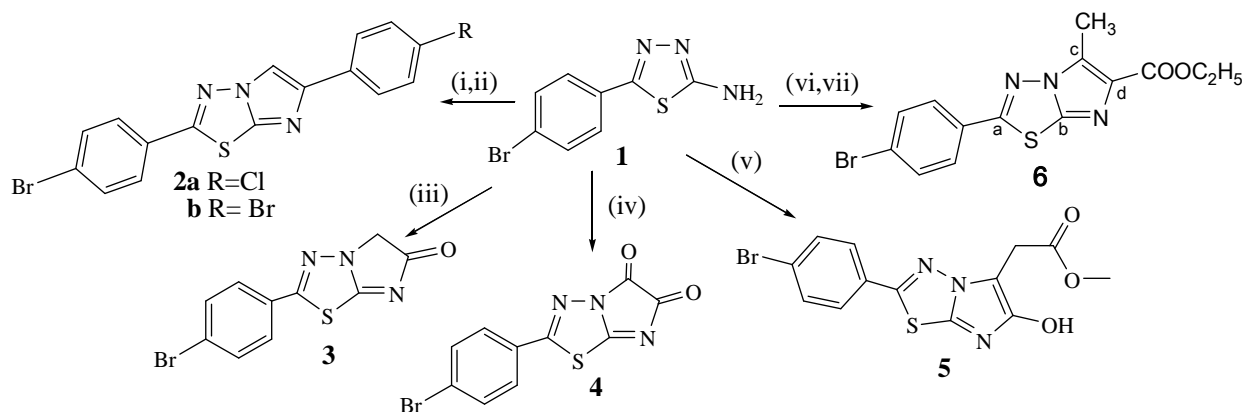
Prior to the docking of the thiadiazole derivatives, redocking of the native ligand bound in the fibroblast stromelysin-1 active site was performed to validate the docking protocol. The generated most stable conformer of each compound was virtually docked into the predefined active site of Fibroblast stromelysin-1. The developed docked models were energetically minimized and then used to predict the interaction of the ligand with the amino acids in the active site of the enzyme.



## RESULTS AND DISCUSSION

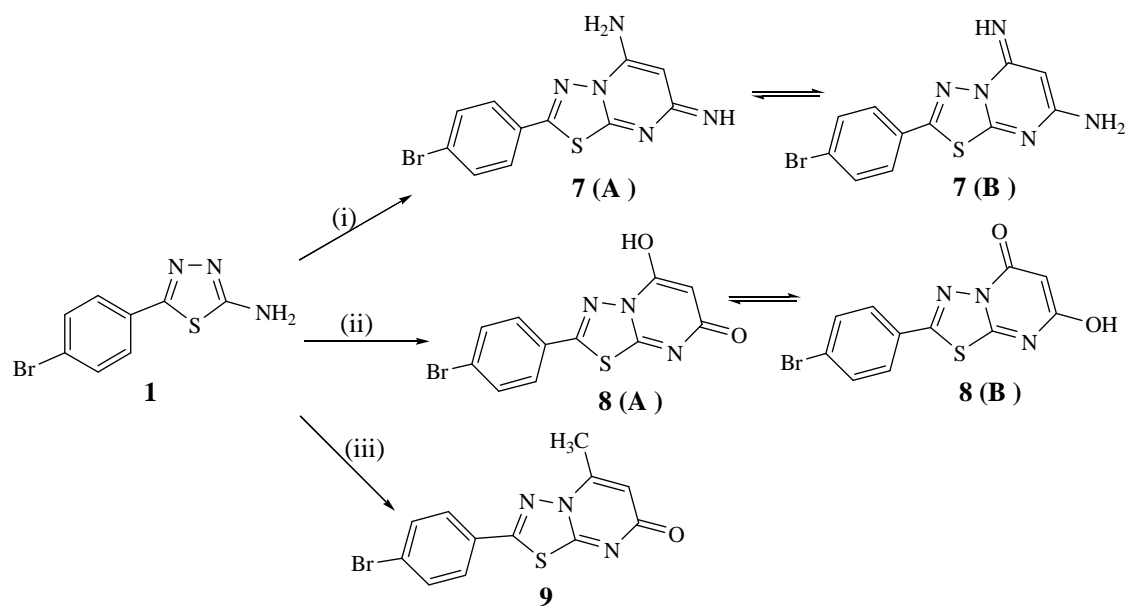
## 3.1. Chemistry

The target compounds substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles **2a,b-6**, substituted 1,3,4-thiadiazolo[3,2-*a*]pyrimidines **7-9** and 1,3-disubstituted thioureas **10a-e** were synthesized as depicted in Schemes 1-3. The starting compound 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine **1** was prepared as reported in literature [39].



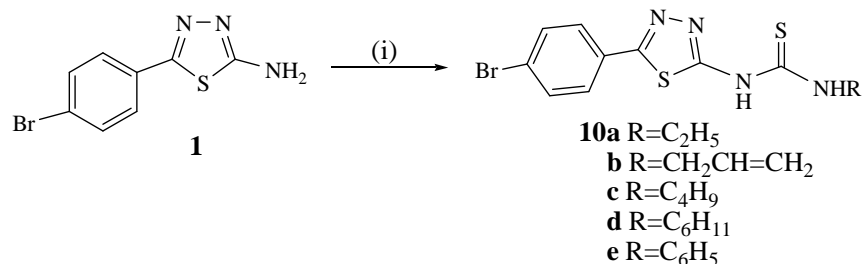
**Scheme 1.** Reagents and conditions: (i) Appropriate phenacylbromide, dioxane, reflux 5 h, (ii) Saturated sodium acetate solution, (iii) Chloroacetyl chloride, toluene, anhydrous potassium carbonate, reflux 8 h, (iv) Oxalyl chloride, toluene reflux 6 h, (v) Maleic anhydride, triethylamine, methanol, reflux 6 h, (vi) Ethyl  $\alpha$ -chloroacetoacetate, ethanol, reflux 24 h, (vii) 3N ammonium hydroxide.

Reaction of compound **1** with appropriate phenacylbromide derivatives yielded compounds **2a,b** (Scheme 1). Structures of compounds **2a,b** were confirmed by spectral and analytical data. Disappearance of bands at 3433 and 3286  $\text{cm}^{-1}$  corresponding to  $\text{NH}_2$  group in IR spectra and increased number of aromatic protons in  $^1\text{H}$  NMR spectra confirmed reaction of amino groups. Reacting compound **1** with chloroacetyl chloride resulted in compound **3** (Scheme 1). Disappearance of bands corresponding to  $\text{NH}_2$  group in IR spectrum and appearance of a band at 1712  $\text{cm}^{-1}$  was assigned to  $\text{C}=\text{O}$  group. In addition, presence of a singlet signal at  $\delta = 3.88$  ppm attributed to  $\text{CH}_2$  protons in  $^1\text{H}$  NMR spectrum confirmed compound **3** structure. Compound **4** was achieved upon reaction of compound **1** with oxalyl chloride (Scheme 1). Disappearance of bands corresponding to  $\text{NH}_2$  group in IR spectrum and appearance of bands at 1766 and 1685  $\text{cm}^{-1}$  were assigned to two  $\text{C}=\text{O}$  groups confirmed structure. Subjecting **1** to reaction with maleic anhydride under basic condition yielded **5** following Michael's addition [43] (Scheme 1). IR spectra showed appearance of a band at 3437 and 1700  $\text{cm}^{-1}$  corresponding to OH and  $\text{C}=\text{O}$  groups, respectively.  $^1\text{H}$  NMR spectrum showed two singlet signals at  $\delta = 3.14$  and 3.52 and singlet signal at 11.46 ppm exchanged with  $\text{D}_2\text{O}$  corresponding to  $\text{CH}_2$ ,  $\text{CH}_3$  and OH protons, respectively. Reaction of compound **1** with ethyl  $\alpha$ -chloroacetoacetate yielded compound **6**. IR spectra showed appearance of a band at 1681  $\text{cm}^{-1}$  corresponding to  $\text{C}=\text{O}$  group.  $^1\text{H}$  NMR spectrum showed triplet and quartet signals at  $\delta = 1.27$  and 4.21 assigned to ethyl protons and singlet signal at 2.08 ppm corresponding to  $\text{CH}_3$  protons.  $^{13}\text{C}$  NMR spectrum showed signal at 14.22 ppm assigned to  $\text{CH}_3$ , signals at 16.90 and 60.06 ppm assigned to ethyl carbons, signals at 133.25, 143.22, 161.77 and 169.00 ppm corresponding to C2 thiadiazole (b), C5 imidazole (c), C5 thiadiazole (a) and  $\text{C}=\text{O}$ . In addition, signals at 122.88-131.77 assigned to 7 aromatic carbons. MS spectra showed their molecular ion peaks.



**Scheme 2.** Reagents and conditions: (i) Malononitrile, triethylamine, ethanol, reflux 10 h, (ii) Diethyl malonate, sodium metal, methanol, reflux 6 h, (iii) Ethyl acetoacetate, 160 °C, 8 h.

1,3,4-Thiadiazolo[3,2-*a*]pyrimidine derivatives **7-9** were achieved via reaction of compound **1** with malononitrile or diethyl malonate or ethyl acetoacetate, respectively. Reaction with ethyl acetoacetate under the same condition used for diethyl malonate gave low yield and impure product (Scheme 2). The structures of compounds **7-9** were deduced from elemental analyses and spectral data. IR spectra showed bands at 3336, 3261 and 3155  $\text{cm}^{-1}$  for  $\text{NH}_2$  and  $\text{NH}$  groups in compound **7** or a new band at 1680  $\text{cm}^{-1}$  assigned to  $\text{C}=\text{O}$  group in compounds **8** and **9**.  $^1\text{H}$  NMR spectrum showed singlet signal at 7.20-7.34 assigned to pyrimidine proton. MS spectra showed their molecular ion peaks.



**Scheme 3.** Reagents and conditions: (i) Appropriate isothiocyanate, ethanol, reflux 24 h.

Refluxing compound **1** with appropriate isothiocyanate yielded thiourea derivatives **10a-e** (Scheme 3). Structures of compounds **10a-e** were confirmed from spectral and analytical data. IR spectra revealed appearance of a new band at 1286-1260  $\text{cm}^{-1}$  corresponding to  $\text{C}=\text{S}$  group.  $^1\text{H}$  NMR spectra showed added aliphatic protons for compounds **10a-d** or additional aromatic protons for compound **10e**.  $^{13}\text{C}$  NMR spectrum showed signals at 13.70 and 36.73  $\text{ppm}$  assigned to ethyl carbons, signals at 144.97  $\text{ppm}$  corresponding to C2 thiadiazole, 155.07  $\text{ppm}$  corresponding to C5 thiadiazole and 179.31  $\text{ppm}$  corresponding to  $\text{C}=\text{S}$ . In addition, signals at 122.95-133.61  $\text{ppm}$  assigned to 6 aromatic carbons. MS spectra showed their molecular ion peaks.

### 3.2. Antitumor activity

All newly synthesized compounds were tested against the tumor cell line A549 (Non-Small Cell Lung Cancer Cell Line) at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University using the Sulfo-Rhodamine B stain (SRB) assay by the method of Skehan et al [40].

Table 1. IC<sub>50</sub> of Tested Compounds for Antitumor Screening against A549 (Non-Small Cell Lung Cancer Cell Line)

Comp. No.	IC <sub>50</sub> μM
<b>1</b>	9.92
<b>2a</b>	12.69
<b>2b</b>	69.80
<b>3</b>	60.98
<b>4</b>	6.47
<b>5</b>	4.74
<b>6</b>	46.30
<b>7</b>	16.44
<b>8</b>	2.58
<b>9</b>	36.19
<b>10a</b>	44.66
<b>10b</b>	4.82
<b>10c</b>	5.44
<b>10d</b>	4.34
<b>10e</b>	28.24
<b>Doxorubicin</b>	0.411

*In vitro* antitumor activity for all newly synthesized compounds in addition to starting compound 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine **1** and reference drug Doxorubicin was performed utilizing Sulfo-Rodamine B (SRB) standard method [41] against the tumor cell line A549 (Non-Small Cell Lung Cancer Cell Line) as literature survey predicted 1,3,4-thiadiazole derivatives to be active compounds. In this work, cell line was inoculated and incubated in plate for 24 h. Test compounds were then added with different concentrations (0.01, 0.1, 1, 10 and 100 μM) and incubated for 48 h. The IC<sub>50</sub> values (concentration that reduce the surviving fraction to 50%) were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

From the observed antitumor activity data of substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles derivatives **2a,b-6**, only compounds **4** and **5** showed good activity with IC<sub>50</sub> values 6.47 and 4.74 μM, respectively and compound **2a** showed moderate activity (IC<sub>50</sub> 12.69 μM). In case of 1,3,4-thiadiazolo[3,2-*a*]pyrimidine derivatives **7-9**, only compound **8** exhibited promising activity with IC<sub>50</sub> values 2.58 μM, while compound **7** showed moderate activity (IC<sub>50</sub> 16.44 μM). Regarding 1,3-disubstituted thiourea derivatives **10a-e**, activity was good for **10b-d** with IC<sub>50</sub> values 4.82, 5.44 and 4.34 μM, respectively. Small alkyl substitution or aromatic substitution on thiourea gave inactive compounds. Active compounds **4, 5, 8** and **10b-d** gave improved activity compared with starting compound **1** (9.92 μM), Table 1.

### 3.3. Molecular docking

Unregulated or over expressed matrix metalloproteinases (MMPs), including stromelysin, collagenase and gelatinase, were implicated in several pathological conditions including arthritis and cancer. MMPs represent a potentially important class of therapeutic targets for the treatment of diseases such as cancer, therefore, small-molecule MMP inhibitors may have therapeutic value in the treatment of cancer [44, 45]. Selective inhibition of MMPs will be required attributed to the discovery that individual MMPs also regulate the natural angiogenesis inhibitor angiostatin [45]. MMPs had been found to be over expressed in tumors in squamous cell cancer [46], human uterine cervical cancer [47], breast cancer [48] and lung cancer [49]. It was found that, the thiadiazole inhibitors extend into the left side of the active site of fibroblast stromelysin-1 and interact with the more shallow S1-S3 binding pockets [44]. The S1 and S3 subsites are actually combined into one large open space (S1/S3) bounded by backbone and side-chain atoms of His 166, Ala 167, and Tyr 168 at the back, and the side chain of Tyr 155 at the top. Side chains of His 205, Phe 210, and Phe 86 assemble to form a shallow depression that is the S2 “pocket”. A similar depression at the intersection of Phe 86, Pro 87, Pro 90, and Ala 169 side chains is S4 [42].



Table 2. Docking results

Compound	Energy score S (kcal/mol)	Amino acid interactions	Interacting groups	H-bond Length (Å)
PNU-142372	-12.1272	Tyr 155 (arene arene) Ala 167 Ala 167 His 205 (arene arene)	Phenyl ring N(3) Thiadiazole N(4) Thiadiazole Thiadiazole ring	3.00 3.02
<b>4</b>	-12.9885	Ala 167	CO at C(5)	2.84
<b>5</b>	-12.8736	Ala 167	CO	3.05
<b>8 (A)</b>	-12.6173	Ala 167	OH	1.43
<b>(B)</b>	-12.8523	Ala 167	OH	2.61
		Ala 167	OH	3.15
<b>10b</b>	-13.4638	Ala 167	N(3) Thiadiazole	3.01
		Glu202	NH	1.48
		Glu202	NH	1.76
		His 205 (arene arene)	Thiadiazole ring	
<b>10c</b>	-15.3297	Ala 167	NH	1.84
		Ala 167	NH	2.05
		His 205 (arene arene)	Thiadiazole ring	
		Zn 257 (arene cation)	Phenyl ring	
<b>10d</b>	-13.5393	Ala 167	NH	1.67
		His 205 (arene arene)	Thiadiazole ring	
		Zn 257 (arene cation)	Phenyl ring	

The binding affinity of the ligand was evaluated with energy score (S, Kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand-enzyme complex. Length of the hydrogen bond, arene arene and arene cation interaction were also used to assess the binding models. The results of docking study; dock score, involved fibroblast stromelysin-1 active site amino acid interacting ligand moieties and hydrogen bond length for each active compound and reference inhibitor, PNU-142372, are listed in Table 2, Fig. 2-7.

*Analysis of the docking results revealed that:*

(i) The inhibitor (PNU-142372)-fibroblast stromelysin-1-complex was precisely reproduced by the docking procedure as demonstrated by low root mean square deviation, rmsd (0.9454) and dock score (-12.1272 kcal/mol, Table 2), i.e. the docking protocol was valid. As shown in Fig. 2 and 3, the inhibitor PNU-142372 nearly fits in the active site forming two hydrogen bonding interactions with the active site residues. N3 thiadiazole and N4 thiadiazole with Ala 167 (3.00 and 3.02 Å, respectively) in addition to arene arene interaction between phenyl and thiadiazole ring with Tyr 155 and His 205, respectively.

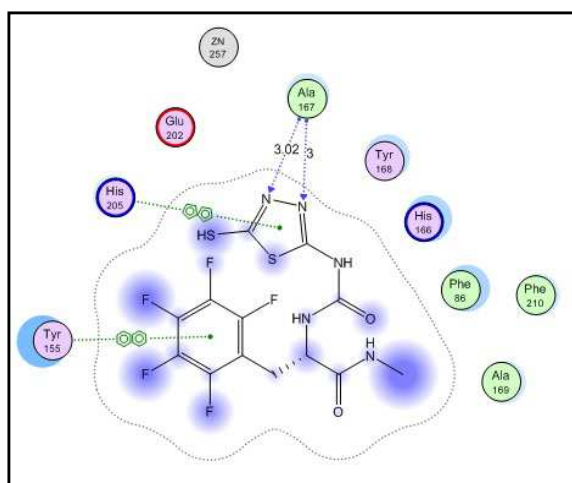


Fig. 2. 2D interactions of PNU-142372 on the active site of Fibroblast stromelysin-1

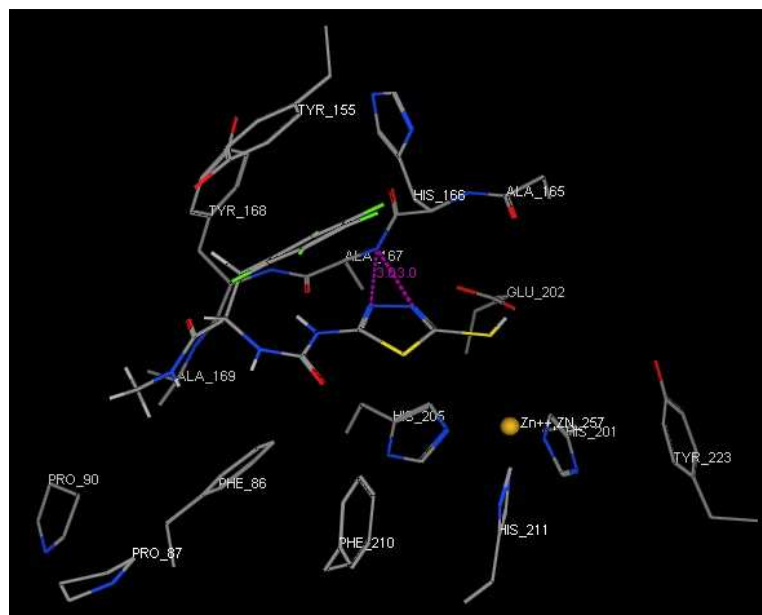


Fig. 3. 3D interactions of PNU-142372 on the active site of Fibroblast stromelysin-1

(ii) The docking scores for compounds **4**, **5**, **8**, **10b**, **10c** and **10d** were all in the range -15.3297 to -12.6173 kcal/mol.

For imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives **4** and **5** (dock scores, -12.9885 and -12.8736 kcal/mol, respectively). The dock scores were comparable correlated with comparable  $IC_{50}$  where  $IC_{50}$  for compound **5** was 4.74  $\mu$ M and for compound **4** was 6.47  $\mu$ M.

For compound **8**, 2-(4-bromophenyl)-5-hydroxy-7*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-7-one (tautomer A) or 2-(4-bromophenyl)-7-hydroxy-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (tautomer B), the most active analogue ( $IC_{50}$  2.58  $\mu$ M) with dock score -12.6173 (for tautomer A) or -12.8523 (for tautomer B) kcal/mol.

For 1,3-disubstituted thioureas **10b-d** (dock scores, -13.4638, -15.3297 and -13.5393 kcal/mol, respectively). The dock scores were comparable correlated with comparable  $IC_{50}$  (4.82, 5.44 and 4.34, respectively)

(iii) Inspection of the binding mode also demonstrated that, all compounds showed one to three hydrogen bonds and/or arene arene or arene cation interactions with the enzyme active site residue. Tyr 155, Ala 167, Glu 202, His 205 and Zn 257 are the amino acids residue involved in these interactions. In common with PNU-142372 fibroblast stromelysin-1 inhibitor, all the docked compounds interacted with amino acid residue Ala 167 present in the active site of enzyme (as native ligand PNU-142372) with at least one hydrogen bond.

Compound **8** (dock score, 12.6173 (A) or 12.8523 (B) kcal/mol.), the most active compound, showed one strong hydrogen bond of Ala 167 (1.43 Å) with OH for tautomer A or two hydrogen bonds of Ala 167 (2.61 and 3.15 Å) with OH for tautomer B (Fig. 4-6).

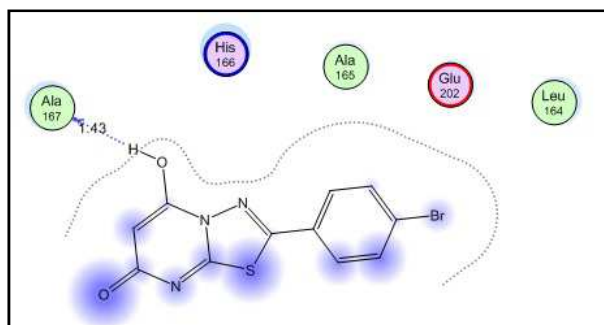


Fig. 4. 2D interactions of Compound 8 (A) on the active site of Fibroblast stromelysin-1

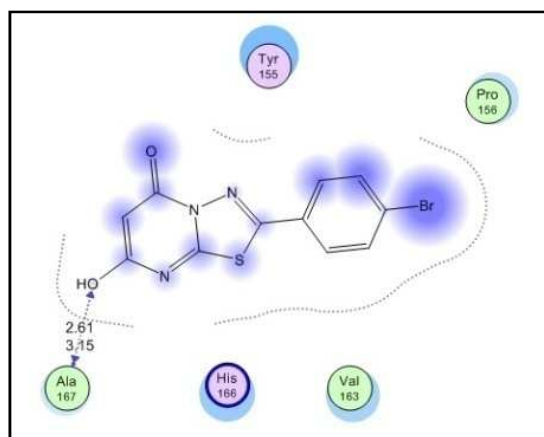


Fig. 5. 2D interactions of Compound 8 (B) on the active site of Fibroblast stromelysin-1

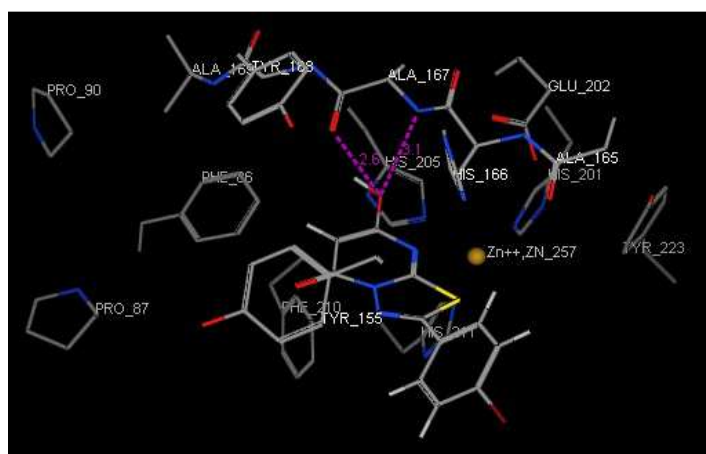


Fig. 6. 3D interactions of Compound 8 (B) on the active site of Fibroblast stromelysin-1

Furthermore, the second most active thiourea compound **10d** (energy score: -13.5393 kcal/mol) mediated one strong hydrogen bonds of NH with Ala 167 (1.67 Å), arene arene interaction between thiadiazole ring and His 205 and arene cation interaction between phenyl ring and Zn 257 (Fig. 7).

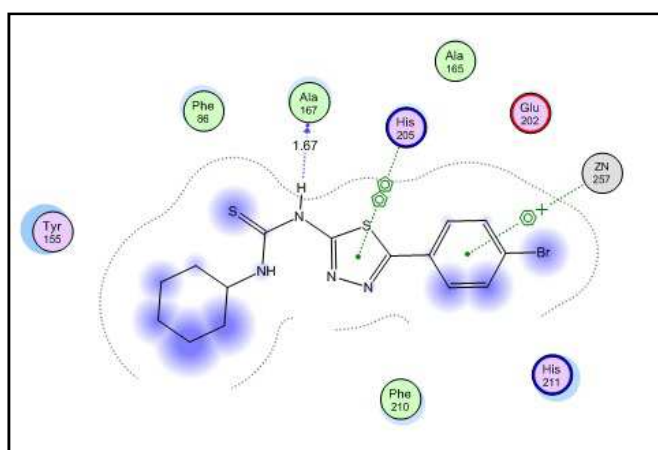


Fig. 7. 2D interactions of Compound 10d on the active site of Fibroblast stromelysin-1

## CONCLUSION

The newly synthesized compounds of condensed heterocyclic substituted imidazo[2,1-*b*]-1,3,4-thiadiazoles **2a,b-6**, substituted-1,3,4-thiadiazolo[3,2-*a*]pyrimidines **7-9** and a structure hybrid compounds comprised of thiourea and 1,3,4-thiadiazole as 1,3-disubstituted thioureas **10a-e** were evaluated for the cytotoxic activity against human tumor

cell line (A549 “Lung” cancers). The obtained results revealed compounds **4**, **5**, **8**, **10b-d** with lower IC<sub>50</sub> values than their precursor compound **1** (more active) but higher than that of the reference drug Doxorubicin. Dock scores of the docked compounds were slight different and close to that of the native ligand (PUN-142372) as cytotoxic activity expressed as IC<sub>50</sub> was comparable to each other. All the docked compounds shared some binding interactions with fibroblast stromelysin-1 similar to those of the native ligand inhibitor. This suggests that these compounds might possibly act as fibroblast stromelysin-1 inhibitors, and this may contribute at least in part to their antitumor activity.

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