Original
ISSN 2413-0516

Molecular Docking, Synthesis, Characterization, and Preliminary Cytotoxic Evaluation of New 1,3,4-Thiadiazole Derivatives as Alpha-Estrogen Receptor Modulator

Zainab M. Abdulkhaleq[1,*], Mohammed Hassan Mohammed[1], Jaafar Suhail Wadi[2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
[2]Department of Pharmacy, AL-RAFIDAIN University College, Baghdad, Iraq.
*Correspondence to: Zainab M. Abdulkhaleq (E-mail: zainab.khaliq1102@copharm.uobaghdad.edu.iq)
(Submitted: 16 February 2022 – Revised version received: 05 March 2022 – Accepted: 23 March 2022 – Published online: 26 June 2022)

Abstract

Objective: This study concluded that these newly synthesized analogs may be represented as an exploitable source of new anticancer agents to fight breast cancer.

Methods: Using The crystal structure of Human α Estrogen Receptor (α-ER) with a 4-HYDROXYTAMOXIFEN (standard) as a co-crystalized ligand was gained from the protein data-bank (PDB code 3ERT) as a result of docking the 1,3,4-thiadiazole derivatives compounds (M1-M3) give good docking scores compared to the standard. Compounds M1-M3 was synthesized by multistep procedures from the reaction of intermediate (benzyloxy)phenyl)-1,3,4-thiadiazole-2(3H)-thione derivatives (D1, D2) and the 4-bromo-1-(cyclic amine1-yl)butan-1-one derivatives intermediate (A1, A2). The chemical structures of the target compounds and their intermediates were confirmed by FT-IR, ^{1}H NMR, and ^{13}C NMR.

Results: The *in-vitro* cytotoxicity assay (MTT assay) demonstrated that compounds M1-M3 showed good inhibition ratios in Breast cancer cell line (MCF-7) comparable with drug control Tamoxifen.

Conclusion: From the docking study, it was concluded that piperidine and methyl-piperazine moiety were very successful to bind tightly to alpha estrogen receptors by making numerous interaction modes.

Keywords: Anticancer, MCF-7, piperidine, methyl-piperazine, docking study

Introduction

Cancer is one of the most serious-clinical problems in the world and its incidence, is increasing in both developing and developed-countries and has been considered one of the major fatal diseases in human history.[1] Cancer can generally be pronounced as the uninhibited growth and banquet of irregular cells in the body the cancer cell can disruption absent from any malignant tumor and pass in the bloodstream or the lymphatic system, and thus cancer can escape, banquet to other parts of the body.

When cancer is established, analysts face a formidable set of trials. Treatment generally comprises various combinations of surgery, chemotherapy, and radiation therapy, but despite these treatment selections, cancer remains related-with a high death rate.[2,3] Some estrogen-responsive genes have both direct ER binding sites and binding sites to which ERs are tethered. The vascular endothelial growth factor gene, for example, contains a variant estrogen receptor element (ERE) that preferentially binds ERs triggered by 17-estradiol and G/GC-rich sequences that bind ER-Sp1 and ER-Sp3 complexes, as well as G/GC-rich sequences that bind ER-Sp1 and ER-Sp3 complexes.[4]

Aromatase, which is found in both epithelial cell components of lung tumors as well as infiltrating macrophages, produces estradiol locally in Non–small cell lung carcinoma (NSCLC). In some animal models, aromatase is even exclusively confined to inflammatory cells infiltrated in the pre-neoplastic and neoplastic areas.[5] Patients with higher levels of aromatase and ER expression in their tumors had a poorer survival rate, especially in postmenopausal women.[6]

Breast cancer is the second most often diagnosed cancer worldwide, with an estimated 2.1 million new diagnoses and about 627,000 breast cancer-related deaths in 2018. Breast cancer is a physiologically and clinically heterogeneous illness with multiple identified histotypes and molecular subtypes, each with its own etiology, risk factor profiles, treatment responses, and prognoses. Around 75 percent of breast cancers are detected in postmenopausal women in high-income nations, with just 5–7% occurring in women younger than 40 years of age.[7] Furthermore, the presence or absence of estrogen receptors (ER) in breast cancers gives prognostic information and is the primary target for endocrine therapy. Selected estrogen receptor modulators (SERMs) and anti-estrogens compete with estrogen for binding to its receptor, whereas third-generation aromatase inhibitors (AIs) are more effective than tamoxifen in postmenopausal women in neoadjuvant and adjuvant contexts.[8] Figure 1 shows some breast cancer medications currently available, including tamoxifen, raloxifene, toremifene, and fulvestrant.[9]

Materials and Methods

Materials

Methyl-3-hydroxy benzoate, 4-bromobutyric acid, benzyl bromide, 4-methyl benzyl bromide, piperidine, and 1-methyl piperazine were purchased from HyperChem China. Other chemicals were purchased from Sigma–Aldrich. All chemicals are of analytical grade, and they were used as received without further purification.

Characterization of Compounds (A-D) and Compounds (M1-M3)

Melting points, Fourier transform infrared spectroscopy: FTIR, NMR: ^{1}H-NMR, and ^{13}C-NMR spectra, were performed for compound characterization.

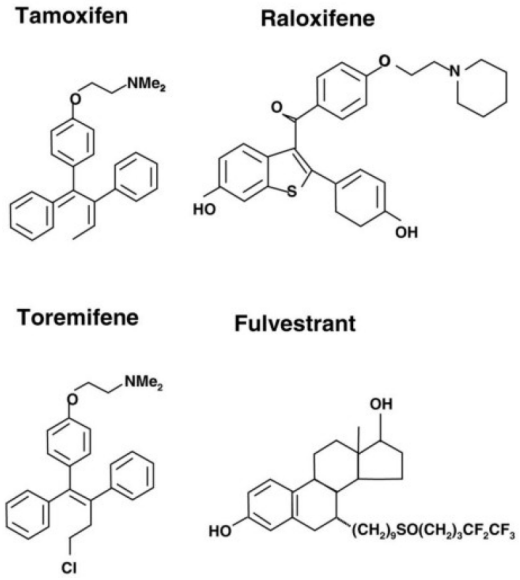


Fig. 1 **Chemical structures of marketed drugs for breast cancer.**

Molecular Docking

3-hydroxytamoxifen was used to validate the docking process because the PDB obtained has a crystal structure of the protein and bound 3-hydroxytamoxifen.

Alpha Estrogen Receptor[10,11]

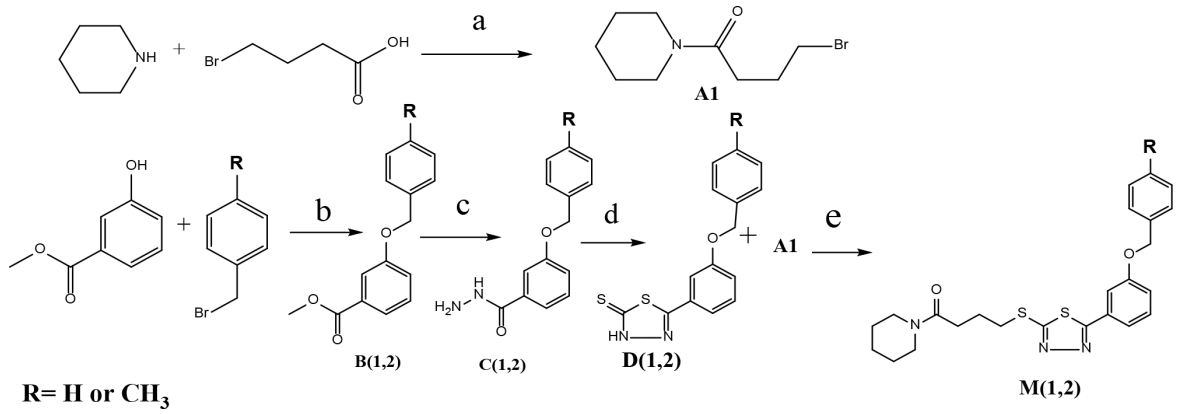
The crystal structure of Human α Estrogen Receptor (α-ER) with a 3-HYDROXYTAMOXIFEN as a co-crystalized ligand was gained from the protein data-bank (PDB code 3ERT). All ions and water molecules were deleted before docking, the docking was carried by Autodock vina and the visualization by chimera, the charge of the protein molecules was modeled by AMBERff14SB force field, while the small molecules (Ligands) by AM1-BCC.

Chemical Synthesis

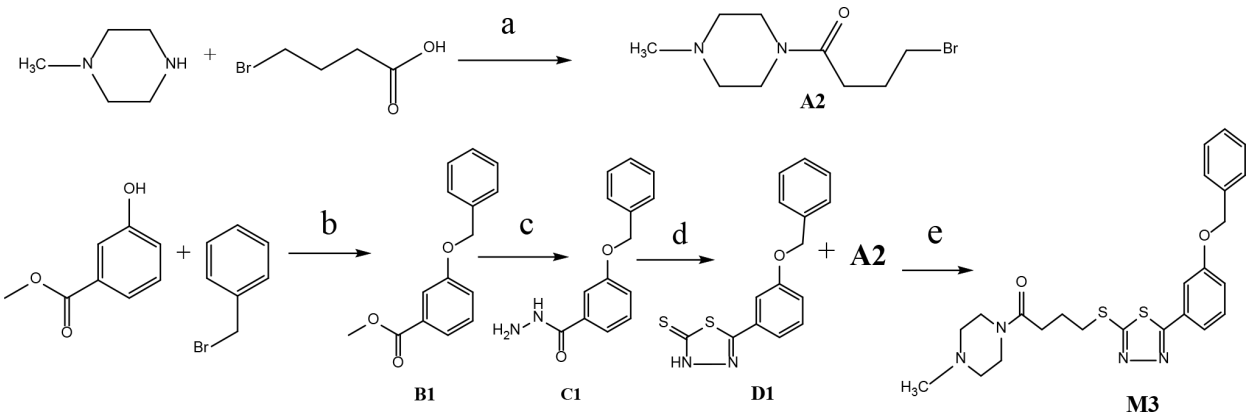
The target compounds were synthesized by multi-step reactions, as shown in Schemes 1 and 2.

Synthesis of 4-bromo-1-(cyclic amine1-yl) butan-1-one (Compound A1, A2)

To a cool the mixture of 4-bromobutyric acid (0.001 mol) in DMF (50 ml) HOBt (0.0012 mol) was added and stirred for 15 min. EDC (0.0012 mol) was added to the reaction mixture



Scheme 1. **Synthesis schematics of compound M(1,2). Reagents and conditions: (a) HoBt, EDC/0–50C, DMF (b) DMF, reflux, K_2CO_3 (c) NH_2NH_2. H_2O, ethanol, reflux (d) CS2, KOH, ethanol, reflux, HCl (e) TEA, ethanol, reflux.**



Scheme 2. **Synthesis schematics of compound M3. Reagents and conditions; (a) HoBt, EDC/0–50C, DMF (b) DMF, reflux, K_2CO_3 (c) NH_2NH_2. H_2O, ethanol, reflux (d) CS2, KOH, ethanol, reflux, HCl (e) TEA, ethanol, reflux.**

and stirred for 1 hr at (0°C) (became slurry-like suspension/activated ester) (checked by TLC). Then, the amine (0.002 mol) DMF (5 ml) was added gradually (at 0°C) to the reaction mixture, & stirring was continuous at r.t. for 24–30 hrs. Termination of reaction was approved by Thin Layer Chromatography (TLC). DMF was evaporated under reduced pressure using a rotary evaporator and the resulted compound was washed by diluted HCL (5%), sodium bicarbonate (5%) and recrystallization from methanol[12,13] the title 4-bromo-1-(piperidin-1-yl)butan-1-one compound (A1) was obtained as a pale off-white powder, mp. 92–94°C, yield 75%. The FT-IR for A1, 2935 cm^{-1} C-H Asymmetric str. Vibration of (CH2-CH2), 1650 cm^{-1} Carbonyl str. vibration band of amide, 1014 cm^{-1} C-N str. Vibration, 648 cm^{-1} C-Br str. Vibration. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.44-3.46(t, J = 5.0 Hz, 2H), 3.29-3.30(t, J = 4.1 Hz, 4H), 2.38-2.41(t, J = 7.2 Hz, 2H), 2.08-2.12(p, J = 5.0 Hz, 2H), 1.55-1.60(m, 6H). And 4-bromo-1-(4-methylpiperazin-1-yl)butan-1-one compound (A2) was obtained as a Pale yellow powder mp. 109–111°C, yield 78%. The FT-IR for A2 2935 cm^{-1} C-H Asymmetric str. Vibration of (CH2-CH2), 1650 cm^{-1} Carbonyl str. vibration band of amide, 1168 cm^{-1} C-N str. Tertiary amine, 1068 cm^{-1} C-N str. Vibration, 644 cm^{-1} C-Br str. Vibration.

^{1}H NMR (400 MHz, DMSO-d6) δ 3.44-3.46 (t, J = 5.0 Hz, 2H), 3.38-3.39 (t, 4H), 2.37-2.41 (t, J = 7.3 Hz, 2H), 2.28-2.29 (t, 4H), 2.23 (s, 3H), 2.09-2.12 (p, 2H).

Synthesis of methyl 3-(benzyloxy)benzoate Derivatives (Compound B1, B2)

A mixture of methyl-3-hydroxybenzoate (0.3 g, 0.002 moles), benzyl bromide (0.7 g or 0.004 mol) or 4-methyl benzyl bromide (0.74 g or 0.004 moles) and K_2CO_3 (0.8 g, 0.006 moles) in DMF (12 ml) were refluxed at room temp. for (9–12 hrs.). Termination of the reaction was supported by (TLC). The solution was then transferred into ice water. The solid gained was filtered & recrystallized from ethanol for (compound B1) & methanol for (compound B2).[14] The title methyl 3-(benzyloxy) benzoate compound (B1) was obtained as a pale-yellow powder, mp. 103-105°C, yield 82%. The FT-IR for B1, 3012 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2951 cm^{-1} C-H Asymmetric str. Vibration of CH_2, 2817 cm^{-1} C-H Asymmetric & symmetric str. Vibration of CH_3, 1708 cm^{-1} C=O str. vibration band of ester, 1589 cm^{-1} C=C Symmetric str. Vibration of aromatic ring, 1431 cm^{-1} C-H bending of CH3, 1230 cm^{-1} C-O str. vibration band of ester, 1072 cm^{-1} C-O-C str. vibration band of ether. ^{1}HNMR (400 MHz, DMSO-d6) δ 7.64-7.66 (d, J = 10.1 Hz, 1H), 7.42-7.44 (m, 4H), 7.28-7.35 (m, 3H), 7.01-7.02 (d, J = 6.4 Hz, 1H), 5.06 (s, 2H), 3.87 (s, 3H). And methyl 3-((4 methylbenzyl)oxy)benzoate compound (B2) was obtained as a white powder mp. 115–117°C, yield 80%. The FT-IR for B2, 3024 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2951 cm^{-1} C-H Asymmetric str. Vibration of CH2, 2870 cm^{-1} C-H Asymmetric & symmetric str. Vibration of CH3, 1720 cm^{-1} C=O str. vibration band of ester, 1585 cm^{-1} C=C Symmetric str. Vibration of aromatic ring, 1442, 1489 cm^{-1} C-H bending of CH3, 1211 cm^{-1} C-O str. vibration band of ester, 1099 cm^{-1} C-O-C str. vibration band of ether. ^{1}H NMR (400 MHz, DMSO-d6) δ7.65–7.66 (d, 1H), 7.44 (s, 1H), 7.33–7.34 (t, J = 8.0 Hz, 1H), 7.27–7.29 (d, 2H), 7.17–7.18 (d, J = 8.2 Hz, 2H), 7.01–7.03 (d, J = 8.0 Hz, 1H), 4.97 (s, 2H), 3.87 (s, 3H), 2.30 (s, 3H).

Synthesis of 3-(benzyloxy)benzohydrazide Derivatives (Compound C1, C2)

A combination of the corresponding ester (B1, B2) (0.48 g, 0.5 g, 0.002 moles), and hydrazine hydrate (N_2H_4) 85% (0.1 ml, 0.002 moles) in EtOH (35 mL) was refluxed for 10–14 h (compound C1 & compound C2 respectively). Next to that, the solution was transferred into ice water. The solid was filtered & recrystallized from methanol,[14] the title 3-(benzyloxy)benzohydrazide compound (C1) was obtained as a white powder, mp. 133–135°C, yield 83%. The FT-IR for C1, 3282, 3190 cm^{-1} NH_2 str. Vibration of amine, 3062 cm^{-1} NH str. Vibration of amide, 3039 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 1643 cm^{-1} C=O str. vibration band of amide, 1600 cm^{-1} N-H bending of amine (NH_2), 1454 cm^{-1} C-H bending of CH2, 1246 cm^{-1} C-O str. vibration band of ester, 1080 cm^{-1} C-O-C str. vibration band of ether. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.07–10.08(t, J = 4.2 Hz, 1H), 7.62–7.64(d, 1H), 7.38–7.39(d, 2H), 7.30–7.34(td, J = 7.6, 3.5 Hz, 3H), 7.24–7.27 (m, 2H), 7.00-7.02 (d, 1H), 5.02 (s, 2H), 4.75-4.76 (d, J = 4.3 Hz, 2H). The title 3-((4-methylbenzyl)oxy)benzohydrazide compound (C2) was obtained as a white powder, mp. 146–148°C, yield 77%. The FT-IR for C2, 3305, 3213 cm^{-1} NH_2 str. Vibration of amine, 3059 cm^{-1} NH str. Vibration of amide, 3012 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 1662 cm^{-1} C=O str. vibration band of amide, 1616 cm^{-1} N-H bending of amine (NH_2), 1454 cm^{-1} C-H bending of CH2, 1234 cm^{-1} C-O str. vibration band of ester, 1149 cm^{-1} C-O-C str. vibration band of ether. ^{1}H NMR (400 MHz, DMSO-d6) δ ^{1}H NMR (500 MHz, DMSO-d6) δ 10.10-10.12 (t, J = 4.2 Hz, 1H), 7.66-7.67 (d, 1H), 7.34-7.37 (t, J = 8.0 Hz, 1H), 7.27-7.31 (m, 3H), 7.17-7.19 (d, 2H), 7.04-7.06 (d, J = 8.1 Hz, 1H), 4.96 (s, 2H), 4.79-4.80 (d, J = 4.3 Hz, 2H), 2.30 (s, 3H).

Synthesis of 1,3,4-Thiadizole-2-thione Derivatives (Compounds D1, D2)

A mixture of CS2 (0.08 ml, 0.0012 moles), KOH (0.03 gm, 0.0005 mol), and compounds (C1 or C2) (0.001 moles) in EtOH (120 mL) was refluxed for 16–18 hour (compound D1 & compound D2 respectively). At the end of the reaction (as indicated by TLC), the solvent concentrated, and the remainder dissolved in water, after that drop-wise addition of 10% HCl, to appear as a white solid. Then it was filtered and washed with cold water[15,16] the title 5-(3-(benzyloxy)phenyl)-1,3,4-thiadiazole-2-thione compound (D1) was obtained as a white powder mp. 150–151°C, yield 79%. The FT-IR for D1, 3062 cm^{-1} N-H str. Vibration of secondary amine, 2924 cm^{-1} C-H Asymmetric str. Vibration of CH2, 2596 cm^{-1} SH Thiols stretching, 1701 cm^{-1} C=N str. Vibration, 1593 cm^{-1} C=C Symmetric str. Vibration of aromatic ring, 1519 cm^{-1} N-H bending of secondary amine, 1242 cm^{-1} C-N str. Vibration of secondary amine, 1195 cm^{-1} C=S str. Vibration, 1080 cm^{-1} C-O-C str. vibration band of ether, 686 cm^{-1} C-S-C str. Vibration. ^{1}H NMR (400 MHz, DMSO-d6) δ 14.76 (s, 1H), 7.48 (s, J = 5.6 Hz, 1H), 7.38-7.46 (m, 4H), 7.32-7.35 (m, 3H), 7.21-7.23 (d, 1H), 5.18 (s, 2H). The 5-(3-((4-methylbenzyl)oxy)phenyl)-1,3,4-thiadiazole-2-thiol compound (D2) was obtained as a Pale off-white powder mp. 162–163°C, yield 75%. The FT-IR for D2, 3062 cm^{-1} N-H str. Vibration of secondary amine, 3035 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2889 cm^{-1} C-H symmetric str. Vibration of CH2, 2596

cm^{-1} SH Thiols stretching, 1975 cm^{-1} -NCS str. Vibration, 1701 cm^{-1} C=N str. Vibration, 1593 cm^{-1} C=C Symmetric str. Vibration of aromatic ring, 1242 cm^{-1} C-N str. Vibration of secondary amine, 1195 cm^{-1} C=S str. Vibration, 1080 cm^{-1} C-O-C str. vibration band of ether, 686 cm^{-1} C-S-C str. Vibration. ^{1}H NMR (400 MHz, DMSO-d6) δ 14.79 (s, 1H), 7.45-7.49 (t, J = 7.9 Hz, 1H), 7.33-7.39 (m, 4H), 7.22-7.25 (m, J = 8.2, 2.3 Hz, 3H), 5.17 (s, 2H), 2.30 (s, 3H).

Synthesis of 1,3,4-Thiadiazole Derivatives Compounds (Thiol alkylation) (Compounds M1-M3)

Intermediate (D1, D2) (3.7 mmol) and TEA (triethylmine) (7.7 mmol) were added into 30 mL of pyridine. The reaction mixture was stirred for 0.5 hrs. At that point, Intermediate (A1, A2) (3.9 mmol.) was add to the reaction mixture and then was refluxed at room temp. for 6–9 hrs. Later the reaction was finished (as indicated by TLC), 100 mL of deionized water (D.W.) was added and left to stand for 10 min. The mixture then filtered, & the solid washed with D.W., dried & recrystallized from ethanol.[16,17] The title 4-((5-(3-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl)thio)-1-(piperidin-1-yl) butan-1-one Compound (M1) was obtained as a off-white powder mp. 168–170°C, yield 75%. The FT-IR for M1, 3010 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2939, 2850 cm^{-1} C-H asymmetric and symmetric str. Vibration of CH2, 1635 cm^{-1} C=O str. vibration band of amide, 1593 cm^{-1} C=N str. Vibration, 1203 cm^{-1} C-N str. Vibration of amide, 1068 cm^{-1} C-O-C str. vibration band of ether, 709, 699 cm^{-1} C-S-C str. Vibration. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.70-7.72 (d, 1H), 7.42-7.43 (d, J = 1.2 Hz, 2H), 7.33-7.38 (m, 4H), 7.29-7.30 (d, 1H), 6.91-6.92 (d, J = 1.2 Hz, 1H), 5.07 (s, 2H), 3.29-3.31 (t, J = 5.3, 4.1 Hz, 4H), 3.10-3.13(t, J = 7.3 Hz, 2H), 2.37-2.40 (t, J = 7.5 Hz, 2H), 1.95-2.01 (p, J = 7.4 Hz, 2H), 1.55-1.59 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.874, 168.170, 165.518, 159.215, 136.462, 131.142, 130.295, 128.687, 128.182, 127.589, 120.678, 118.076, 113.212, 77.376, 77.059, 76.741, 70.242, 46.569, 33.915,31.527, 25.579, 24.847, 24.561. The title 4-((5-(3-((4-methylbenzyl)oxy)phenyl)-1,3,4-thiadiazol-2-yl) thio)-1-(piperidin-1-yl)butan-1-one compound (M2) was obtained as a white powder mp. 180–182°C, yield 70%. The FT-IR for M2, 3010 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2939 cm^{-1} C-H asymmetric and symmetric str. Vibration of CH3, 2850 cm^{-1} C-H Asymmetric & symmetric str. Vibration of CH2, 1631 cm^{-1} C=O str. vibration band of amide, 1593 cm^{-1} C=N str. Vibration, 1365 cm^{-1} C-C str. Vibration of CH3, 1199 cm^{-1} C-N str. Vibration of amide, 1138 cm^{-1} C-O-C str. vibration band of ether, 705, 682 cm^{-1} C-S-C str. Vibration. The ^{1}H NMR (400 MHz, DMSO-d6) δ 7.70-7.71 (d, J = 5.1 Hz, 1H), 7.33-7.38 (m, 2H), 7.27-7.27 (d, J = 1.0 Hz, 2H), 7.17-7.19 (d, J = 9.2 Hz, 2H), 6.90-6.92 (d, J = 9.0 Hz, 1H), 5.01 (s, 2H), 3.29-3.31 (t, J = 5.2, 4.1 Hz, 4H), 3.10-3.13 (t, J = 7.3 Hz, 2H), 2.37-2.41 (t, J = 7.5 Hz, 2H), 2.30 (s, 3H), 1.95-2.01 (p, J = 7.4 Hz, 2H), 1.55-1.59 (m, 6H). The ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.882, 168.213, 165.489, 159.263, 138.001, 133.398, 131.088, 130.267, 129.366, 127.744, 120.569, 118.082, 113.215, 77.397, 77.080, 76.763, 70.173, 46.570, 42.738, 33.900, 31.579, 25.578, 24.845, 24.559, 21.287. The title 4-((5-(3-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl)thio)-1-(4-methylpiperazin-1-yl)butan-1-one compound (M3) was obtained as a pale yellow powder mp. 185–187°C, yield 70%. The FT-IR for M3, 3062, 3024 cm^{-1} C-H Asymmetric str. Vibration of aromatic ring, 2939, 2862 cm^{-1} C-H asymmetric and symmetric str. Vibration of CH2, 2799 cm^{-1} C-H Asymmetric & symmetric str. Vibration of CH3, 1643 cm^{-1} C=O str. vibration band of amide, 1593 cm^{-1} C=N str. Vibration, 1199, 1172 cm^{-1} C-N str. Vibration of tertiary amine and amide, 1138, 1072 cm^{-1} C-O-C str. vibration band of ether, 694, 675 cm^{-1} C-S-C str. Vibration. ^{1}H NMR (400 MHz, DMSO-d6) δ7.70-7.71 (d, J = 5.1 Hz, 1H), 7.42-7.43 (d, J = 5.8 Hz, 2H), 7.33-7.38(m, 4H), 7.29-7.29 (d, J = 0.7 Hz, 1H), 6.90-6.91 (d, J = 4.6 Hz, 1H), 5.07 (s, 2H), 3.38-3.40(t, 4H), 3.10-3.13(t, J = 7.3 Hz, 2H), 2.38-2.41 (t, J = 7.5 Hz, 2H), 2.28-2.30 (t, 4H), 2.23 (s, 3H), 1.95-2.01(p, J = 7.4 Hz, 2H). The ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.125, 168.227, 165.407, 159.209, 136.439, 131.092, 130.317, 128.694, 128.195, 127.595, 120.670, 118.080, 113.215, 77.407, 77.089, 76.771, 70.231, 54.705, 46.105, 45.369, 33.767, 31.470, 24.736.

In Vitro Cytotoxicity Assay[18-20]

The *in vitro* cytotoxicity of compounds M1, M2, and M3 were evaluated by MTT assay on human breast cancer cells (MCF-7). MTT was performed to determine the cytotoxic effect of the samples at various concentrations. The results were given as the mean of three independent experiments and the IC_{50} values were then calculated.

Statistical Analysis

The results of the experimental work were demonstrated as the standard error of the mean (SEM) for triplicate data by using nonlinear regression analysis (Prism Pad 8.1).

Results

Molecular Docking

The binding site of a Estrogen Receptor (α-ER)

The binding site is formed like a jar with a narrow neck by a volume of approximately 4,105 Å3. The binding pocket comprises Leu391, Leu428, Phe404, Meth421, Leu346, Leu346, Met343, Leu391, Arg394, Leu394, Leu391, Leu387, Met388, Glu353, Leu387, Ala350, Thr347, Leu384, Trp383.

According to the result of docking as shown in Table 1 and Figures (2 to 6), M1 and M2 which are derivatives of piperidine ring, both give good docking scores compared to the standard (Tamoxifen) since the synthetic compounds bind to the residues within the binding site (mostly hydrophobic interaction).

In the case of M3 derivatives of the methyl-piperazine ring, give very good docking score compared with standard (tamoxifen), as seen M3 bind with the most amino acids residue in the active site (mostly hydrophobic interaction).

Synthesis of Compounds (A-D) and (M1-M3)

Compound (A1, A2) was synthesized by the reaction of carboxylic acid(4-bromobutyric acid) with amine(piperidine and methyl-piperazine) using EDC and HOBt as coupling agents. The FTIR for both compounds were characterized by disappearance of secondary amine band in the region around 3267 cm^{-1} and the appearance of C=O amide bond band at a region

Table 1. **The results of interactions of the ligands with the α-ER**

Compound ID	Docking score (kcal/mol)	H-bond interaction	Non-H-bond interaction
3-hydroxytamoxifen	–9.8	Non	Met343, Met421, Met388, Leu346, Leu387, Thr347, Leu525 (extensive), Trp383 (extensive)
Tamoxifen	–8.6		
M1	–8.9	Thr347 (3.05)	Leu536, Thr347 (extensive), Leu384 (extensive), Leu391, Leu387
M2	–9	Thr347 (3.00)	Trp383 (extensive), Leu525 (extensive) Thr347 (extensive), Leu391, Arg394, Met421, Leu346
M3	–8.9	Non	Leu539, Leu525 (extensive), Leu536 Met343, Leu346, Benzene ring has interacted mainly with Met388

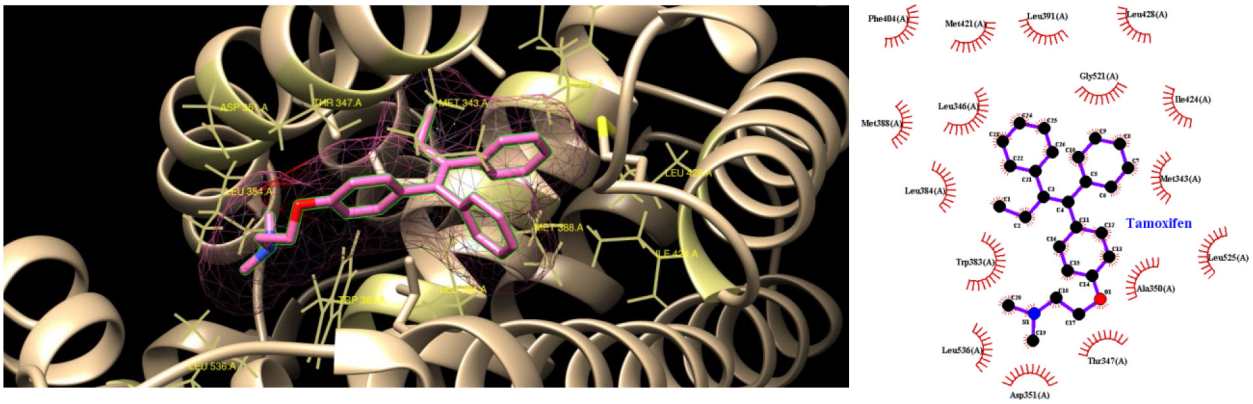


Fig. 2 **Tamoxifen binding mode.**

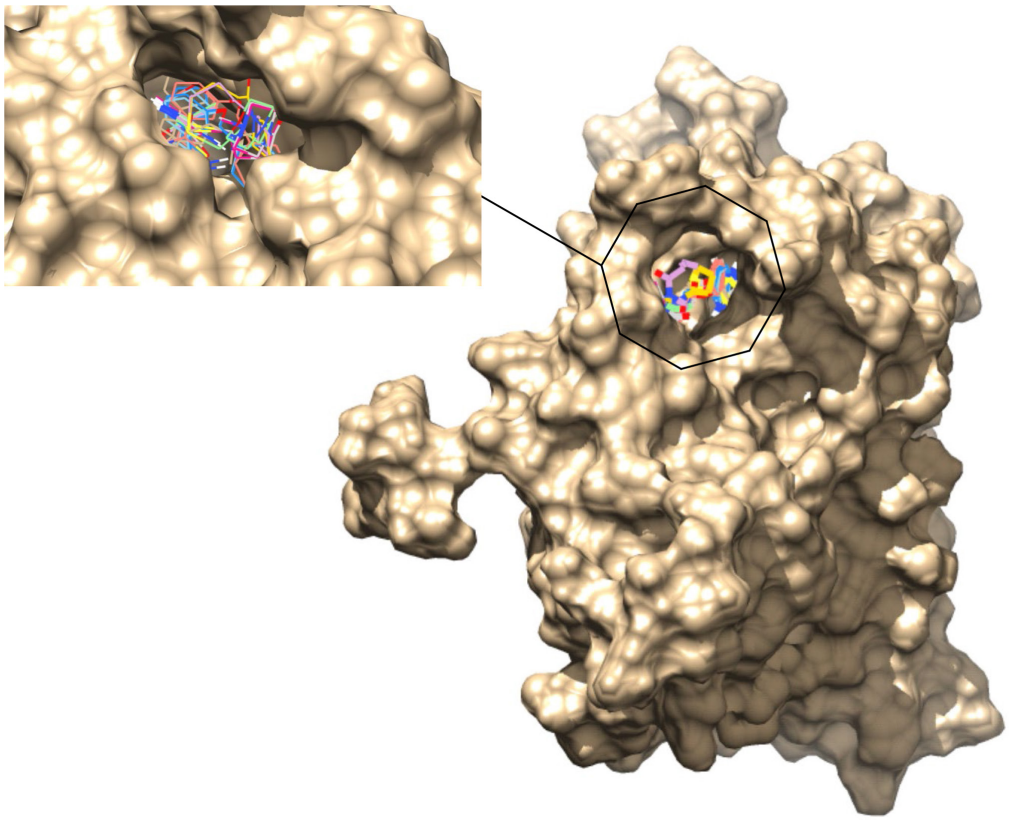


Fig. 3 **Demonstrate the conformational occupancy of the binding pocket of Human α-ER by all ligands (M1-M3) alongside the standard (3-Hydroxytamoxifen).**

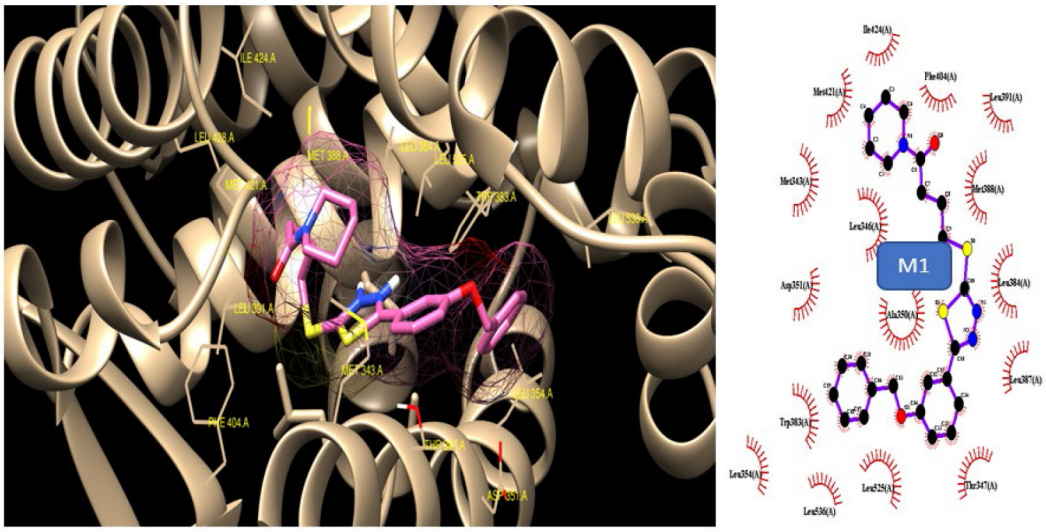


Fig. 4 **The best scored pose of M1 inside the binding side while being matched with 3-Hydroxytamoxifen.**

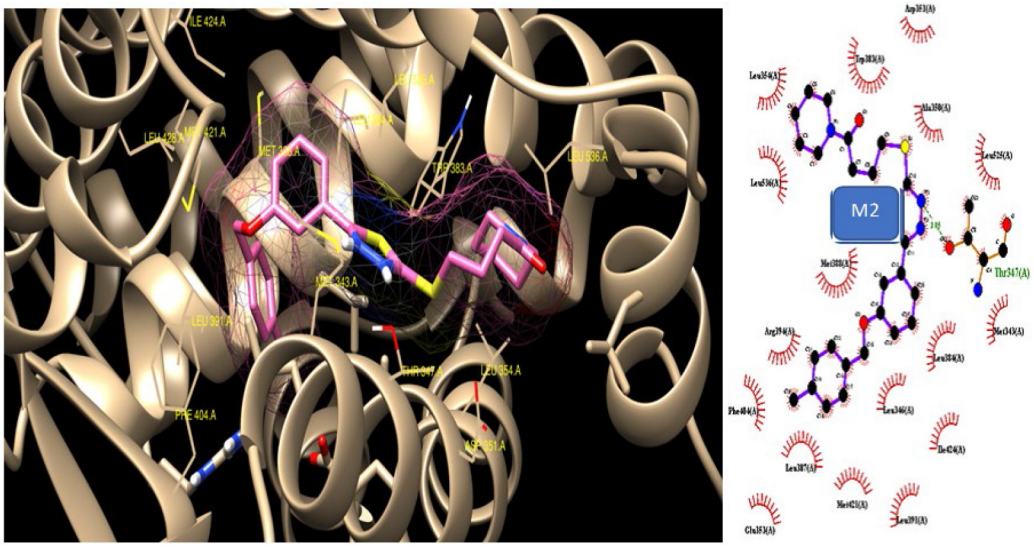


Fig. 5 **The best scored pose of M2 inside the binding side while being matched with 3-Hydroxytamoxifen.**

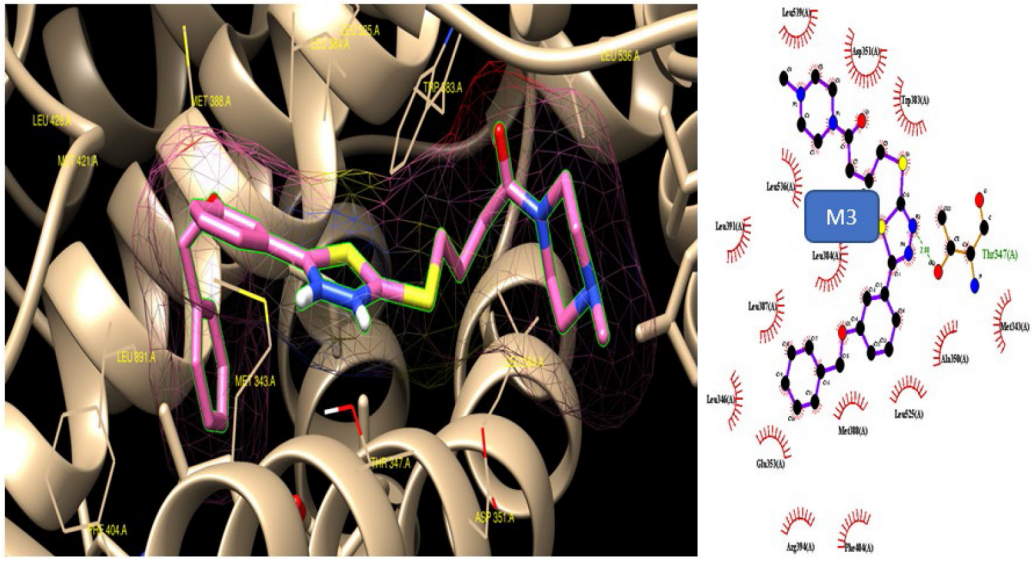


Fig. 6 **The best scored pose of M3 inside the binding side while being matched with 3-Hydroxytamoxifen.**

around 1660 cm^{-1}. The ^{1}H-NMR for compounds (A1, A2), amides analogs were characterized by the appearance of triplet signal due to the proton at the C alpha to the carbonyl of amide near 2.3δ, and appearance of multiplate signal due to the proton at C alpha to N of heterocyclic rings near 3.3δ.

Compound (B1, B2) was synthesized by the reaction of methyl-3-hydroxybenzoate with benzyl bromide or 4-methyl benzyl bromide in presence of K_2CO_3. The Williamson ether reaction follows an SN2 bimolecular nucleophilic substitution mechanism. The FTIR for both compounds were characterized by disappearance of OH band in the region around 3371 cm^{-1} and appearance of C-O-C ether band in the region around 1072 cm^{-1} (B1) and 1099 cm^{-1} (B2). The ^{1}H-NMR for compounds (B1, B2), ethers analogs were characterized by the appearance of aromatic protons showed 5 protons for mono-substituted and 4 protons for disubstituted at 6-8δ region in addition to the appearance of protons of methyl of ester and methylene near 3.8 and 5 respectively.

Compound (C1, C2) was synthesized by the reaction of corresponding ester (B1, B2) and hydrazine hydrate. The general feature of the hydrazine reagent that is reacts with methyl ester (B1, B2) employed in Wolff-Kishner reductions. The FTIR for both compounds were characterized by disappearance of C=O band of ester in the region around 1720 cm^{-1} and appearance of a NH a primary amine band at 3282, NH of secondary amide band at 3062 and C=O band of amide in the region around 1643 cm^{-1} (C1) and 1662 cm^{-1} (C2). The ^{1}H-NMR for compounds (C1, C2), amino-lysis of ester analogs were characterized by the disappearance the signal of proton of CH3 of ester and appearance of proton signal of NH_2 at the region of above 4δ, signal NH proton at the region of 10δ.

Compound (D1, D2) was synthesized by the reaction of CS2, KOH, and compounds (C1 or C2), the benzo-hydrazide compounds (C1 or C2) were subjected to intramolecular cyclization. The FTIR for both compounds were characterized by disappearance of C = O of amide band in the region around 1643 cm^{-1} (C1) and 1662 cm^{-1} (C2) and appearance of SH thiol band in the region around 2596 cm^{-1}, and appearance of C=N band in the region around 1701 cm^{-1}. The ^{1}H-NMR for compounds (D1, D2), 1,3,4-Thiadizole-2-thione derivatives characterized by the disappearance of NH_2, NH signal and apparent of SH proton as a signal at the region of above 14δ.

Compound (M1-M3) was synthesized by the reaction of intermediate (D1, D2), with intermediate (A1, A2) In the presence of triethylamine. 1,3,4-Thiadizole-2-thione (D1, D2) can be alkylated at thiol group with alpha-haloester of intermediate (A1, A2). Triethylamine or potassium carbonate act as a base that Converts the thiol to thiolate anion which acts as nucleophile that attacks the intermediate (A1, A2) which have a bromide group that is a good leaving group. The FTIR for compounds (M1-M3) were characterized by disappearance of SH bands and apparent of proton signal CH2 of heterocyclic rings appeared at the region of above 1.5 and 3.2δ indicating the successful synthesis of the final products. The ^{1}H-NMR for compounds (M1-M3), were characterized by disappearance of SH bands and apparent of proton signal CH2 of heterocyclic rings appeared at the region of above 1.5 and 3.2δ indicating the successful synthesis of the final products.

In Vitro Cytotoxicity Assay

The anticancer activity of compound M1, M2, and compound M3 were examined in the dose-response curve generated by Prism Pad 8.1 using nonlinear regression analysis for compounds in MCF-7 cells is shown below figures. The IC_{50} values were obtained to a range of concentrations of compounds from (100–1.56 μM) by MTT assay (Figures 7–10).

IC_{50} of (M1) in Breast Cancer Cell Line (MCF-7) = 34.09 μM Vs Tamoxifen (Control) in the same cell line = 18.02 μM.

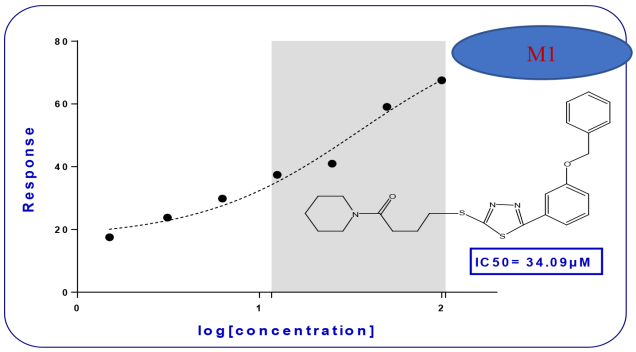


Fig. 7 **Dose-response curves of IC_{50} for (M1). MCF-7 cells were treated for 72 h with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 μM dose ranges of M1. The dose-response for M1 was plotted over log-transformed M1 concentrations.**

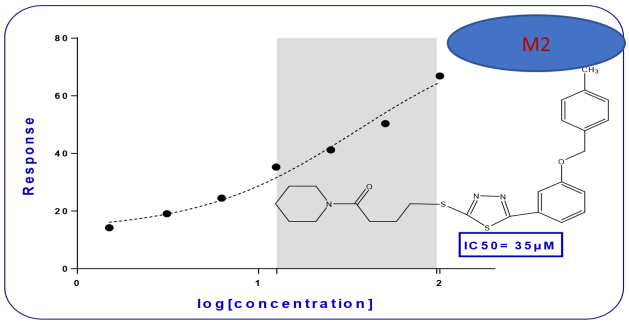


Fig. 8 **Dose-response curves of IC_{50} for (M2). MCF-7 cells were treated for 72 h with 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 μM dose ranges of M2. The dose-response for M2 was plotted over log-transformed M2 concentrations.**

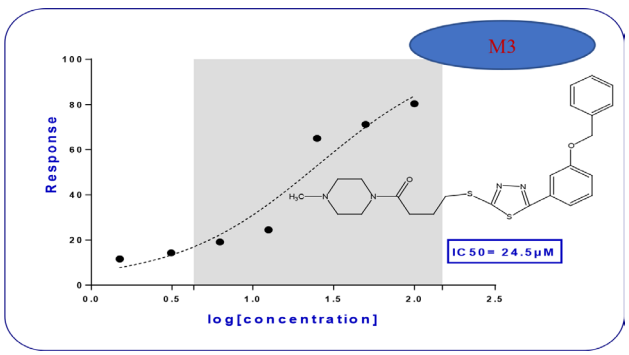


Fig. 9 **Dose-response curves of IC_{50} for (M3). MCF-7 cells were treated for 72 h with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 μM dose ranges of M3. The dose-response for M3 was plotted over log-transformed M3 concentrations.**

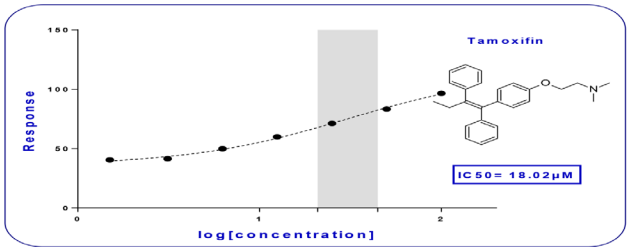


Fig. 10 **Dose-response curves of IC_{50} for Tamoxifen (Control). MCF-7 cells were treated for 72 h with 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µM dose ranges of Tamoxifen. The dose-response for Tamoxifen was plotted over log-transformed Tamoxifen concentrations.**

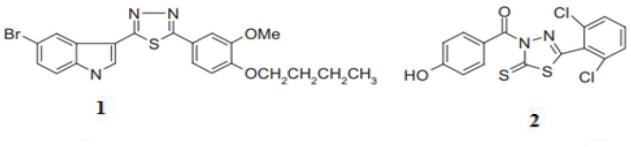


Fig. 11 **Structure of compounds 1 and 2.**

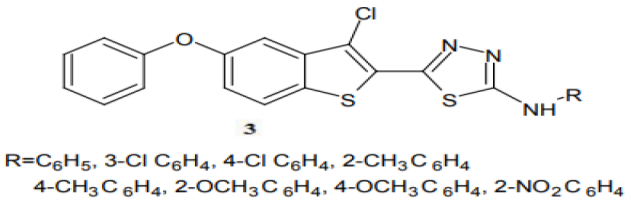


Fig. 12 **Structure of compounds 3.**

IC_{50} of (M2) in Breast Cancer Cell Line (MCF-7) = 35.5 µM Vs Tamoxifen (Control) in the same cell line = 18.02 µM

IC_{50} of (M3) in Breast Cancer Cell Line (MCF-7) = 24.5 µM Vs Tamoxifen (Control) in the same cell line = 18.02 µM.

Discussion

According to the above-mentioned results, the synthetic compounds (M1-M3) are useful for the treatment of breast cancer, as they inhibit the alpha-estrogen receptor through the presence of ring 1,3,4–thiadiazoles moiety, which has a wide range of biological activities moiety such as antidiabetic, anticancer, anti-inflammatory, anticonvulsant, antiviral, antihypertensive, and antimicrobial.[21] Also, the other important rings used piperidine and 1-methyl piperazine due to the fact that heterocyclic compounds exhibit exciting medicinal properties including anticancer.[22]

Kumar et al.[23] synthesized and tested 5-(3-indolyl)-1,3,4-thiadiazoles for anti-cancer activity. It was discovered that the C-2 position of the 1,3,4-thiadiazole ring plays a vital role in the compound's cytotoxic effect. Compounds **1** and **2** demonstrated a wide range of growth inhibitory effects against human tumor cells and outstanding cytotoxic action against non-small lung cancer, colon cancer, breast cancer, and prostate cancer, Figure 11.

While H.S Joshi, et al.[24] had synthesized a series of 2-(3'-chloro-5'-phenoxy-benzo[b]thiophen-2'-yl)-5-arylamino-1,3,4-thiadiazoles **3** and tested their antimicrobial activity, Figure 12.

Conclusion

In the recent decade, there have been several drugs to treat breast and lung cancer. However, there is still an unmet need to develop different types of drugs to reduce systemic toxicity and improve therapeutic efficacy. In the present study, we synthesized three compounds (M1-M3). The chemical structures of the synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, and ^{13}C-NMR. MTT assay demonstrated *In vitro* cytotoxicity study against MCF-7, for compounds M1, M2 & M3 show a good inhibition ratio to this cell line compared to tamoxifen. Therefore, these newly synthesized analogs may be represented as an exploitable source of new anticancer agents fighting breast cancer.

Acknowledgments

We thank the Department of Pharmaceutical Chemistry/ College of the Pharmacy/University of Baghdad for their official support in this study.

Conflicts of Interest Disclosure

There are no conflicts of interest.

Competing Interests

The authors declare that they have no competing interests.

Source of Funding

Self-funded project.

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