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Synthesis and molecular modeling of MetAP2 of thiosemicarbazides, 1,2,4-triazoles, thioethers derived from (S)-Naproxen as possible breast cancer agents

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ABSTRACT

New thiosemicarbazides (**3, 5-6**), 1,2,4-triazoles (**14-15**) and thioethers (**22-68**) from derived (S)-Naproxen were synthesized in this study. The structure of these compounds were elucidated by spectral (FT-IR, ^{1}H NMR, ^{13}C NMR) methods, besides elemental analysis and TLC. The molecular binding of the compounds on MetAP-2 was performed. Anticancer effects of the synthesized compounds were studied by using MTT assay method on MCF-7 (includes oestrogene and progesterone receptors) and MDA-MB-231 (lacks estrogen and progesterone receptors) adenocarcinoma cell lines at 0, 10, 25, 50, 75 and 100 μM concentrations for 24 h. The IC_{50} values of novel (S)-Naproxen derivatives were determined between from 5 to 100 μM on MCF-7 breast cancer cell line and MDA-MB-231 cell lines. The apoptotic activity of selected compounds **22** and **42** were first analyzed by Annexin V staining using Tali Image-Based Cytometer. Mitochondrial membrane potential changes determined in fluorescence plate reader following JC-1 stain for compounds **22** and **42** in MCF-7 and MDA-MB-231 cells. The effect of these compounds on the cell viability 4T1 mouse mammary tumor cell line was tested at 1 to 5 times of calculated IC_{50} value (IC_{50}x1, IC_{50}x2, IC_{50}x3, IC_{50}x4, and IC_{50}x5). Next in order to determine the toxicity of the combination of compound **51** and Docetaxel, WST-1 cell viability and proliferation assay was performed with 4T1.

1. Introduction

Breast cancer is the second most common cancer in the world after lung cancer. Most of the developed and developing countries are of the neoplasm type, the most common cancer type for female sex. Because breast cancer has a relatively better prognosis, it is ranked 5th in cancer-related deaths.

In our country, one of the two types of cancer in which national screening program is established for early diagnosis is breast cancer. Breast cancer is a heterogeneous group of different histological subtypes. While risk factors for breast cancer are estrogen exposure, familial genetic predisposition (BRCA1 / 2 mutation) and salivary proliferative breast lesions are major endogenous factors in terms of breast cancer, hormone replacement therapy, obesity, sedanter life, dietary elements, geographical region conditions and environmental carcinogen exposure constitute the main ecogenetic factors.

Breast cancer is divided into five classes according to the expression of estrogen (ER), progesterone (PR) and Her-2 oncogene. Of these, luminal A and luminal B type breast cancers are ER-PR positive; Her-2, basal-like and normal breast-like breast cancers are ER negative. Apart from these groups, the type in which all three of ER, PR and Her-2 are negative is called TNBC (triple negative breast cancer). Triple negative breast cancer (TNBC) is characterized by the absence of estrogen receptor, progesterone receptor and human epidermal growth factor 2 expression and constitutes 15–20% of all breast cancers. TNBC is resistant to many chemotherapy drugs because it does not carry ER / PR / HER2 receptors. It also shows aggressive spread due to its unresponsiveness to systemic

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endocrine therapy and poor prognosis. In particular, neoadjuvant therapy has gained importance in the treatment of TNC, usually as locally advanced (locally advanced) breast chemotherapy. Neoadjuvant chemotherapy offers the opportunity to treat both the main tumor and invisible metastases (micrometastases), especially in locally spread tumors with high metastasis potential.

The cancer cell's formation of new vascular extensions from existing vessels is called angiogenesis. It has an important role in the formation of diseases such as angiogenesis, inflammation and cancer. The formation of new blood vessels appears to be the potential area for inhibiting the biological transformations involved in angiogenesis, particularly macromolecular targets, solid tumor growth / metastasis. Methionine aminopeptidase type 2 (MetAP2) has been identified as a potent antiangiogenesis intracellular target. MetAP2 is a bifunctional protein that plays a critical role in the growth of different types of tumours [1]. Triazole derivatives [2] and thioether compounds [3–6] have been reported by researchers as the MetAP2 inhibitor class. In recent years, there are studies showing that some nonsteroidal anti-inflammatory drugs (NSAIDs) can also be used as anticancer agents. These drugs are used to treat inflammation and prevent inflammation by inhibiting COX-1 and COX-2, which catalyze the conversion of arachidonic acid to prostaglandins to a different extent. Naproxen ((S)-2-(6-methoxy-2-naphthyl) propanoic acid) is a non-steroidal anti-inflammatory drug (NSAID), also known to have anticancer activities [7–13]. Besides, thiosemicarbazides [14,15], 1,2,4-triazole-3-thiones [16–21] and thioethers [3–6] have been reported to have anticancer activity on different cancer cell lines. On the light of the foregoing, new naproxen derivatives were synthesized. The aim of this study was to investigate the anticancer effects of the synthesized naproxen compounds on various breast cancer cells. In particular, its use with doxataxel in neoadjuvant therapy in TNC cells has also been investigated.

2. Material and methods

2.1. General

All the chemicals were purchased from Merck (Darmstadt, Germany), Sigma Aldrich (St. Louis, MO, USA) or Alfa Aesar (Karlsruhe, Germany). The reactions were monitored by thin layer chromatography (TLC) on silica gel using solvent systems M1: petroleum ether/dichloromethane/ethyl acetate (25:50:25, v/v) at 25 °C, M2: petroleum ether/ethyl acetate (60:40 v/v) at 25 °C. TLC spots were marked under UV light (254 nm, t = 25 °C). Melting point of the synthesized compound were determined in a Stuart SMP 20 (Staffordshire, UK) melting point apparatus, expressed in degrees centigrade (°C) and uncorrected. The optical rotation angle of the naproxen was performed according to European Pharmacopoeia with Rudolph Autopol V Plus. The purity of the compounds were proven by TLC and elemental analysis. Elementel analysis was performed on CHNS-932 (LECO, St. Joseph MI, USA). FT-IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX (Massachusetts, USA) over the range 4000–500 cm^{-1} spectrophotometer. ^{1}H NMR spectra were recorded as solutions DMSO-d_6 on BRUKER 300 (Billerica, MA, USA) at 300 MHz. ^{1}H NMR shifts were expressed in ppm relative to tetramethylsilan (TMS) as an internal standart data reported as respectively: chemical shift, multiplicity (s: singlet, d: dublet, dd: double dublet, t: triplet, q: quartet, m: multiplet) coupling constants (Hz), integration. All elemental analysis and ^{1}H NMR spectra analyses were performed by Inonu University Scientific and Technological Research Center (IBTAM). ^{13}C NMR shifts were expressed in ppm relative to tetramethylsilan (TMS) as an internal standard. All ^{13}C NMR spectra measurements were carried out DMSO-d_6 on Varian 600 Spectrometer at 150 MHz.

2.2. Chemistry

2.2.1. Preparation of (S)-methyl 2-(6-methoxy-2-naphthyl)propanoate (1, CAS Number: 26,159-35-3)

The concentrated sulfuric acid (1 ml) and methanolic solution of (S)-Naproxen (0.01 mol) were refluxed for 4 h. The reaction monitored by TLC. Neutralization is achieved by treating the reaction medium with sodium bicarbonate solution ($NaHCO_3$) (%10 w/v). The precipitate was washed with water, filtered, dried, and recrystallized one time with methanol. Yield%85. Compound **1** M.p. 92–94 °C [6], lit [13] 88 °C.

2.2.2. Preparation of (S)-2-(6-methoxy-2-naphthyl)propanoic acid hydrazide (2, CAS Number: 57,475-91-9)

Ethanolic solution of compound **1** (50 mL, 0.01 mol) was added hydrazine hydrate (0.7 mL,%100) and refluxed for 2 h, monitored by TLC and then cooled. The resulting product was washed with water, filtered, dried, recrystallized with methanol. Yield%82, Compound **2** M.p. 98–100 °C [6], lit [13] 94 °C.

2.2.3. General procedure for synthesis of (S)-N-(aryl/alkyl)-2-[2-(6-methoxynapthalen-2-yl)propanoyl)hydrazine-1-carbothioamides (3-12)

A solution of compound **2** and equimolar amount of aryl/alkyl isothiocyanates were added in n-butanol (60 mL) and refluxed. The result product was evaporated, filtered, washed with cold ethanol, dried, and recrystallized with ethanol.

2.2.4. General procedure for synthesis of (S)-4-(aryl/alkylsubstitued)-5-[1-(6-methoxynapthalen-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones (13-21)

(S)-Naproxen thiosemicarbazides (**3-12**) (0.01 mol) was added in 4 N sodium hydroxide solution (50 mL) and refluxed for 4 h. After cooling to room temperature, the solution was neutralized with concentrated HCl. The product was precitipated, filtered, washed with water, dried and recrystallized with ethanol.

2.2.5. General procedure for synthesis of (S)-4-(aryl/alkylsubstitued)-5-[1-(6-methoxynapthalen-2-yl)ethyl)-3-(substitutedbenzyl)thio)-4H-1,2,4-triazoles (22-68)

(S)-Naproxen triazoles (0.01 mol) (13-21), equimolar molar of substituted benzyl chlorides and potassium carbonate (K_2CO_3) was added in ethanolic medium refluxed for 24 h. After cooling down at room temperature, the solvent was evaporated. The resulting solid was filtered, washed with water, dried, and recrystallized with ethanol.

- (S)-N-(2-chlorophenyl)-2-(2-(6-methoxynapthalen2-yl)propanoyl)hydrazine-1-carbo thioamide (**3**)

White solid; yield %79.2; Mp 157.6–160 °C; Rf x 100 value (M1): 22; FT-IR (γ_{max} cm^{-1}): 3382, 3362 (Thiosemicarbazide N-H); 3177, 2969, 2935 (C-H, aromatic ring); 2840 (Aliphatic C-H symmetric, asymmetric); 1673 (C=O); 1531, 1504, 1461, 1445 (N-H, C-N, Aromatic C=C); 1393, 1349 (-CH_3 symmetric, asymmetric C-H); 1262 (C=S). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.49–1.50 (d, J = 3.6 Hz, 3H, -CH-C<u>H</u>$_3$); 3.87 (s, 1H, C<u>H</u>-CH_3 and 3H, O-C<u>H</u>$_3$); 7.16–7.80 (m, 10H, Ar-<u>H</u>); 9.31 (s, 1H, -NH-NH-C=S-N<u>H</u>-); 9.78 (s, 1H, -NH-N<u>H</u>-C=S); 10.25 (s, 1H, -C=O-N<u>H</u>-). Elemental Analysis for $C_{21}H_{20}ClN_3O_2S$ (% calculated/found): C: 60.94/61.78 H: 4.87/4.82, N: 10.15/10.30, S: 7.75/6.85; M.W: 413.92 g/mol.

- (S)-N-(3-chlorophenyl)-2-(2-(6-methoxynapthalen2-yl)propanoyl)hydrazine-1-carbothioamide (**4**)

White solid, yield %83.7; Mp 194.7–196^{o}C; Rf x 100 value (M1): 42; FT-IR (γ_{max} cm^{-1}): 3337, 3299, 3234 (Thiosemicarbazide N-H);

3133, 3012, 2957 (C-H, aromatic ring); 2838 (Aliphatic C-H symmetric, asymmetric); 1673 (C=O); 1531, 1502, 1487, 1437 (N-H, C-N, Aromatic C=C); 1392, 1342 (-CH_3 symmetric, asymmetric C-H); 1263 (C=S). ^{1}H-NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.40–1.42 (q, 1H, -CH-C$\underline{H}_3$); 1.48–1.50 (d, J = 7.2 Hz, 2H, -CH-C$\underline{H}_3$); 3.85–3.87 (t, 1H, C$\underline{H}$-CH_3 and 3H, O-C$\underline{H}_3$); 7.13–7.80 (m, 10H, Ar-$\underline{H}$); 9.58 (s,1H, -NH-NH-C=S-N$\underline{H}$-); 9.78 (s, 1H, NH-N$\underline{H}$-C=S-NH-); 10.16 (s, 1H, -C=O-N$\underline{H}$). Elemental Analysis for $C_{21}H_{20}ClN_3O_2S$ (% calculated/found): C: 60.94/61.77, H: 4.87/5.13, N: 10.15/10.30; M. W: 413.9 g/mol.

- (S)-N-benzyl-2-(2-(6-methoxynapthalen-2-yl)propanoyl)hydrazine-1-carbothioamide (**5**)

White solid, yield %78; Mp 137.5.-139.5°C; Rf x 100 value (M1): 35; FT-IR (γ_{max} cm^{-1}): 3660 (O-H); 3420, (Thiosemicarbazide N-H); 3156, 3061, 2981, 2950 (C-H, aromatic ring); 2839 (Aliphatic C-H symmetric, asymmetric); 1688 (C=O); 1531, 1496, 1464, 1415 (N-H, C-N, Aromatic C=C.); 1350, 1339 (-CH_3 symmetric, asymmetric C-H); 1264 (C=S). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.45–1.47 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.77–3.86 (m, 1H, C$\underline{H}$-CH_3 and 3H, O-C$\underline{H}_3$); 4.64–4.82 (m, 2H, Triazole-N-C$\underline{H}_2$-Ar); 7.13–7.78 (m, 11H, Ar-$\underline{H}$); 8.33 (s, 1H, -NH-NH-C=S-N$\underline{H}$); 9.35 (s, 1H, NH-N$\underline{H}$-C=S-NH-); 10.01 (s, 1H, -C=O-N$\underline{H}$). Elemental Analysis for $C_{22}H_{23}N_3O_2S.H_2O$ (% calculated/found): C: 64.21/64.25, H: 6.12/6.16, N: 10.21/10.19, S: 7.79/8.27; M. W: 411.5 g/mol.

- (S)-N-ethyl-2-(2-(6-methoxynapthalen-2-yl)propanoyl)hydrazin-1-carbothioamide (**6**)

White solid, yield %82.9; Mp 182–184 °C, Rf x 100 value (M1): 42; FT-IR (γ_{max} cm^{-1}): 3347, (Thiosemicarbazide N-H); 3175, 2973, 2937 (C-H, aromatic ring); 2839 (Aliphatic C-H symmetric, asymmetric); 1690 (C=O); 1544, 1504, 1481, 1393 (N-H e.b., C-N g.b., Aromatic C=C g.b.); 1335, 1319 (-CH_3 symmetric, asymmetric C-H e.b.); 1262 (C=S). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.00–1.02 (t, J = 3.6 Hz, J = 3.6 Hz, 3H, CH_2-C$\underline{H}_3$); 1.46–1.47 (d, 3H, CH-C$\underline{H}_3$); 3.41–3.46 (m, 2H, C$\underline{H}_2$-CH_3) 3.74–3.79 (q, 1H, -C$\underline{H}$-CH_3); 3.87 (s, 3H, O-C$\underline{H}_3$); 7.68 (s, 1H, -NH-C=S-N$\underline{H}$-); 7.12–7.80 (m, 6H, Ar-$\underline{H}$) 9.16 (s, 1H, -NH-N$\underline{H}$-C=S); 9.92 (s, 1H, -C=O-N$\underline{H}$); Elemental Analysis for $C_{17}H_{21}N_3O_2S$ (% calculated/found): C: 61.61/61.77, H: 6.39/6.25, N: 12.68/12.19; M. W: 331.4 g/mol.

- (S)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione (**13**)

White solid, yield %97.3; Mp 205.3–207.9 °C; Rf x 100 value (M2):54.7; FT-IR (γ_{max} cm^{-1}): 3093 (C-H, aromatic ring), 3038, 2933 (Aliphatic C-H symmetric, asymmetric), 2775 (Triazole S-H), 1632, 1607, 1565, 1487, 1388 (triazole C=N, aromatic -C=C-), 818, 764 (Ar-Cl), 687 (C-S). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.57–1.58 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.10 (q, 1H, -C$\underline{H}$-CH_3); 6.98–7.66 (m, 10H, Ar-$\underline{H}$); 13.95 (s, 1H, -N$\underline{H}$). Elemental Analysis for $C_{21}H_{18}ClN_3O_2S$ (% calculated/found): C: 63.71/62.83, H: 4.58/5.05, N: 10.61/10.01, S: 8.10/7.69; M. W: 395.91 g/mol.

- (S)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione (**14**)

White solid, yield %91.6; Mp 185.3–186.6 °C, Rf x 100 value (M2): 37; FT-IR (γ_{max} cm^{-1}): 3503 (O-H) 3083 (C-H, aromatic ring), 3022, 2932 (Aliphatic C-H symmetric, asymmetric), 2762 (Triazole S-H), 1633, 1558, 1454, 1389 (triazole C=N, aromatic -C=C-), 692 (C-S). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.50–1.52 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.09–4.18 (q, 1H, -C$\underline{H}$-CH3); 4.76–5.31 (dd, J = 16.2 Hz, J = 15.9 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar); 7.12–7.73 (m, 11H, Ar-$\underline{H}$); 13.90 (s, 1H, -NH). Elemental Analysis for $C_{21}H_{18}ClN_3O_2S.½H_2O$ (% calculated/found): C: 68.72/68.68, H: 5.77/6.15, N: 10.93/10.14, S: 8.34/7.84; M. W: 384.5 g/mol.

- (S)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione (**15**)

White solid, yield %84.7; Mp 138.5–140.3 °C; Rf x 100 value (M2): 65; FT-IR (γ_{max} cm^{-1}): 3349 (Triazole N-H), 3102 (C-H, aromatic ring), 3052, 2936 (Aliphatic C-H symmetric, asymmetric), 2753 (Triazole S-H), 1633, 1572, 1448, 1379 (triazole C=S, aromatic -C=C-), 678 (C-S e.b.). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.71–0.73 (t, J = 3.6 Hz, J = 3.6 Hz, 3H, CH_2-C$\underline{H}_3$); 1.60–1.62 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 3.65–3.71 (m, 1H, Triazole-N-C$\underline{H}_2$-CH_3); 3.84–3.90 (m, 1H, Triazole-N-C$\underline{H}_2$-CH_3 and 3H, O-C$\underline{H}_3$) 4.46–4.49 (q, 1H, -C$\underline{H}$-CH_3); 7.16–7.82 (m, 6H, Ar-$\underline{H}$); 13.71 (s, 1H, -N$\underline{H}$). Elemental Analysis for $C_{17}H_{19}N_3OS.½H_2O$ (% calculated/found): C: 63.33/62.96, H: 6.25/6.24, N: 13.03/12.88, S: 9.94/9.40; M. W: 322.4 g/mol.

- (S)-3-((3-fluorobenzyl)thio)-4-(4-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**22**)

White solid, yield %28.8; Mp 141.3oC; Rf x 100 value (M2): 29; FT-IR (γmax cm-1): 3093, 3030 (C-H, aromatic ring), 2963, 2937, 2843 (Aliphatic C-H symmetric, asymmetric), 1627, 1617, 1504, 1463, 1389 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1229 (S-CH_2), 1175 (C-F), 1096 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.66 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.09–4.16 (q, 1H, -C$\underline{H}$-CH_3); 4.30 (s, 2H, S-C$\underline{H}_2$); 6.77–7.66 (m, 14H, Ar-H); Elemental Analysis for $C_{28}H_{23}ClFN_3OS$ (% calculated/found): C: 66.72/66.18, H: 4.60/4.55, N: 8.34/8.35, S: 6.36/6.46; M. W: 504.02 g/mol.

- (S)-3-((2,4,6-trimethylbenzyl)thio)-4-(4-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**23**)

White solid, yield %45.5; Mp 146.3–150.8 °C; Rf x 100 value (M2): 30; FT-IR (γ_{max} cm^{-1}): 3047 (C-H, aromatic ring), 2987, 2936, 2839 (Aliphatic C-H symmetric, asymmetric), 1633, 1603, 1493, 1456, 1393 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1233 (S-CH_2), 1088 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 2.08 (9H, s, Ar-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.10–4.14 (m, 1H, -C$\underline{H}$-CH_3 and 1H, S-C$\underline{H}_2$); 4.26 – 4.28 (d, J = 6.3 Hz, 1H, S-C$\underline{H}_2$,); 6.70–7.67 (m, 12H, Ar-$\underline{H}$); Elemental Analysis for $C_{31}H_{30}ClN_3OS$.½H_2O (% calculated/found): C: 69.32/69.33, H: 5.82/5.66, N: 7.82/7.69, S: 5.97/5.47; M. W.: 537.1 g/mol.

- (S)-3-(benzylthio)-4-(4-fluorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**24**)

White solid; yield: %19; Mp 119.2–120.1 °C; Rf x 100 value (M2): 23; FT-IR (γ_{max} cm^{-1}): 3385 (O-H), 3077, 3042 (C-H, aromatic ring), 3007, 2957, 2934, 2834 (Aliphatic C-H symetric, asymetric), 1628, 1600, 1456, 1390 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1259 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.66 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.06–4.13 (q, 1H, -C$\underline{H}$-CH_3); 4.28 (s, 2H, S-C$\underline{H}_2$,); 6.99–7.66 (m, 15H, Ar-$\underline{H}$); ^{13}C NMR (150 MHz) (DMSO-d_6/TMS) δ ppm: 164.43–161.12 (C-25), 160.20 (C-2), 153.13 (C-17), 140.20 (C-14), 136.15 (C-9), 132.93 (C-28), 132.87(C-5), 132.23 (C-4), 132.10 (C-22), 131.98 (C-7), 131.51(C-10), 131.33 (C-27, C-23), 130.48 (C-30, C-32), 130.10 (C-6), 128.84 (C-29, C-33), 128.32 (C-31), 121.77 (C-8), 119.29 (C-1), 108.80 (C-3), 58.24 (C-12), 39.96 (C-13), 39.35 (C-21), 23.87 (C-15). Elemental Analysis for $C_{28}H_{24}FN_3OS$.½H_2O (% calculated/found): C: 70.27/69.67, H: 5.27/5.08, N: 8.78/8.71, S: 6.70/6.10; M. W: 478.6 g/mol.

- (S)-3-(benzylthio)-4-(4-methylphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**25**)

White solid; yield: %38.7; Mp 134–135 °C; Rf x 100 value (M2): 40; FT-IR (γ_{max} cm^{-1}): 3038 (C-H, aromatic ring), 3002, 2954, 2935, 2885, 2836 (Aliphatic C-H symetric, asymetric), 1628, 1601, 1514, 1480 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1264 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.64 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 2.29 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.06–4.09 (q, 1H, -C$\underline{H}$-CH_3); 4.29 (s, 2H, S-C$\underline{H}_2$,); 7.04–7.67 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{27}N_3OS$ (% calculated/found): C: 74.81/74.20 H: 5.84/5.76, N: 9.02/8.93, S: 6.89/6.48; M. W: 465.62 g/mol.

- (S)-3-((4-chlorobenzyl)thio)-4-(4-methylphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**26**)

White solid, yield: %45.1; Mp 138.2 °C; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3065 (C-H, aromatic ring), 3042, 3022, 2959, 2935, 2839 (Aliphatic C-H symetric, asymetric), 1628, 1602, 1513, 1454, 1391 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1265 (S-CH_2); 840 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.62–1.65 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 2.29 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.04–4.11 (q, 1H, -C$\underline{H}$-CH_3); 4.27 (s, 2H, S-C$\underline{H}_2$,); 6.73–7.67 (m, 14H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{26}ClN_3OS$ (% calculated/found): C: 69.65/68.75, H: 5.24/5.11, N: 8.40/8.39, S: 6.41/6.42; M. W: 500.06 g/mol.

- (S)-3-((4-florobenzyl)thio)-4-(4-methylphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**27**)

White solid, yield %15.7; Mp 128.6–130.2 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm-1): 3036 (C-H, aromatic ring), 3000, 2964, 2940, 2841 (Aliphatic C-H symetric, asymetric), 1628, 1602, 1508, 1417, 1392 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1265 (S-CH_2); 1033 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.64 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 2.30 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.10 (q, 1H, -C$\underline{H}$-CH_3); 4.29 (s, 2H, S-C$\underline{H}_2$,); 7.04–7.66 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (150 MHz) (DMSO-d_6/TMS) δ ppm: 163.70 – 161.11 (C-31), 160.19 (C-2), 153.01 (C-17), 142.57 (C-14) 140.23 (C-9), 136.63 (C-28), 136.14 (C-25), 134.02 (C-5), 133.27 (C-4), 132.98 (C-22), 132.12 (C-7), 131.33 (C-10), 130.18 (C-30, C-32), 130.04 (C-29, C-33), 128.92 (C-6), 128.31 (C-27, C-23), 121.73 (C-24, C-26), 118.31 (C-8), 118.17 (C-1), 108.80 (C-3), 58.24 (C-12), 39.20 (C-13), 38.76 (C-21), 23.98 (C-34), 23.76 (C-15). Elemental Analysis for $C_{29}H_{26}FN_3OS$ (% calculated/found): C: 72.02/71.17 H: 5.42/5.36, N: 8.69/8.54, S: 6.63/6.49; M. W: 483.6 g/mol.

- (S)-3-((2,4,6-trimethylbenzyl)thio)-4-(4-methylphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**28**)

White solid, yield %37.8; Mp 181 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} cm^{-1}): 3049 (C-H, aromatic ring), 3009, 2942, 2912, 2847 (Aliphatic C-H symetric, asymetric), 1633, 1605, 1561, 1484, 1393 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1258 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 2.12 (s, 9H, Ar-C$\underline{H}_3$); 2.30 (s, 3H, Ar-C$\underline{H}_3$) 3.85 (s, 3H, O-C$\underline{H}_3$); 4.09–4.32 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 6.72–7.69 (m, 12H, Ar-$\underline{H}$). Elemental Analysis for $C_{32}H_{33}N_3OS$.$2H_2O$ (% calculated/found): C: 70.69/70.68 H: 6.86/6.03, N: 7.73/7.52, S: 5.90/6.62; M. W: 543.72 g/mol.

- (S)-3-((2-chlorobenzyl)lthio)-4-(4-methoxyphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**29**)

White solid, yield %54.1; Mp 70–72 °C; Rf x 100 value (M2): 25; FT-IR (γ_{max} cm^{-1}): 3057 (C-H, aromatic ring), 2973, 2933, 2837 (Aliphatic C-H symetric, asymetric), 1633, 1605, 1511, 1484, 1373 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1231 (S-CH_2), 1084 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.62–1.65 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.73 (s, 3H, Ar-O-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.04–4.11 (q, 1H, -C$\underline{H}$-CH_3); 4.35 (s, 2H, S-C$\underline{H}_2$,); 6.83–7.67 (m, 14H, Ar-$\underline{H}$); Elemental Analysis for $C_{29}H_{26}ClN_3O_2S$. ⅓H_2O (% calculated/found): C: 66.72/66.89, H: 5.15/4.87, N: 8.05/7.92, S: 6.14/5.80; M. W: 522.06 g/mol.

- (S)-3-((2,6-dichlorobenzyl)thio)-4-(4-methoxyphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**30**)

White solid, yield %66.4; Mp 128.3–130.1 °C; Rf x 100 value (M2): 24; FT-IR (γ_{max} cm^{-1}): 3076, 3059 (C-H, aromatic ring), 3009, 2977, 2933, 2909, 2847 (Aliphatic C-H symetric, asymetric), 1629, 1603, 1561, 1513, 1434, 1391 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1226 (S-CH_2); 1022 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.67 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.73 (s, 3H, Ar-O-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.14 (q, 1H, -C$\underline{H}$-CH_3); 4.32 – 4.37 (dd, J = 12.9 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$,); 6.81–7.69 (m, 13H, Ar-$\underline{H}$). ^{13}C NMR (150 MHz) (DMSO-d_6/TMS) δ ppm: 162.78 (C-25), 161.91 (C-2), 160.3 (C-28), 151.84 (C-17), 140.23 (C-14) 139.15 (C-29, C-33), 137.94 (C-9), 136.17 (C-5), 135.42 (C-4), 133.36 (C-22), 132.12 (C-7), 131.77 (C-6), 131.34 (C-30, C-32),130.04 (C-10), 130.03(C-27, C-23), 129.01 (C-26), 128.42 (C-24), 128.39 (C-31), 121.76 (C-8), 117.59 (C-1), 108.83 (C-3), 58.58 (C-35), 58.24 (C-12), 39.43 (C-13), 36.74 (C-21), 23.94 (C-15). Elemental Analysis for $C_{29}H_{25}Cl_2N_3O_2S$ (% calculated/found): C: 63.27/62.96, H: 5.15/5.05, N: 8.95/8.85, S: 6.83/6.85; M. W: 550.5 g/mol.

- (S)-3-((3-fluorobenzyl)lthio)-4-(4-methoxyphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**31**)

White solid, yield %69; Mp 105.3–106 °C, Rf x 100 value (M2): 22; FT-IR (γ_{max} cm-^{1}): 3076, 3050 (C-H, aromatic ring), 2965, 2935, 2840 (Aliphatic C-H symetric, asymetric), 1631, 1604, 1512, 1484 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1251 (S-CH_2), 1121 (C-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.62–1.65 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.73 (s, 3H, Ar-O-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.05–4.12 (q, 1H, -C$\underline{H}$-CH_3); 4.31 (s, 2H, S-C$\underline{H}_2$,); 6.84–7.67 (m, 13H, Ar-$\underline{H}$); Elemental Analysis for $C_{29}H_{26}FN_3O_2S$.½H_2O (% calculated/found): C: 68.48/68.91 H: 5.35/4.94, N: 8.26/8.32, S: 6.30/6.14; M. W: 508.6 g/mol.

- (S)-3-(benzylthio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**32**)

White solid, yield %35.3; Mp 148.7–150.6 °C; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3060 (C-H, aromatic ring), 3001, 2982, 2935, 2905 (Aliphatic C-H symetric, asymetric), 1631, 1606, 1497, 1390 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1262 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.14 (q, 1H, -C$\underline{H}$-CH_3); 4.26 (s, 2H, S-C$\underline{H}_2$,); 6.98–7.66 (m, 15H, Ar-$\underline{H}$); Elemental Analysis for $C_{28}H_{24}ClN_3OS$ 0.2/3H_2O (% calculated/found): C: 67.52/67.05, H: 5.13/4.74, N: 8.44/8.38, S: 6.44/6.40; M. W: 498 g/mol.

- (S)-3-((2-chlorobenzyl)thio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**33**)

White solid, yield %58.3; Mp 84–85.6 °C; Rf x 100 value (M2): 29; FT-IR (γ_{max} cm^{-1}): 3061 (C-H, aromatic ring), 3012, 2983, 2934, 2870 (Aliphatic C-H symetric, asymetric), 1633, 1605, 1477, 1390 (aromatic -C=C- and C=N, -CH_3 symmetric, asymmetric), 1263 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.10–4.11 (d, J = 3.3 Hz, 2H, S-C$\underline{H}_2$); 4.29 – 4.34 (q, 1H, -C$\underline{H}$-CH_3); 6.98–7.66 (m, 14H, Ar-$\underline{H}$). Elemental Analysis for $C_{28}H_{23}Cl_2N_3OS$ (% calculated/found): C: 64.61/64.40, H: 4.45/4.15, N: 8.07/7.86, S: 6.16/5.90; M. W: 520.47 g/mol.

- (S)-3-((3-fluorobenzyl)thio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**34**)

White solid, yield %62.8; Mp 113.6–116.8 °C; Rf x 100 value (M2): 31; FT-IR (γ_{max} cm-1): 3063 (C-H, aromatic ring), 2981, 2932, 2873 (Aliphatic C-H symetric, asymetric), 1635, 1604, 1439, 1394 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1249 (S-CH_2), 1123 (C-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.08–4.15 (q, 1H, -C$\underline{H}$-CH_3); 4.28 (s, 2H, S-C$\underline{H}_2$,); 6.98–7.66 (m, 14H, Ar-$\underline{H}$); Elemental Analysis for $C_{28}H_{23}ClFN_3OS$.½H_2O (% calculated/found): %C: 65.55/65.48 %H: 4.72/4.37, %N: 8.19/8.29, %S: 6.25/5.44; M. W: 513 g/mol.

- (S)-3-((3-methylbenzyl)thio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**35**)

White solid, yield %52.3; Mp 136–138.2 °C, Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3050 (C-H, aromatic ring), 2986, 2935, 2808 (Aliphatic C-H symetric, asymetric), 1634, 1592, 1450, 1383 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1261 (S-CH_2), 1077 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 2.18 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.13 (q, 1H, C$\underline{H}$-CH_3); 4.21 (s, 2H, S-C$\underline{H}_2$,); 6.93–7.66 (m, 14H, Ar-$\underline{H}$); Elemental Analysis for $C_{29}H_{26}ClN_3OS$ ½H_2O (% calculated/found): %C: 68.42/68.26 %H: 5.35/4.88, %N: 8.25/8.33, %S: 6.30/5.34; M. W: 509.05 g/mol.

- (S)-3-((4-methylbenzyl)lthio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**36**)

White solid, yield %45.7; Mp 142.8 °C; Rf x 100 value (M2): 0.39; FT-IR (γ_{max} cm^{-1}): 3068, 3044 (C-H, aromatic ring), 2993, 2957, 2936, 2840 (Aliphatic C-H symetric, asymetric), 1631, 1605, 1494, 1378 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1227 (S-CH_2), 864 (C-Cl).^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 2.07 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.03–4.20 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 6.89–7.68 (m, 14H, Ar-$\underline{H}$); Elemental Analysis for $C_{29}H_{26}ClN_3OS$ (% calculated/found): C: 69.65/68.72, H: 5.24/5.13, N: 8.40/8.38, S: 6.41/6.31; M. W: 500.1 g/mol.

- (S)-3-((4-methoxybenzyl)thio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**37**)

White solid, yield %71.6; Mp 121.6–123.4 °C, Rf x 100 value (M2): 22; FT-IR (γ_{max} cm^{-1}): 3458 (O-H), 3075 (C-H, aromatic ring), 2960, 2932, 2833 (Aliphatic C-H), 1631, 1606, 1508, 1418, 1390 (aromatic -C=C, triazole C=N, -CH_3), 1264 (S-CH_2), 1078 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.58 (s, 3H, Ar-O-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.05–4.36 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 6.70–7.68 (m, 14H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{26}ClN_3O_2S$. ⅓H_2O (% calculated/found): C: 66.72/66.01, H: 5.15/5.20, N: 8.05/7.81, S: 6.14/6.08; M. W: 522.05 g/mol.

- (S)-3-((3-(trifluoromethyl)benzyl)thio)-4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**38**)

White solid, yield %35.5; Mp 159.8–162.7 °C; Rf x 100 value (M2): 29; FT-IR (γ_{max} cm^{-1}): 3066, 3050 (C-H, aromatic ring), 2985, 2938, 2845 (Aliphatic C-H symetric, asymetric), 1634, 1609, 1582, 1449, 1393 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1276 (S-CH_2), 1163 (C-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.65 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.84 (s, 3H, O-C$\underline{H}_3$); 4.08–4.14 (q, 1H, -C$\underline{H}$-CH_3); 4.37 (s, 2H, S-C$\underline{H}_2$); 6.97–7.65 (m, 14H, Ar-$\underline{H}$); Elemental Analysis for $C_{29}H_{23}ClF_3N_3OS$ (% calculated/found): C: 62.87/62.50, H: 4.18/4.16, N: 7.58/7.57, S: 5.79/5.95; M. W: 554.03 g/mol.

- (S)-3-((2-chlorobenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**39**)

White solid, yield %50.3; Mp 64.7–67.3 °C, Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3057, 2976 (C-H, aromatic ring), 2976, 2933, 2866, 2838 (Aliphatic C-H symetric, asymetric), 1633, 1605, 1498, 1437, 1390 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1263 (S-CH_2), 1030 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.66 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.84 (s, 3H, O-C$\underline{H}_3$); 4.07–4.13 (q, 1H, -C$\underline{H}$-CH_3); 4.35 (s, 2H, S-C$\underline{H}_2$,); 6.99–7.65 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{28}H_{24}ClN_3OS$ (% calculated/found): C: 69.19/68.81, H: 4.98/4.65, N: 8.65/8.44, S: 6.60/6.19; M. W: 486.03 g/mol.

- (S)-3-((4-chlorobenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**40**)

White solid, yield %29.3; Mp 155.8–158.3 °C; Rf x 100 value (M2): 32; FT-IR (γ_{max} cm^{-1}): 3058, 3007 (C-H, aromatic ring), 2984, 2934, 2835 (Aliphatic C-H), 1632, 1605, 1500, 1417, 1390 (aromatic -C=C, triazole C=N, -CH_3), 1262 (S-CH_2), 1073 (C-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.62–1.63 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 3.83 (s, 3H, O-C$\underline{H}_3$); 4.07–4.10 (q, 1H, -C$\underline{H}$-CH_3); 4.27 (s, 2H, S-C$\underline{H}_2$,); 6.98–7.63 (m, 15H, Ar-$\underline{H}$); Elemental Analysis for $C_{28}H_{24}ClN_3OS$. ⅓H_2O (% calculated/found): C: 68.35/68.11, H: 5.05/4.74, N: 8.54/8.53, S: 6.52/6.42; M.W: 492 g/mol.

- (S)-3-((3-fluorobenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**41**)

White solid, yield %59; Mp 107.8–111.1 °C, Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3048, 3028 (C-H, aromatic ring), 3010, 2935, 2880, 2845 (Aliphatic C-H), 1628, 1601, 1500, 1437, 1346 (aromatic -C=C, triazole C=N, -CH_3), 1259 (S-CH_2), 1148 (C-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.65 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.84 (s, 3H, O-C$\underline{H}_3$); 4.07–4.15 (q, 1H, -C$\underline{H}$-CH_3); 4.31 (s, 2H, S-C$\underline{H}_2$); 6.99–7.65 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{28}H_{24}FN_3OS$ (% calculated/found): C: 71.26/70.99, H: 5.15/5.05, N: 8.95/8.85, S: 6.83/6.85; M. W: 469.6 g/mol.

- (S)-3-((4-methylbenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**42**)

White solid, yield %31.1; Mp 134.6–136.2 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3056, 3006 (C-H, aromatic ring), 2985, 2935, 2834 (Aliphatic C-H), 1633, 1605, 1499, 1431, 1389 (aromatic -C=C, triazole C=N, -CH_3), 1269 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 2.18 (s, 3H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.07–4.10 (q, 1H, -C$\underline{H}$-CH_3); 4.21–4.24 (dd, J = 6.3 Hz, J = 6.3 Hz, 2H, S-C$\underline{H}_2$); 7.00–7.65 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{27}N_3OS$. ½H_2O (% calculated/found): C: 73.39/72.77, H: 5.95/5.69, N: 8.85/8.79, S: 6.76/6.35; M. W: 474.6 g/mol.

- (S)-3-((4-methoxybenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**43**)

White solid, yield %35.5; Mp 142.3–146.2 °C; Rf x 100 value (M2): 22; FT-IR (γ_{max} cm^{-1}): 3057 (C-H, aromatic ring), 2968, 2934, 2837 (Aliphatic C-H), 1632, 1606, 1509, 1421, 1389 (aromatic -C=C, triazole C=N, -CH_3), 1263 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.64–1.66 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 3.65 (s, 3H, Ar-O-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.08–4.12 (q, 1H, -C$\underline{H}$-CH_3); 4.22–4.28 (dd, J = 6.3 Hz, J = 6.3 Hz, 2H, S-C$\underline{H}_2$); 6.77–7.65 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{27}N_3O_2S$.½H_2O (% calculated/found): C: 70.99/70.43, H: 5.75/5.39, N: 8.5/8.55, S: 6.54/6.43; M. W: 490.6 g/mol.

- (S)-3-((2,4,6-trimethylbenzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**44**)

White solid, yield %40.3; Mp 178.3–181.4 °C; Rf x 100 value (M2): 27; FT-IR (γ_{max} cm^{-1}): 3058, 3015 (C-H, aromatic ring),

2984, 2958, 2938, 2854 (Aliphatic C-H), 1631, 1604, 1507, 1416, 1389 (aromatic -C=C, triazole C=N, -CH_3), 1264 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.67 (d, J = 3.6 Hz, 3H, -CH-C$\underline{H}_3$); 2.09 (s, 9H, Ar-C$\underline{H}_3$); 3.85 (s, 3H, Ar-O-C$\underline{H}_3$); 4.09–4.13 (q, 1H, -C$\underline{H}$-CH_3); 4.16–4.27 (dd, J = 6 Hz, J = 6 Hz, 2H, S-C$\underline{H}_2$,); 6.71–7.66 (m, 13H, Ar-$\underline{H}$). Elemental Analysis for $C_{31}H_{31}N_3OS$. ⅓H_2O (% calculated/found): C: 74.52/74.36, H: 6.39/6.16, N: 8.41/8.36, S: 6.42/6.10; M. W: 499.7 g/mol.

- (S)-3-((3-(trifluoromethyl)benzyl)thio)-4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**45**)

White solid, yield %58; Mp 85.2–87.1 °C; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3091, 3057 (C-H, aromatic ring), 2984, 2936, 2842 (Aliphatic C-H), 1633, 1606, 1498, 1434, 1327 (aromatic -C=C, triazole C=N, -CH_3), 1264 (S-CH_2), 1148 (C-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.62–1.64 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.84 (s, 3H, O-C$\underline{H}_3$); 4.06–4.13 (q, 1H, -C$\underline{H}$-CH_3); 4.38 (s, 2H, S-C$\underline{H}_2$); 6.98–7.65 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{24}F_3N_3OS$ (% calculated/found): C: 67.04/66.61, H: 4.66/4.79, N: 8.09/7.98, S: 6.17/6.14; M. W: 519.6 g/mol.

- (S)-3-(benzylthio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**46**)

White solid, yield %43.3; Mp 128.3–129.6 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} cm^{-1}): 3054, 3020 (C-H, aromatic ring), 2967, 2955, 2925, 2844 (Aliphatic C-H symetric, asymetric), 1633, 1601, 1495, 1445, 1305 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1233 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.22–4.32 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.67–4.92 (dd, J = 16.5 Hz, J = 16.5 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.74–7.74 (m, 16H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{27}N_3OS$ .1H_2O (% calculated/found): C: 72.02/72.61, H: 6.04/5.88, N: 8.69/8.69, S: 6.63/6.68; M. W: 483.63 g/mol.

- (S)-3-((2-chlorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**47**)

White solid, yield %75.7; Mp 115.3–116.5 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3057, 3030 (C-H, aromatic ring), 2973, 2927, 2862, (Aliphatic C-H symetric, asymetric), 1634, 1607, 1508, 1497, 1389, (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1262 (S-CH_2), 1052 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.63 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.24–4.40 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.69–4.94 (dd, J = 16,8 Hz, J = 16,8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.74–7.74 (m, 15H, Ar-$\underline{H}$). M Elemental Analysis for $C_{29}H_{26}ClN_3OS$ (% calculated/found): C: 69.65/68.87, H: 5.24/5.48, S: 6.41/6.36; M. W: 500.06 g/mol.

- (S)-3-((3-chlorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**48**)

White solid, yield %68.2; Mp 103–106 °C; Rf x 100 value (M2): 22; FT-IR (γ_{max} cm^{-1}): 3087, 3043 (C-H, aromatic ring), 2982, 2955, 2832, (Aliphatic C-H symetric, asymetric), 1632, 1605, 1505. 1478, 1390, (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1228 (S-CH_2), 1028 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.25–4.34 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.76–5.02 (dd, J = 16.5, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar); 6.73–7.73 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{26}ClN_3OS$.½H_2O (% calculated/found): C: 68.42/68.32, H: 5.35/4.90, N: 8.25/8.31, S: 6.30/5.44; M. W.: 509.06 g/mol.

- (S)-3-((4-chlorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**49**)

White solid, yield %68.1; Mp 103.4–104.4 °C,; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 33,990 (O-H); 3054 (C-H, aromatic ring), 2978, 2952, 2933, 2838 (Aliphatic C-H symetric, asymetric), 1673, 1633, 1505, 1461, 1390 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1226 (S-CH_2), 1032 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.63 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.22–4.32 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.70–4.94 (dd, J = 16.8 Hz, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar); 6.73–7.74 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{26}ClN_3OS$.½ H_2O (% calculated/found): C: 68.42/68.06, H: 5.35/5.42, N: 8.25/8.14, S: 6.30/6.29; M. W: 509.07 g/mol.

- (S)-3-((2,6-dichlorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**50**)

White solid, yield %53.0; Mp 78–80 °C; Rf x 100 value (M2): 31; FT-IR (γ_{max} cm^{-1}): 3321 (O-H), 3060, 3025 (C-H, aromatic ring), 2975, 2932, 2900, 2866 (Aliphatic C-H symetric, asymetric), 1633, 1605, 1562, 1451, 1391, (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1210 (S-CH_2), 1121 (Ar-Cl). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.63–1.65 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.26–4.35 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.79–5.08 (dd, J = 16.5 Hz, J = 16.5 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.82–7.76 (m, 14H, Ar-$\underline{H}$). ^{13}C NMR (150 MHz) (DMSO-d_6/TMS) δ ppm: 161.60 (C-2), 160.29 (C-28′), 151.09 (C-17), 140.03 (C-14) 138.33 (C-33′, C-29′), 137.94 (C-9), 136.37 (C-5), 135.59 (C-4), 133.36 (C-31), 132.19 (C-7), 131.73 (C-6), 130.76 (C-30′, C-32′), 130.03 (C-10), 130.34 (C-32), 129.54 (C-33),129.36 (C-34, C-35), 129.06 (C-31′), 128.59 (C-36), 121.83 (C-8, C-1), 108.85 (C-3), 58.28 (C-12), 49.33 (C-30), 39.24 (C-13), 37.53 (C-21), 24.45 (C-15). Elemental Analysis for $C_{29}H_{25}Cl_2N_3OS$.¾ H_2O (% calculated/found): C: 63.56/63.25, H: 4.87/4.50, N: 7.67/7.39, S: 5.85/5.75; M. W.: 548.01 g/mol.

- (S)-3-((3-fluorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**51**)

White solid, yield %67.7; Mp 135–136 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3055, 3035 (C-H, aromatic ring), 2973, 2951, 2930, 2866 (Aliphatic C-H symetric, asymetric), 1630, 1603, 1506, 1459, 1391, (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1206 (S-CH_2), 1075 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.26–4.35 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.75–5.01 (dd, J = 16.8 Hz, J = 16.5 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.74–7.73 (m, 15H, Ar-$\underline{H}$). ^{13}C NMR (150 MHz) (DMSO-d_6/TMS) δ ppm: 165.50, 164.20 (C-32′), 161.17 (C-2), 160.27 (C-28′), 152.37 (C-17), 140.18 (C-14) 138.24 (C-33′, C-9), 136.33 (C-5), 133.37 (C-4, C-29), 132.18 (C-31′, C-7), 131.62 (C-6), 130.67 (C-10), 129.31 (C-32, C-33), 128.89 (C-29′, C-30′), 128.51 (C-1), 128.10 (C-30, C-31), 121.79 (C-34), 118.72 (C-8), 117.36 (C-1), 108.83 (C-3), 58.28 (C-12), 49.25 (C-28), 39.67 (C-13), 39.03 (C-21), 24.50 (C-15). Elemental Analysis for $C_{29}H_{26}FN_3OS$ 0.3/2 H_2O (% calculated/found): C: 68.21/68.08, H: 5.72/5.04, N: 8.23/8.15, S: 6.28/6.32; M. W: 510.64 g/mol.

- (S)-3-((4-fluorobenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**52**)

White solid, yield %67.7; Mp 97–100 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3057, 3025 (C-H, aromatic ring), 2978, 2936, 2840 (Aliphatic C-H symetric, asymetric), 1633, 1603, 1508, 1439, 1391, (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1223 (S-CH_2), 1121 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.22–4.32 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.70–4.95 (dd, J = 16.8 Hz, J = 16.5 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.74–7.74 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{29}H_{26}FN_3OS$ (% calculated/found): C: 72.02/71.45, H: 5.42/5.66, N: 8.69/8.52, S: 6.63/6.49; M. W: 483.61 g/mol.

- (S)-3-((3-methylbenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**53**)

White solid, yield %74.9, Mp 108 °C; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3055, 3017 (C-H, aromatic ring), 2975, 2955, 2930, 2855, (Aliphatic C-H symetric, asymetric), 1633, 1603, 1505. 1446, 1391 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1229 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 2.19 (s, 3H, Ar-C$\underline{H}_3$) 3.85 (s, 3H, O-C$\underline{H}_3$); 4.19–4.33 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.73–4.97 (dd, J = 16.8 Hz, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.74–7.73 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{30}H_{29}N_3OS$ .1H_2O (% calculated/found): C: 72.40/72.47, H: 6.28/5.88, N: 8.44/8.48, S: 6.44/6.46; M. W: 497.65 g/mol.

- (S)-3-((4-methylbenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**54**)

White solid, yield %62; Mp 105.4–106 °C,; Rf x 100 value (M2): 30; FT-IR (γ_{max} cm^{-1}): 3462 (O-H), 3021 (C-H, aromatic ring), 2971, 2934 (Aliphatic C-H symetric, asymetric), 1632, 1603, 1514, 1454, 1389, (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1261 (S-CH_2).^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 2.14 (s, 3H, Ar-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.15–4.28 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.62–4.85 (dd, J = 16.8 Hz, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.75–7.76 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{30}H_{29}FN_3OS$.½H_2O (% calculated/found): C: 73.74/73.43, H: 6.19/6.01, N: 8.60/8.50, S: 6.56/6.6.38; M. W: 488.64 g/mol.

- (S)-3-((3-methoxybenzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**55**)

White solid, yield %70.9; Mp 79.3–80.6 °C; Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3391 (O-H), 3050, 3031 (C-H, aromatic ring), 2976, 2950, 2837, (Aliphatic C-H symetric, asymetric), 1632, 1597, 1488. 1449, 1320, (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1264 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.64 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.66 (s, 3H, Ar-O-C$\underline{H}_3$) 3.85 (s, 3H, O-C$\underline{H}_3$); 4.21–4.33 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.74– 4.99 (dd, J = 16.8 Hz, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.75–7.73 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{30}H_{29}N_3O_2S$.½H_2O (% calculated/found): C: 71.40/71.15, H: 5.99/6.21, N: 8.33/8.27, S: 6.35/6.46; M. W: 504.64 g/mol.

- (S)-3-((3-(trifluoromethyl)benzyl)thio)-4-benzyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**56**)

White solid, yield %85.2; Mp 116–118 °C; Rf x 100 value (M2): 32; FT-IR (γ_{max} cm^{-1}): 3055, 3034 (C-H, aromatic ring), 2991, 2962, 2902, 2841 (Aliphatic C-H symetric, asymetric), 1630, 1605, 1505. 1448, 1389, (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1225 (S-CH_2), 1094 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.61–1.63 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.26–4.40 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 4.76–5.02 (dd, J = 16.8 Hz, J = 16.8 Hz, 2H, Triazole-N-C$\underline{H}_2$-Ar) 6.70–7.71 (m, 15H, Ar-$\underline{H}$). Elemental Analysis for $C_{30}H_{26}F_3N_3OS$ (% calculated/found): C: 67.53/67.15, H: 4.91/4.96, N: 7.87/7.84, S: 6.01/6.01; M. W: 533.6 g/mol.

- (S)-3-(benzylthio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**57**)

White solid, yield %62.3; Mp 146.2–148.3 °C; Rf x 100 value (M2): 21; FT-IR (γ_{max} cm^{-1}): 3315 (O-H); 3083, 3054, 3028 (C-H, aromatic ring), 2989, 2933, 2872 (Aliphatic C-H symetric, asymetric), 1631, 1603, 1466, 1445, 1349 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1213 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.61–0.65 (t, J = 7.2 Hz, J = 7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 1.66–1.69 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.43–3.50 (m, 1H, -C$\underline{H}_2$-CH_3, under DMSO peak); 3.58–3.66 (m, 1H, -C$\underline{H}_2$-CH_3); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.30–4.35 (dd, J = 12.9 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$); 4.40–4.47 (q, 1H, -C$\underline{H}$-CH_3); 7.13–7.79 (m, 11H, Ar-$\underline{H}$). Elemental Analysis for $C_{24}H_{25}N_3OS$ 0.1/3H_2O (% calculated/found): C: 70.38/70.29, H: 6.32/5.77, N: 10.26/10.30, S: 7.83/8.14, M. W: 409.55 g/mol.

- (S)-3-((4-chlorobenzyl)thio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**58**)

White solid, yield %77.9; Mp 162–164.3 °C; Rf x 100 value (M2): 21; FT-IR (γ_{max} cm^{-1}): 3059, (C-H, aromatic ring), 2988, 2976, 2932, 2873 (Aliphatic C-H symetric, asymetric), 1630, 1603, 1511, 1485, 1389 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1250 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.61–0.66 (t, J = 7.2 Hz, J = 7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 1.66–1.69 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.43 – 3.59 (C$\underline{H}_2$-CH_3, under the DMSO-d_6 water peak.) 3.61–3.70 (m, 1H, C$\underline{H}_2$-CH_3); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.27–4.42 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$); 7.13–7.80 (m, 10H, Ar-$\underline{H}$); Elemental Analysis for $C_{24}H_{24}ClN_3OS$.½H_2O (% calculated/found): C: 64,49/64.51 H: 5.64/5.15, N: 9.40/9.45, S: 7.17/7.40; M. W: 447 g/mol.

- (S)-3-((3-florobenzyl)thio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**59**)

White solid, yield %72.3; Mp 110.5–113 °C; Rf x 100 value (M2): 31; FT-IR (γ_{max} cm^{-1}): 3054, 3033 (C-H, aromatic ring), 2983, 2966, 2934, 2873 (Aliphatic C-H symetric, asymetric), 1631, 1618, 1505, 1486, 1381 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1262 (S-CH_2), 1030 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.59–0.64 (t, J = 7.2 Hz, J = 6.9 Hz, 3H, CH_2-C$\underline{H}_3$); 1.65–1.68 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.30 (1H Triazole-N-C$\underline{H}_2$-CH_3 peak is under the DMSO-d_6 water peak); 3.67–3.76 (m, 1H, C$\underline{H}_2$-CH_3); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.30–4.47 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$,); 6.99–7.78 (m, 10H, Ar-$\underline{H}$). Elemental Analysis for $C_{24}H_{24}FN_3OS$.½H_2O (% calculated/found): C: 66.95/66.87 H: 5.85/5.43, N: 9.76/9.59, S: 7.45/6.96; M. W: 430.54 g/mol.

- (S)-3-((4-florobenzyl)thio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**60**)

White solid, yield %50.6; Mp 159.7–161.0 °C; Rf x 100 value (M2): 21; FT-IR (γ_{max} cm^{-1}): 3073, 3026, (C-H, aromatic ring), 2991, 2971 2918, 2877 (Aliphatic C-H symetric, asymetric), 1630, 1603, 1505, 1484, 1339 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1263 (S-CH_2), 1026 (Ar-F). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.61–0.66 (t, J = 7.2 Hz, J = 7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 1.66–1.69 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.44 – 3.54 (C$\underline{H}_2$-CH_3 1H under the DMSO-d_6 water peak.) 3.62–3.72 (m, 1H, C$\underline{H}_2$-CH_3); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.28–4.43 (m, 1H, -C$\underline{H}$-CH_3 and 2H, S-C$\underline{H}_2$,); 6.99–7.79 (m, 10H, Ar-$\underline{H}$). Elemental Analysis for $C_{24}H_{24}FN_3OS$ (% calculated/found): C: 68.68/67.92 H: 5.74/5.53, N: 9.97/10.00, S: 7.61/8.02; M. W: 421.53 g/mol.

- (S)-3-((4-methylbenzyl)thio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**61**)

White solid, yield %69.7; Mp 155.2–158.6 °C; Rf x 100 value (M2): 23; FT-IR (γ_{max} cm^{-1}): 3051, 3026, (C-H, aromatic ring), 2987, 2973, 2929, 2870 (Aliphatic C-H symetric, asymetric), 1629, 1602, 1511, 1483, 1389 (aromatic -C=C, triazole C=N, $-CH_3$ symmetric, asymmetric), 1264 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.62–0.67 (t, J = 7.2 Hz, J = 7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 1.67–1.69 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 2.12 (s, 3H, Ar-C$\underline{H}_3$) 3.39 -3.49 (C$\underline{H}_2$-CH_3 1H,under the DMSO-d_6 water peak.); 3.54–3.64 (m, 1H, C$\underline{H}_2$-CH_3); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.21–4.30 (t, J = 12.9 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$), 4.39–4.45 (q, 1H, -C$\underline{H}$-CH_3); 6.92–7.80 (m, 10H, Ar-$\underline{H}$). Elemental Analysis for $C_{25}H_{27}N_3OS$.¼H_2O (%

calculated/found): C: 71.14/70.91 H: 6.57/6.50, N: 9.96/10.03, S: 7.60/7.97; M. W: 422.07 g/mol.

- (S)-3-((4-methoxybenzyl)thio)-4-ethyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**62**)

White solid, yield %69; Mp 144.4–148 °C; Rf x 100 value (M2): 33; FT-IR (γ_{max} cm^{-1}): 3059, 3019, (C-H, aromatic ring), 2984, 2933, 2909, 2836 (Aliphatic C-H symetric, asymetric), 1628, 1601, 1510, 1488, 1389 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1256 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 0.60–0.65 (t, J = 7.2 Hz, J = 7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 1.66 – 1.68 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.55 (2H, Triazole-N-C$\underline{H}_2$-CH_3 and 3H, Ar-OC$\underline{H}_3$ under the DMSO-d_6 water peak); 3.85 (s, 3H, O-C$\underline{H}_3$); 4.22–4.26 (dd, J = 12.6 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$) 4.39–4.46 (q, 1H, -C$\underline{H}$-CH_3); 6.66–7.80 (m, 10H, Ar-$\underline{H}$). Elemental Analysis for $C_{25}H_{27}N_3O_2S$ 0.5/4 H_2O (% calculated/found): C: 65.84/65.65 H: 6.52/5.47, N: 9.21/8.86, S: 7.03/6.62; M. W: 456.07 g/mol.

- (S)-3-(benzylthio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**63**)

White solid, yield %62.8; Mp 116–118.8 °C, Rf x 100 value (M2): 35; FT-IR (γ_{max} cm^{-1}): 3059, 3032, 3002, (C-H, aromatic ring), 2968, 2926, 2834 (Aliphatic C-H symetric, asymetric), 1629, 1604, 1519, 1453, 1385 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1255 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 2.98 (s, 3H, Triazole-N-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.17–4.23 (dd, J = 12.9 Hz, J=12.9 Hz, 2H, S-C$\underline{H}_2$), 4.37–4.44 (q, 1H, -C$\underline{H}$-CH_3); 7.08–7.80 (m,11H, Ar-$\underline{H}$). Elemental Analysis for $C_{23}H_{23}N_3OS$ 2/3 H_2O (% calculated/found): C: 68.80/68.66, H: 6.11/6.28, N: 10.47/10.39, S: 7.99/7.60; M. W: 401.53 g/mol.

- (S)-3-((4-chlorobenzyl)thio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**64**)

White solid, yield %22.7; Mp 137–139 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} cm^{-1}): 3059, 3027, (C-H, aromatic ring), 2992, 2972, 2929, 2841 (Aliphatic C-H symetric, asymetric), 1631, 1604, 1518, 1445, 1389 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1261 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 2.99 (s, 3H, Triazole N-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.16–4.23 (dd, J = 13.2 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$), 4.38–4.45 (q, 1H, -C$\underline{H}$-CH_3); 7.09–7.81 (m,10H, Ar-$\underline{H}$). Elemental Analysis for $C_{23}H_{22}ClN_3OS$ .1H_2O (% calculated/found): C: 62.50/62.48, H: 5.47/4.86, N: 9.51/9.16, S: 7.25/6.59; M. W: 441,97 g/mol.

- (S)-3-((3-fluorobenzyl)thio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**65**)

White solid, yield %57.1; Mp 104.7–106.3 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} cm^{-1}): 3057, 3030, 3001, (C-H, aromatic ring), 2973, 2903, 2870 (Aliphatic C-H symetric, asymetric), 1630, 1605, 1586, 1470, 1420, 1386 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1258 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.65–1.68 (d, J = 6.9 Hz, 3H, -CH-C$\underline{H}_3$); 3.08 (s, 3H, Triazole-N-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.22–4.28 (dd, J = 13.2 Hz, J = 13.2 Hz, 2H, S-C$\underline{H}_2$), 4.39–4.46 (q, 1H, -C$\underline{H}$-CH_3); 6.97–7.79 (m, 10H, Ar-$\underline{H}$). Elemental Analysis for $C_{23}H_{22}FN_3OS$.½H_2O (% calculated/found): C: 66.32/66.48 H: 5.57/4.97, N: 10.09/10.12, S: 7.70/7.64; M. W: 416.51 g/mol.

- (S)-3-((4-fluorobenzyl)thio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**66**)

White solid, yield %59.6; Mp 143.4–144.9 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} in cm-1): 3076, 3048, 3006, (C-H, aromatic ring), 2955, 2902, 2830 (Aliphatic C-H symetric, asymetric), 1629, 1605, 1507, 1417, 1387 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1260 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 3.01 (s, 3H, Triazole N-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.17–4.24 (dd, J = 13.2 Hz, J = 13.2 Hz, 2H, S-C$\underline{H}_2$), 4.38–4.45 (q, 1H, -C$\underline{H}$-CH_3); 6.88–7.80 (m,10H, Ar-$\underline{H}$). Elemental Analysis for $C_{23}H_{22}FN_3OS$.½H_2O (% calculated/found): C: 66.32/66.69 H: 5.57/5.48, N: 10.09/10.17, %S: 7.70/7.54; M. W: 416.51 g/mol.

- (S)-3-((4-methylbenzyl)thio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**67)**

White solid, yield %54.2; Mp 127–130 °C; Rf x 100 value (M2): 30; FT-IR (γ_{max} in cm-1): 3055, 3026, (C-H, aromatic ring), 2969, 2926, 2841 (Aliphatic C-H symetric, asymetric), 1630, 1603, 1517, 1461, 1388 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1261 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.66–1.68 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 2.00 (s, 3H, Ar-C$\underline{H}_3$); 2.89 (s, 3H, Triazole-N-C$\underline{H}_3$); 3.87 (s, 3H, O-C$\underline{H}_3$); 4.06–4.14 (dd, J = 12.9 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$), 4.35–4.42 (q, 1H, -C$\underline{H}$-CH_3); 6.75–7.82 (m,10H, Ar-$\underline{H}$). Elemental Analysis for; $C_{24}H_{25}N_3OS$.½H_2O (% calculated/found): C: 69.87/69.77 H: 6.35/5.93, N: 10.19/10.10, S: 7.77/7.60; M. W.: 412.5 g/mol.

- (S)-3-(3-((2,4,6-trimethylbenzyl)thio)-4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole (**68**)

White solid yield %45.5; Mp 154–160 °C; Rf x 100 value (M2): 37; FT-IR (γ_{max} in cm-1): 3062, (C-H, aromatic ring), 2994, 2984, 2912, 2838 (Aliphatic C-H symetric, asymetric), 1632, 1605, 1577, 1466, 1391 (aromatic -C=C, triazole C=N, -CH_3 symmetric, asymmetric), 1299 (S-CH_2). ^{1}H NMR (300 MHz) (DMSO-d_6/TMS) δ ppm: 1.67–1.69 (d, J = 7.2 Hz, 3H, -CH-C$\underline{H}_3$); 1.85 (s, 3H, Ar-C$\underline{H}_3$); 2.00 (s, 6H, Ar-C$\underline{H}_3$); 2.76 (s, 3H, Triazole-N-C$\underline{H}_3$); 3.86 (s, 3H, O-C$\underline{H}_3$); 4.02–4.18 (dd, J = 12.9 Hz, J = 12.9 Hz, 2H, S-C$\underline{H}_2$), 4.32–4.39 (q, 1H, -C$\underline{H}$-CH_3); 6.39–7.83 (m,8H, Ar-$\underline{H}$). Elemental Analysis for $C_{26}H_{29}N_3OS$ ½ H_2O (% calculated/found): C: 70.88/71.03 H: 6.86/6.30, N: 9.54/9.49, S: 7.28/6.84; M. W: 440.6 g/mol.

2.3. Biologicial assay

2.3.1. Cell culture

MCF-7 and MDA-MB-231 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10% Fetal Bovine Serum (FBS), 1% glutamine and penicillin/streptomycin at 37 C, 5% CO_2 atmosphere.

4T1 mouse breast cancer cells were obtained from American Type Culture Collection (ATCC®, #CRL-2539). 4T1 cells were maintained in DMEM/F12 (Dulbecco's Modified Eagle's Medium: Nutrient Mixture F12, Gibco™), containing 10% (v/v) FBS (fetal bovine serum) (Gibco™) and 100 units/mL penicillin/streptomycin (Gibco™), in a humidified incubator at 37 °C and 5% CO_2.

2.3.2. Cell-based screening of compounds

Compound screening was performed for analyzing 50% lethal effect of 54 compounds against MCF-7 and MDA-MB-231 cell lines. To analyze cell viability and growth inhibition, cells were incubated with different concentrations of compounds for 24 h at 37 °C, 5% CO_2 atmosphere with DMEM medium supplemented with 10% FBS, 1% glutamine and penicillin/streptomycin and MTT colorimetric assay was performed according to the instructions of manufacturer (Cell Proliferation Kit I (MTT), 11465007001, Roche, Indianapolis, USA). Briefly, cells were seeded into 96-well plates and left to grow in the presence and absence of agents for indicated time points (24 h). At the end of incubation period, 10 μL of MTT was added to each well and incubated for 4 h, at 37 °C in 5% CO_2 humidified incubator. The absorbance of the formazan was read at 570 nm

using a multi-mode plate reader (Synergy H1, BioTek Instruments Inc., Vermont, USA).

The effect of these compounds on the cell viability 4T1 mouse mammary tumor cell line was tested at 1 to 5 times of calculated IC_{50} value (IC_{50}x1, IC_{50}x2, IC_{50}x3, IC_{50}x4, and IC_{50}x5). Next in order to determine the toxicity of the combination of compound **51** and Docetaxel, WST-1 cell viability and proliferation assay was performed with 4T1. For this purpose, 4T1 cells were seeded into 96-well plates at a density of 5×10^3 cells per well and allowed to attach overnight. While 4T1 cells were seeded into 96-well plates at a density of 2.5×10^3, 5×10^3, 7.5×10^3, 10×10^3, 15×10^3, 20×10^3 cells per well for the generation of standard curve. The very next day, the cells were treated with 48 µg/ml of the compound **51** or **32** and 25 µg/ml of Docetaxel, followed by incubation for 72 h. WST-1 reagent ((4-(3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1.3-benzene disulfonate) (Sigma Aldrich, #5015944001) was then added to measure the cell viability, according to the manufacturer's instructions. Shortly, the cells were treated with WST-1 reagent (10% (v/v) WST-1 reagent in DMEM/F-12 complete media), then incubated for 1 h in the dark at 37 °C. One hour later, the absorbance value was measured at 450 nm using 630 nm as reference wavelength with a microplate reader, and% cell viabilities were calculated by normalizing non-treated cells to 100%. Cell numbers were extrapolated from the linear equation obtained from the standard graph that was constructed using the absorbance values obtained for 2.5×10^3, 5×10^3, 10×10^3, 15×10^3 and 20×10^3 cells.

2.3.3. Annexin V and propidium iodide (PI) staining

The cultured cells (5×10^5 cells/ml) with different concentrations of compounds were stained with Annexin-V/PI and analyzed using the Tali image-based cytometer. Incubated cells were centrifuged and resuspended with 1x Annexin binding buffer. To each 100 µL sample, 5 µL of Annexin V–Alexa Fluor® 488 conjugate was added and incubated 20 min at the dark. After the centrifugation and resuspension of cells, 1 μL of Propidium Iodide was added to each well for 1 min and 25 μL of stained cells were loaded into the Tali slides. The fluorescence was detected using Tali Imaged-Based Cytometer equipped with two fluorescence filters for green and red emissions. The Annexin–V positive / PI negative cells were recognized as apoptotic / dead cells, respectively (Molecular Probes A10788). The stained cells were also monitored using inverted microscope with fluorescence at- tachment system (Olympus CKX-41).

2.3.4. Mitochondrial membrane potential assay

Apoptosis was further evaluated by mitochondrial membrane potential (MMP) changes. MMP is an important parameter for cell health. JC-1 is a lipophilic, cationic dye that can selectively enter mitochondria and its fluorescence emission shifts from green to red as the membrane potential increases. Cells were stained with JC-1 probe according to the protocol of JC-1 mitochondrial membrane potential kit (Abnova KA1324). The ratio of fluorescence from J-monomers to J-aggregates was determined in fluorescence microplate reader with appropriate filters capable of measuring excitation/emission wavelengths of 485/535 nm and 535/595 nm (Synergy H1, BioTek Instruments Inc., Vermont, USA).

2.4. Docking studies

2.4.1. Protein preparation

Structure of human methionine aminopeptidase-2 complexed with spiroepoxytriazole inhibitor (+)-31a (PDB ID; 5CLS) was retrieved from the protein data bank (https://www.rcsb.org). The co-crystallized ligands were subsequently removed from the structure. Missing hydrogen atoms were added based on the protonation state of the titratable residues at a pH of 7.4 using Biovia Discovery Studio 4.5 (Dassault Systèmes BIOVIA, 2017).

2.4.2. Ligand preparation

All compounds were sketched and their 3D geometry optimized, and prepared at pH of 7.4 using Biovia Discovery Studio 4.5.

2.4.3. Molecular docking

SGK compounds were docked into the binding pocket of methionine amino peptidase- 2 enzyme using Autodock 4.2 (https://autodock.scripts.edu) [22]. The energy grid box of dimensions 60_60_60 Å and of grid map 26.297_21.191_16.855 Å was used. Lamarckian genetic algorithm was used for ligand conformational search. For each compound, 20 independent runs were performed and the distinct ligand conformers were generated and docked randomly into the enzyme's binding pocket. The program randomly assigned torsion angles to rotatable bonds. Ten million energy evaluations were allowed for each ligand. Three independent dockings were run for each complex and the average of free energy of binding was computed. The interaction diagrams were generated using Biovia Discovery Studio 4.5 (Dassault Systèmes BIOVIA, 2017).

3. Results

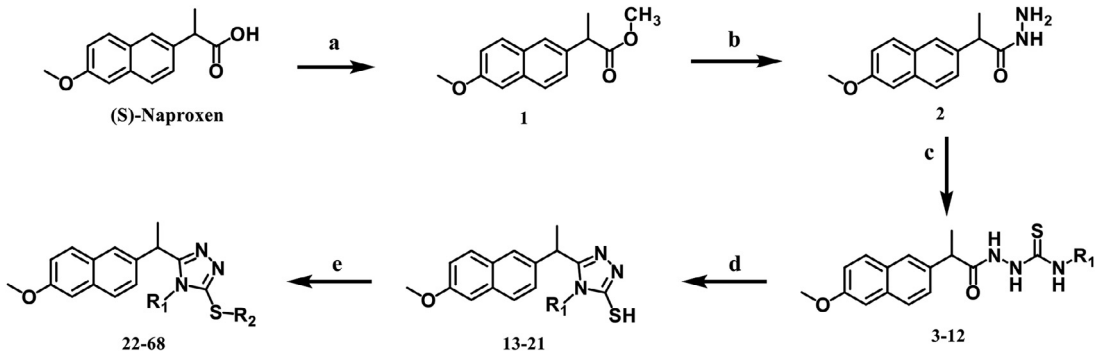
3.1. Chemistry

Naproxen ((S)-2-(6-methoxynapthalen-2yl)propanoic acid), was refluxed in the methanolic medium with catalyst saturated sulfuric acid to obtain Naproxen methyl ester (methyl (S)-2-(6-methoxynaphthalen-2-yl)propanoate) (**1**). Ethanolic medium of naproxen methyl ester and hydrazine hydrate were refluxed to obtain Naproxen hydrazide ((S)-2-(6- methoxynaphthalen-2-yl)propanehydrazide) (**2**) according to literature [6, 13, 19]. Compound **2** and equimolar substituted isothiocyanates were heated in n-butanol medium to yield new (S)-2-(2-(6-methoxynaphthalen-2-yl)propanoyl)-N-(substituted)alkyl/arylhydrazine-1-carbothioamide (**3-12**). Compounds **3-12** was reacted with 4 N NaOH solution and given the 5-(1-(6-methoxynaphthalen-2-yl)ethyl)-4-(4-(substituted)aryl/alkyl)-4H-1,2,4-triazole- 3-thiols (**13-21**). The reaction was then monitored by TLC and neutralized with concentrated hydrochloric acid after its complition. Naproxen thiosemicarbazides and naproxen triazoles were obtained high yield (%80-%95). Last step of this study, thioether compounds ((3-((substituted)arylthio)-5-(1-(6-methoxynaphthalen-2-yl)ethyl)-4-(substitutedalkyl/aryl)- 4H-1,2,4-triazoles) (**22-68**) were obtained by reaction of (S)-naproxen triazoles (**13-21**) dissolved in ethanol with various substitued benzyl chloride in the medium of K_2CO_3. The synthesis of the new (S)-Naproxen derivatives outlined in Scheme 1, according to the literature [23,24]. Novel thiosemicarbazides (**3, 5, 6**), 1,2,4-triazoles (**14-15**) and thioethers (**22-68**) which given in this study are original compounds.

The structures of all compounds were confirmed by FT-IR and ^{1}H NMR spectroscopic methods and their purity were proben by TLC and elementel analysis.

Our starting compound, Naproxen, is synthesized S-enantiomer form [25] and it was proved with Rudolph Autopol V Plus brand / model polarimeter device. This analysis according to EU Pharmacopoeia (EP) monograph [26], 0.5 g Naproxen was dissolved in 25 ml pure ethanol. The result (+59.246°) was in the edge of (S)-Naproxen value which was reported by EP 7.0 as between +59° and +62° [26]. With this result, all the molecules which we synthesized was proved in (S) form.

All of the reaction were monitored by TLC in different mobile phases, M_1: petroleum ether/dichloromethane/ethyl acetate (25:50:25, v/v) at 25 °C, M_2: petroleum ether/ethyl acetate (60:40 v/v) at 25 °C.



Scheme 1. The synthesis of the new (S)-Naproxen derivatives
Reagents and Conditions: **a**: CH_3OH/H_2SO_4; **b**: $NH_2NH_2.H_2O/C_2H_5OH$; **c**: R_1-NCS/n- butanol; **d**: 4 N NaOH, HCl; **e**: Cl-CH_2-C_6H_4-R_2, C_2H_5OH, K_2CO_3.

N-(4-chlorophenyl)-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide (Compound **7**, CAS- Number: 1003001-31-7), *N-(4-fluorophenyl)-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **8**, CAS Number: 2361527-36-6) were synthesized from Amir et al [13].. *N-(4-methylphenyl)-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **9**, CAS Number: 225508-98-5) and *N-(4-methoxyphenyl)-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **10**, CAS Number: 225508-99-6) were reported by Amir et al [19].. *N-phenyl-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **11**, CAS Number: 225508-97-4), *N-methyl-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **12**, CAS Number: 136404-25-5) were synthesized by Yousif et al [20].. It was observed that melting point of the synthesized thiosemicarbazide compounds were 150–152 °C, 158–160 °C, 154–157 °C, 168–171 °C, 165 °C and 158 °C respectively and compatible with the literature. *N-(3-chlorophenyl)-2-[2-(6-methoxynaphthalen-2-yl)propanoyl]hydrazinecarbothioamide* (Compound **4**, CAS Number: 2521777-36-4) were synthesized by Birgül et al. and all spectral data of compound **4** were given in literature [6].

FT-IR Spectral analysis of novel thiosemicarbazides (**3, 5, 6**) showed N-H stretching bands between 3420 and 3234 cm^{-1}. Compound **2**, C=O stretching bonds were shifted from 1633 cm^{-1} to 1673–1688 cm^{-1}. The C=S streching bands were identified at nearly 1263 cm^{-1}, which additionally demonstrated specific thiosemicarbazide C=S stretching bands. The ^1H NMR spectra proven the chemical shifts of N_1, N_2 and N_4 protons between 9.92–10.25 ppm 9.16–9.78, 7.68–9.31 respectively.

The cyclization reaction of thiosemicarbazide to triazole was reported [17], the common method is using an alkaline medium. In this study, we tried different alkaline solutions from 1N NaOH to 4N NaOH. This reaction was carried out with maximum yield in 4N NaOH. As reported before [27], the triazoles could be present in their tautomeric forms and their condition were proven in FT-IR spectral analysis. Compounds **13** and **14** was showed no N-H streching band; instead S-H stretch band was observed between 2600 and 2800 cm^{-1}. FT-IR spectra of compound **15**, molecule showed N-H bands in 3349 cm^{-1}. ^1H NMR analysis of all molecules showed the N-H protons between 13.71 and 13.95 ppm. There was no peak observed between 1.0 and 3.5 ppm for SH protons which proved the thione form of our triazoles. The NH protons of thiosemicarbazide were not detected in triazole samples in ^1H NMR spectra. In the ^1H NMR spectra, all protons of thiosemicarbazide and 1,2,4-triazoles were seen according to the expected chemical shifts and integration values.

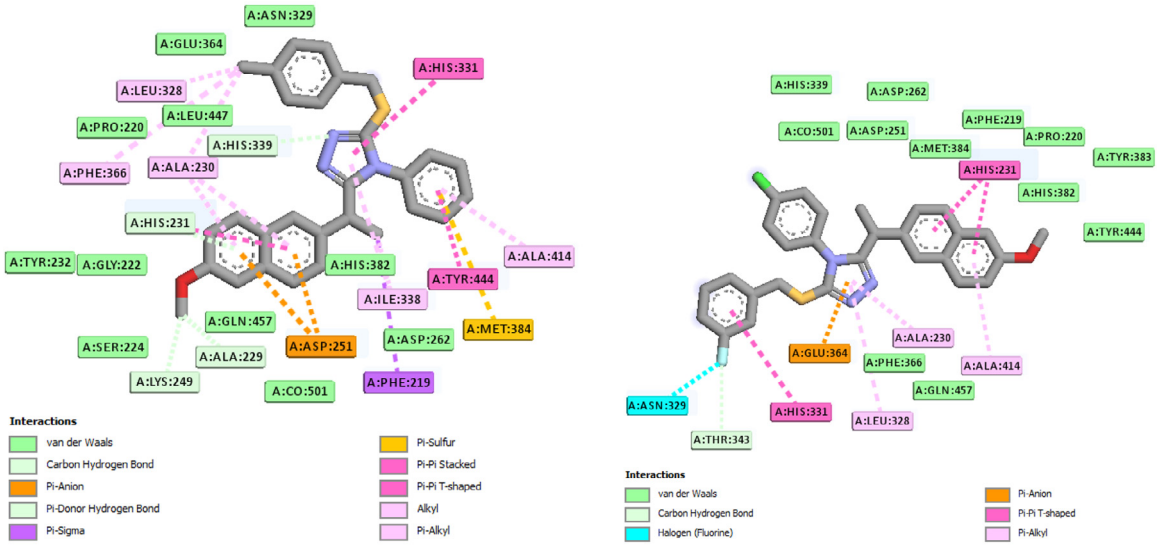
4-(4-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione (Compound **16**, CAS Number: 1003001-43-1) and *4-(4-fluorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **17**, CAS Number: 2395016-10-9) were synthesized by Amir et al [13].. *4-(4-methylphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **18** CAS Number: 225509-05-7), *4-(4-methoxyphenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **19**, CAS Number: 225509-06-8) and *4-phenyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **20**, CAS Number: 225509-04-6) were reported by Amir et al [19].. *4-methyl-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **21**, CAS Number: 136404-29-0) were synthesized by Yousif et al [20].. *4-(3-chlorophenyl)-5-(1-(6-methoxynaphtalen-2-yl)ethyl)-4H-1,2,4-triazole-3-thione* (Compound **7**, CAS Number: 2521777-30-8) were synthesized by Birgül et al. and all spectral data of compound **7** were given in literature [6]. It was observed that melting point of the synthesized 1,2,4-triazole-3-thione/thiol compounds were 146–148 °C, 160 °C, 132 °C, 147 °C, 193–195 °C, 123 °C respectively and the stated melting points of the compounds were also found to be compatible with the literature.

The novel thioethers (**22-68**) were obtained from related triazole with substitued benzyl chloride in potassium carbonate and ethanolic medium. FT-IR studies of naproxen thioethers showed no N-H or S-H strecting bands which were observed in 1,2,4-triazoles compounds. ^1H NMR studies proved the S-CH_2 protons at nearly 4.00 ppm in thioether structure [4].

^1H NMR spectral data of some compounds proved that, S-$C\underline{H}_2$ protons were observed together with $C\underline{H}$-CH_3 protons as multiplet peaks according to the substitution of the compound. Double doublet observation of S-CH_2 protons, showed their coupling consants between 6 and 6.3 Hz. Likewise, substituted triazole-$C\underline{H}_2$-CH_3 or triazole-$C\underline{H}_2$-Ar protons were observed as double doublet; giving the coupling consants of these protons between 16.5 and 16.8 Hz. Observing S-CH_2 carbons in ^{13}C NMR spectra supports the thioether structures. Another finding were supported the synthesis of thioether compounds observation of S-CH_2 carbon atoms in ^{13}C NMR spectra. The S-CH_2 carbons of compounds **24, 27, 30, 50** and **51** were observed between 36.74 and 39.35 ppm. These findings were supported the synthesis of compounds.

3.2. Docking studies

The newly synthesized compounds were docked into the active sites of MetAP2 to predict their biological activity and to rationalize their binding mode using AutoDock 4.2. Consistent with the experimental results, all the compounds were found to be active, especially, compounds **42** and **22** showed good binding affinity against MetAP2 enzyme with free energies of binding ΔG = -11.4 kcal/mol and -11.75 kcal/mol, respectively. The difference in



(A) Compound **42** **(B)** Compound **22**

Fig. 1. (**A**) Compound **42** bound MetAP2 with different binding mode from the other molecules. The Naphthalenyl group formed 2 π-Anion interactions with Asp251 deep inside the active site. Other interactions include π-π Stacked, π-π T-Shaped π-Sigma, a couple of hydrophobic, and several van der Waals interactions. The estimated binding energy was found to be -11.49 which correspond to the Ki value of 3.80 nM **.** (**B**) Compound **22** formed a very strong halogen interaction between the Fluro substituent on the phenyl group and Asn329 deep inside the active site of MetAP2 enzyme. It also formed a π-Anion, π-π T-shaped, few hydrophobic and several van der Waals interactions. The estimated binding energy was estimated to be -11.75 kcal/mol, corresponding to the Ki value of 2.45 nM **.**

the binding energy values was brought about by the difference in the binding orientations of these compounds in the catalytic channel of MetAP2. The interaction diagrams of compounds **42** and **22** were shown in Fig. 1 (**A**) and (**B**).

The structure of all compounds and molecular docking results are given in Table 1.

3.3. Biological activity

Anticancer activities of (S)-Naproxen derivatives were tested with several approaches on breast cancer cell lines MCF-7 and MDA-MB-231 using MTT assay. MCF-7 is the first hormone responsive cell line, with estrogen receptor (ER), progesterone receptor (PR) positive, HER2 negative pattern, while the later is "basal" type and triple negative (ER, PR and HER2 negative) breast cancer cell line with highly aggressive, invasive and poorly differentiated profile. All compounds were applied at 0, 10, 25, 50, 75 and 100 μM concentrations for 24 h. The IC_{50} values of novel (S)-Naproxen derivatives were determined between from 5 to 100 μM (Table 2).

In addition to viability, proliferation was also evaluated by direct cell counts. In general, changes in proliferative capacity were strongly associated with cytotoxicity of drugs. The novel (S)-Naproxen derivatives demonstrating dose-dependence in both cell lines, with IC_{50} values up to 30 μM, were then further examined for their apoptotic effects. The growth inbihition of selected compounds are summarized in Fig. 2A and B.

The apoptotic activity of selected compounds were first analyzed by Annexin V staining using Tali Image-Based Cytometer. For compounds **22** and **42**, apoptotic results were found to be very significant. After addition of 50 μM of compound **42**, we observed 17.6% increase in MCF-7, and 20% increase in MDA-MB-231 cells (Fig. 3A–C). Other derivatives showed apoptotic activity as well, except compound **26**, which displayed only a minor effect.

Decrease in $\Delta\psi$m is also a well-established marker for apoptosis where ratio of apoptotic cells relative to healthy cells was assesed by a shift from red to green fluorescence generated by JC-1 stain [28]. Mitochondrial membrane potential (MMP) assay results were consistent with Annexin V findings in compounds **22** and **42** for both cell lines, but only in MDA-MB-231 cells for compounds **51** and **68**. Compound **26**, on the other hand, did not show any apoptotic effect in none of these cell lines.

As shown in Fig. 4, comparison of 50 μM compound concentration vs control cells revealed an apoptotic induction of 14% in MCF ($p = 0.033$) and 11% in MDA-MB-231 ($p = 0.020$) for Compound **22** using Annexin V staining. The corresponding values in MMP were 21% in MCF ($p = 0.134$) and 22% in MDA-MB-231 ($p = 0.028$) for the same compound. Similarly, for Compound **42**, observed apoptotic induction were 17.5% in MCF-7 ($p = 0.000$) and 20% in MDA-MB-231 ($p = 0.003$) using Annexin V staining, and 27% in MCF-7 ($p = 0.080$) and 16% in MDA-MB-231 ($p = 0.029$) using MMP assay. Strangely, there was a high apoptotic response only in MDA-MB-231 cells for compounds **51** and **68**. Exposure of cells to 50 μM concentration yielded to 10–30% increase in apoptotic ratio for both compunds in this cell line, whereas no effect was observed in MCF-7 cells. Since MDA-MB-231, a triple-negative cell line, was known for its invasive and malignant nature, further examination of these derivatives in molecular level could be highly beneficial.

Similarly, our study also allowed us to determine several additional compounds that are highly effective in one of the cell lines, but not both. According to MTT assay data, compounds **3, 4, 14, 15, 27, 35, 48, 57, 58** showed cytotoxicity with an IC_{50} <10 μM only in MCF-7; whereas **30, 34** and **50** only in MDA-MB-231. All these compounds displayed considerably higher IC_{50} values for the other cell line, which could help to conceive characteristic mechanism of action for each cell type.

Anticancer activities of (S)-Naproxen derivatives were also tested with 4T1 triple negative (ER, PR, and HER2 negative) mouse breast cancer cell line using WST-1 cell viability and proliferation assay. 4T1 cell line was chosen for these studies because tumor growth and the metastatic spread of 4T1 cell line in BALB/c mouse very closely mimics human breast cancer [29–31]. Therefore, this cell line could be used to test effective (S)-Naproxen derivatives in an animal model for stage IV human breast cancer. In order to determine the effect of (S)-Naproxen derivatives on 4T1 cell via-

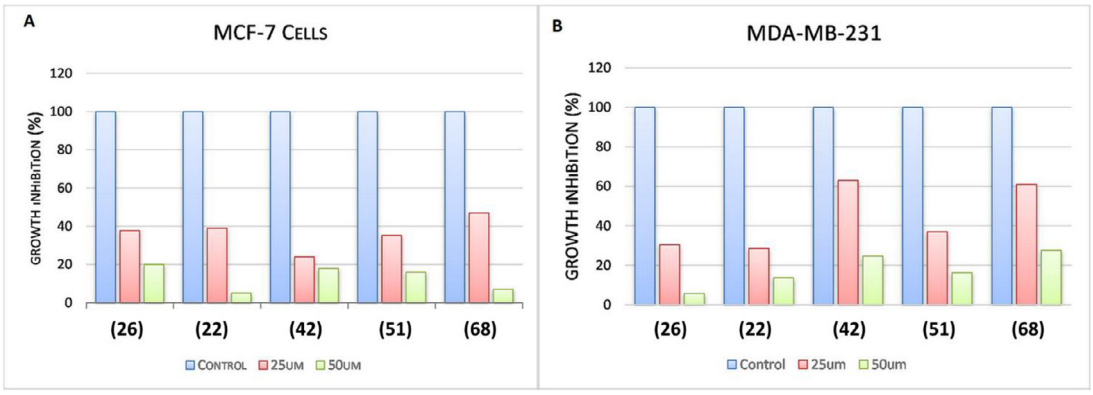


Fig. 2. The growth inbihition of selected compounds (**26, 22, 42, 51** and **68**) (**A**) in MCF-7 and (**B**) MDA-MB-231 cells.

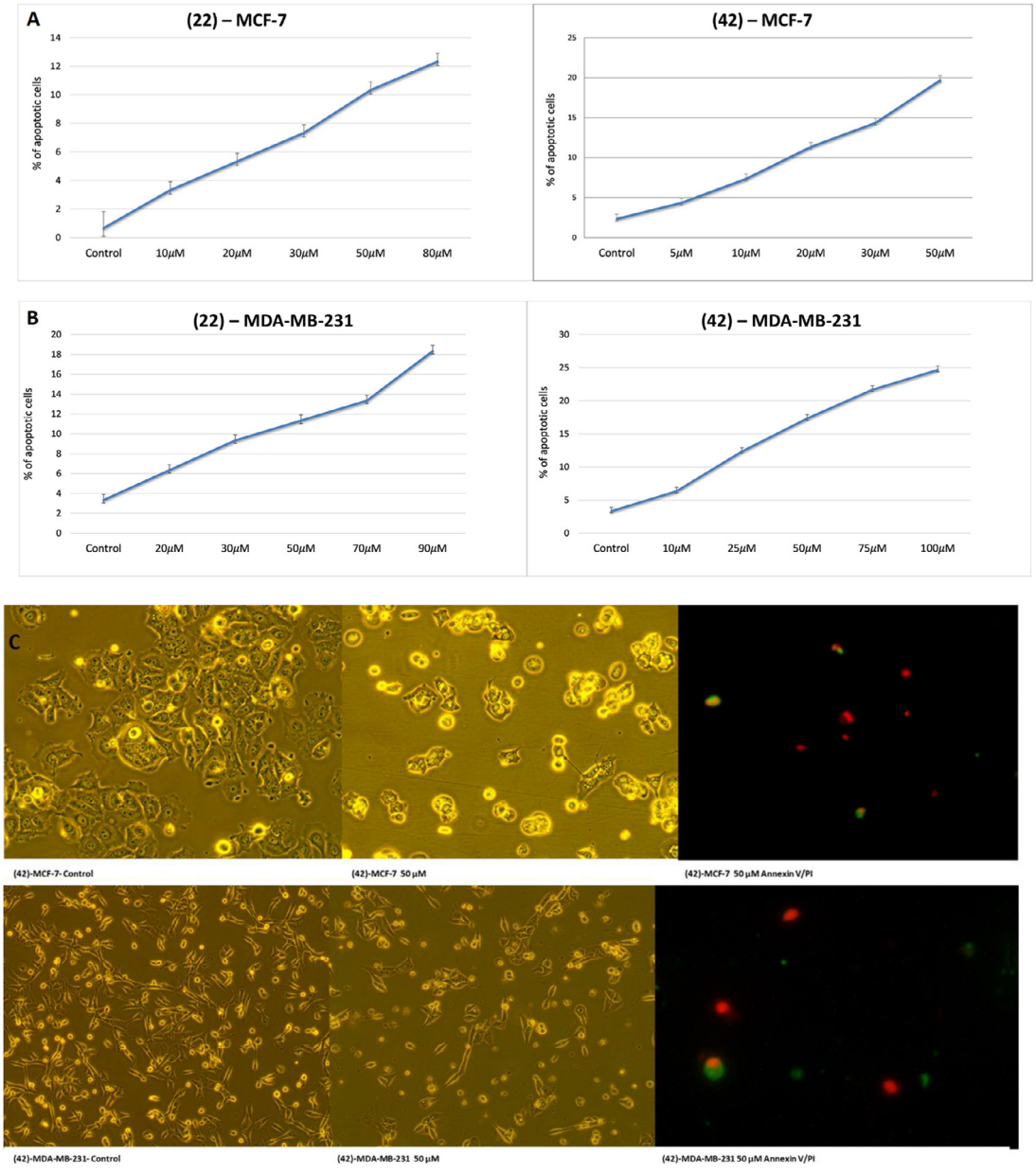
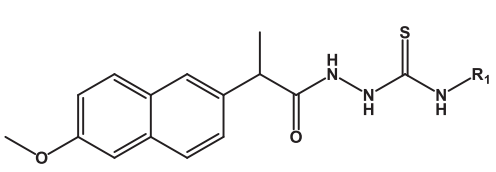
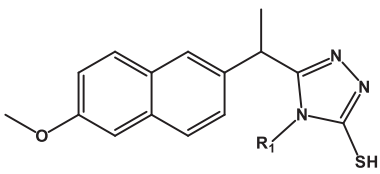


Fig. 3. Percent of apoptotic cells following 24 h exposure to derivatives Compound **22** and Compound **42** (**A**) in MCF-7, (**B**) in MDA-MB-231 cells and (**C**) microscopic images reflecting morphologic changes and green/red fluorescence upon Annexin V/PI staining for control and 50 μM Compound **42**.

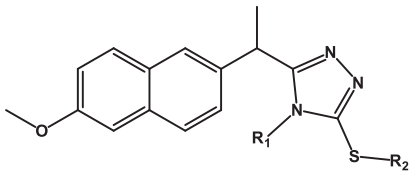
Table 1
Structures and molecular docking scores (**ΔG, Ki**) for novel (S)-Naproxen derivatives.



3-12



13-21



22-68

Compound	Lab Code	R_1	R_2	ΔG (Kcal/mol)	Ki (μ**M**)
3	SGK617	2Cl-C_6H_4-	-	-9.14	200.51
4	SGK624	3Cl-C_6H_4-	-	-11.03	8.27
5	SGK663	C_6H_5-CH_2-	-	-8.46	624.07
6	SGK678	CH_3-CH_2-	-	-7.99	1398
13	SGK625	3-Cl-C_6H_4-	-	-9.1	212.47
14	SGK664	C_6H_5-CH_2-	-	-9.34	149.22
15	SGK679	CH_3-CH_2-	-	-9.34	165.23
22	SGK643	(4)-Cl-C_6H_4-	(3)F-C_6H_4-CH_2-	-11.75	2.45
23	SGK644	(4)-Cl-C_6H_4-	((2,4,6)CH_3)$_3$-C_6H_2-CH_2-	-9.82	63.51
24	SGK612	(4)F-C_6H_4-	C_6H_5-CH_2-	-10.02	45.53
25	SGK618	(4)CH_3-C_6H_4-	C_6H_5-CH_2-	-11.33	5.12
26	SGK619	(4)CH_3-C_6H_4-	(4)Cl-C_6H_4-CH_2-	-11.77	2.36
27	SGK620	(4)CH_3-C_6H_4-	(4)F-C_6H_4-CH_2-	-10.66	16.99
28	SGK692	(4)CH_3-C_6H_4-	((2,4,6)CH_3)$_3$-C_6H_2-CH_2-	-11.96	1.71
29	SGK640	(4)OCH_3-C_6H_4-	(2)Cl-C_6H_4-CH_2-	-10.31	27.88
30	SGK621	(4)OCH_3-C_6H_4-	(2,6)Cl_2-C_6H_3-CH_2-	-0.9.75	71.82
31	SGK638	(4)OCH_3-C_6H_4-	(3)F-C_6H_4-CH_2-	-10.2	33.61
32	SGK637	(3)Cl-C_6H_4-	C_6H_5-CH_2-	-9.81	64.09
33	SGK693	(3)Cl-C_6H_4-	(2)Cl-C_6H_4-CH_2-	-11.53	3.56
34	SGK645	(3)Cl-C_6H_4-	(3)F-C_6H_4-CH_2-	-10.72	13.97
35	SGK646	(3)Cl-C_6H_4-	(3)CH_3-C_6H_4-CH_2-	-10.46	21.47
36	SGK641	(3)Cl-C_6H_4-	(4)CH_3-C_6H_4-CH_2-	-9.31	150.32
37	SGK660	(3)Cl-C_6H_4-	(4) CH_3O-C_6H_4-CH_2-	-9.66	82.78
38	SGK647	(3)Cl-C_6H_4-	(3)CF_3-C_6H_4-CH_2-	-9.34	143.59
39	SGK677	C_6H_5-	(2)Cl-C_6H_4-CH_2-	-9.72	75.48
40	SGK648	C_6H_5-	(4)Cl-C_6H_4-CH_2-	-11.18	6.41
41	SGK661	C_6H_5-	(3)F-C_6H_4-CH_2-	-8.99	256.58
42	SGK649	C_6H_5-	(4)CH_3-C_6H_4-CH_2-	-11.49	3.80
43	SGK650	C_6H_5-	(4) CH_3O-C_6H_4-CH_2-	-10.61	16.73
44	SGK651	C_6H_5-	((2,4,6)CH_3)$_3$-C_6H_2-CH_2-	-9.93	53.02
45	SGK662	C_6H_5-	(3)CF_3-C_6H_4-CH_2-	-10.62	16.31
46	SGK665	C_6H_5-CH_2-	C_6H_5-CH_2-	-9.17	191.06
47	SGK671	C_6H_5-CH_2-	(2)Cl-C_6H_4-CH_2-	-10.82	12.01
48	SGK672	C_6H_5-CH_2-	(3)Cl-C_6H_4-CH_2-	-10.72	13.99
49	SGK666	C_6H_5-CH_2-	(4)Cl-C_6H_4-CH_2-	-10.76	15.22
50	SGK667	C_6H_5-CH_2-	(2,6)Cl_2-C_6H_3-CH_2-	-10.86	11.43
51	SGK670	C_6H_5-CH_2-	(3)F-C_6H_4-CH_2-	-11.29	5.33
52	SGK668	C_6H_5-CH_2-	(4)F-C_6H_4-CH_2-	-10.22	32.16
53	SGK674	C_6H_5-CH_2-	(3)CH_3-C_6H_4-CH_2-	-10.88	11.24
54	SGK669	C_6H_5-CH_2-	(4)CH_3-C_6H_4-CH_2-	-10.32	27.09
55	SGK675	C_6H_5-CH_2-	(3) CH_3O-C_6H_4-CH_2-	-11.22	5.69
56	SGK673	C_6H_5-CH_2-	(3)CF_3-C_6H_4-CH_2-	-10.38	24.54
57	SGK680	CH_3-CH_2-	C_6H_5-CH_2-	-9.17	191.06
58	SGK681	CH_3-CH_2-	(4)Cl-C_6H_4-CH_2-	-9.46	120.07
59	SGK691	CH_3-CH_2-	(3)F-C_6H_4-CH_2-	-9.11	208.97
60	SGK682	CH_3-CH_2-	(4)F-C_6H_4-CH_2-	-9.98	49.43
61	SGK683	CH_3-CH_2-	(4)CH_3-C_6H_4-CH_2-	-8.46	624.07
62	SGK690	CH_3-CH_2-	(4) CH_3O-C_6H_4-CH_2-	-10.05	-43.05
63	SGK684	CH_3-	C_6H_5-CH_2-	-8.78	375.67
64	SGK685	CH_3-	(4)Cl-C_6H_4-CH_2-	-9.46	116.66
65	SGK688	CH_3-	(3)F-C_6H_4-CH_2-	-10.30	28.3
66	SGK686	CH_3-	(4)F-C_6H_4-CH_2-	-9.07	220.33
67	SGK687	CH_3-	(4)CH_3-C_6H_4-CH_2-	-9.23	172.47
68	SGK689	CH_3-	((2,4,6)CH_3)$_3$-C_6H_2-CH_2-	-10.79	12.22

bility, WST-1 assay was used. As Table 2 indicated compounds **48, 50, 51**, and **58** to be the most effective on the cell viability, those compounds were used to treat 4T1 cells at 3x, 4x and 5x of IC_{50} value determined for 4T1 cells at 24 h. Compounds **48, 50, 51**, and **58** led to an average of 43% decrease in 4T1 cell viabity at 3xIC_{50} concentration, while an average of 60% and 69% reduction of cell viability was evident for concentations of 4x and 5x of IC_{50}, respectively (Fig. 5A). In order to determine whether the observed decrease in cell viability was due to cytotoxic or cytostatic effect of these compounds, cell numbers were calculated using the standart curve for the indicated concentrations (Fig. 5B). The average cell number recorded for compounds **48, 50, 51**, and **58** at 3xIC_{50} concentration was 2×10^4, while 1.5×10^4 and 1.1×10^4 cells were found at 4x and 5x of IC_{50} treatments. Given that 5×10^3

Table 2
IC_{50} values (μM) of novel (S)-Naproxen derivatives. (ND: not determined).

Lab Code	Compd.	MCF-7	MDA-MB-231	4T1	Lab Code	Compd.	MCF-7	MDA-MB-231	4T1
SGK617	**3**	4.75	35.4	ND	**SGK649**	**42**	10.76	26	15.4
SGK624	**4**	6.6	ND	ND	**SGK650**	**43**	14.16	19.79	ND
SGK663	**5**	12.33	ND	ND	**SGK651**	**44**	47.31	33.31	ND
SGK678	**6**	ND	ND	ND	**SGK662**	**45**	ND	25.38	ND
SGK625	**13**	ND	ND	ND	**SGK665**	**46**	15.9	45.43	ND
SGK664	**14**	3.24	ND	ND	**SGK671**	**47**	ND	29.42	ND
SGK679	**15**	3.3	22.63	ND	**SGK672**	**48**	9.3	21.86	27.19
SGK643	**22**	22.87	20.55	15.12	**SGK666**	**49**	33.93	42.84	ND
SGK644	**23**	13.81	72.71	ND	**SGK667**	**50**	ND	4.8	5
SGK612	**24**	15.48	10.98	11.27	**SGK670**	**51**	20.3	23.67	18.75
SGK618	**25**	23.58	37.93	ND	**SGK668**	**52**	26.1	33.58	ND
SGK619	**26**	19.17	22.54	9	**SGK674**	**53**	10.95	21.84	ND
SGK620	**27**	9	33.32	12.29	**SGK669**	**54**	15.85	18.76	ND
SGK692	**28**	62	11.26	ND	**SGK675**	**55**	11.0	28.38	ND
SGK640	**29**	29.55	ND	ND	**SGK673**	**56**	21	19.12	ND
SGK621	**30**	110	3.3	5	**SGK680**	**57**	8.9	45.56	12
SGK638	**31**	26.75	48.86	ND	**SGK681**	**58**	6.4	55.18	17.89
SGK637	**32**	26.35	27.41	ND	**SGK691**	**59**	65.71	43.32	ND
SGK693	**33**	10.98	16.4	ND	**SGK682**	**60**	ND	ND	ND
SGK645	**34**	45.72	4.37	19.37	**SGK683**	**61**	18.43	ND	ND
SGK646	**35**	8.6	19.03	11.7	**SGK690**	**62**	48.27	11.65	ND
SGK641	**36**	10.88	ND	ND	**SGK684**	**63**	ND	ND	ND
SGK660	**37**	28.98	15.16	ND	**SGK685**	**64**	70.25	66.35	ND
SGK647	**38**	10.14	22.14	ND	**SGK688**	**65**	66.12	19.0	ND
SGK677	**39**	17.0	18.57	ND	**SGK686**	**66**	60.6	50.02	ND
SGK648	**40**	40.35	21.61	ND	**SGK687**	**67**	26.69	28.47	24.33
SGK661	**41**	20.46	ND	ND	**SGK689**	**68**	10.76	26	ND

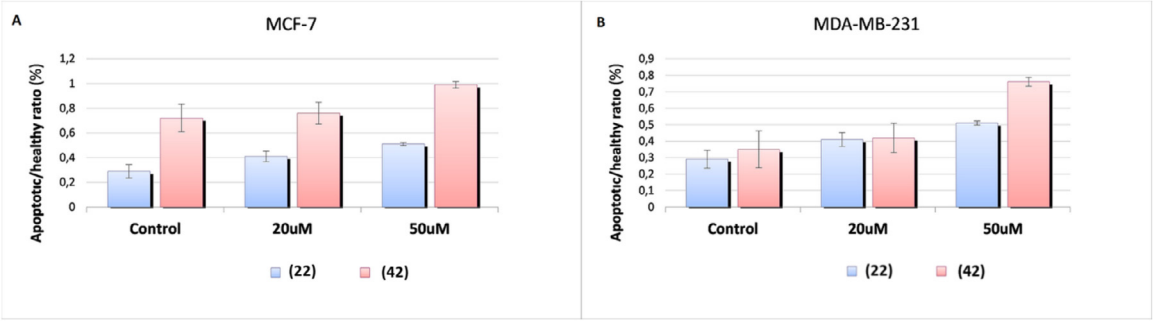


Fig. 4. Mitochondrial membrane potential changes determined in fluorescence plate reader following JC-1 stain for compounds Compound **22**, Compound **42** (A) in MCF-7 and (B) MDA-MB-231 cells.

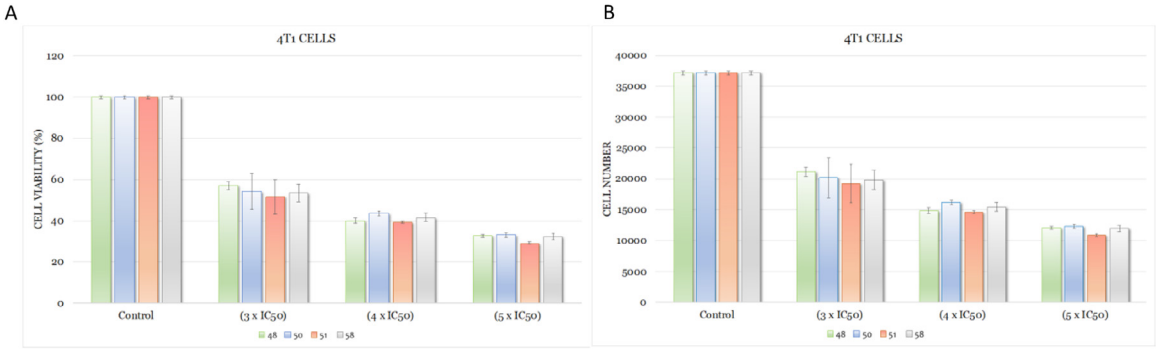


Fig. 5. Cell viability (A) and number of 4T1 (B) cell line under the treatment of Compounds 48, 50, 51, and 58) were shown. Viability was assessed for cells seeded on 96-well plates (5×10^3 cells/well) and treated with 3 x IC_{50} to 5 x IC_{50} doses for 72 h. Cell viability of untreated cells (control) was considered as 100% for all time points. Results were from the average of three independent experiments.

were initially seeded to each well, the effect of compounds **48, 50, 51**, and **58** on 4T1 cells appeared to be cytostatic rather than cytotoxic.

Human epidermal growth factor receptor 2 (HER2) is overexpressed in approximately one-third of breast cancer patients with a strong involvement of the cyclooxygenase-2 (COX-2) enzyme in tumor progression. Because HER2 and COX-2 are potential targets for inhibiting carcinogenesis in breast cancer, Cerón-Carrasco et al [32]. designed dual Herceptin-NSAID drugs. Considering a dual targeting of HER2 with etofenamate, they aimed to reveal the feasibility of improving the antitumor activity of Herceptin. In the light of this information, we aimed to study the anticancer effect of the

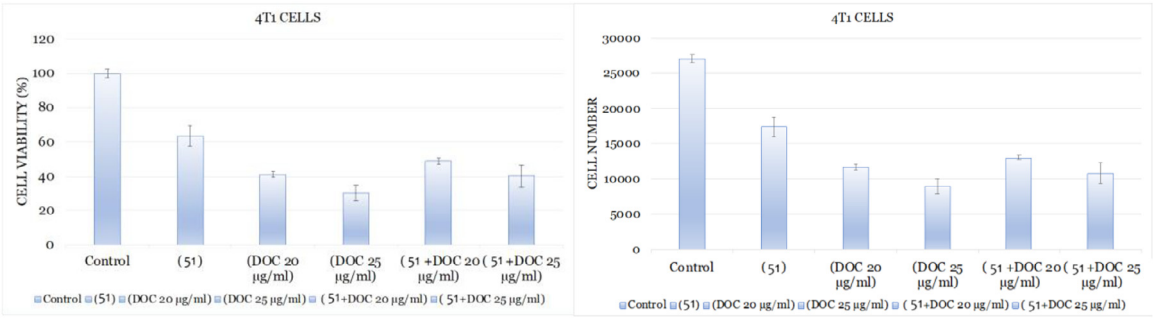


Fig. 6. Cell viability (A) and number of 4T1 cell line (B) under the treatment of compound 51 and docetaxel was shown. Viability was assessed for cells seeded on 96-well plates (5×10^3 cells/well) and treated with 48 µg/ml compound 51 ($5xIC_{50}$), and 20 µg/ml or 25 µg/ml docetaxel for 72 h. Cell viability of untreated cells (control) was considered as 100% for all time points. Results were from the average of three independent experiments.

Docetaxel -naproxen thioether compound. Docetaxel is the active substance in the taxon group used alone to inhibit microtubule polymerization in metastatic breast cancer. Considering that there may be a synergistic effect on the active substance of Docetaxel, an anticancer effect study was conducted on 4T1 cells together with naproxen thioether. It has been suggested that the use of docetaxel in combination therapy could increase the efficacy of docetaxel, resulting in enhanced drug response at lower doses [33]. In order to analyze whether (S)-Naproxen derivatives could enhance the cytostatic effect of low dose docetaxel treatmenton 4T1 cell viability, we have chosen Compound **51** for combination studies with docetaxel. For this purpose, 4T1 cells were treated with 48 µg/ml (5 x IC_{50} value) of Compound **51** combined with 20 or 25 µg/ml of docetaxel for 72 h, and cell viability and number were analyzed for each concentration (Fig. 6). Treatment of 4T1 cell with 20 or 25 µg/ml docetaxel resulted in 59% and 70% reduction in cell viability (Fig. 6A), which corresponded to 1.1×10^4 and 9×10^3 cells (Fig. 6B). When combined with compound **51**, the cytostatic effect of these low doses of docetaxel was not enhanced to cytotoxic effect, as combination of 48 µg/ml (5xIC50) compound **51** with 20 or 25 µg/ml docetaxel resulted in only 51% (1.3×10^4 cells) and 60% (1×10^4) reduction in cell viability.

4. Conclusion

In this study, we designed and synthesized new (S)-naproxen derivatives which could have possible anticancer activity on breast cancer. To understand the mechanism of action, we also performed the docking studies on MetAP2 enzyme. The results were satisfying and promising in terms of anticancer effects of the novel naproxen compounds.

Collectively, these preliminary screening results indicate that derivatives show differential anti-cancer activity depending on the cell type, as could be expected, and compounds with high efficiency and potency for only one cell type could be unique candidates to describe distinctive molecular mechanisms underlying different responses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Kaan Birgül: Investigation, Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Abdullah Ibrahim Uba:** Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft. **Ozan Çuhadar:** Investigation, Visualization, Methodology, Visualization, Writing – original draft. **Sevgi Koçyiğit Sevinç:** Investigation, Methodology, Visualization, Writing – original draft. **Selen Tiryaki:** Investigation, Methodology, Visualization, Writing – original draft. **Pınar Mega Tiber:** Project administration, Writing – original draft, Writing – review & editing. **Oya Orun:** Project administration, Writing – original draft, Writing – review & editing. **Dilek Telci:** Project administration, Writing – original draft, Writing – review & editing. **Özgür Yılmaz:** Project administration, Writing – review & editing. **Kemal Yelekçi:** Project administration, Writing – review & editing. **Ş. Güniz Küçükgüzel:** Project administration, Supervision, Conceptualization, Writing – original draft, Writing – review & editing.

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Supplementary materials

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.132739.

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