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Design and Synthesis of pyrimidine nucleus containing 4,6-disubstituted pyrimidine derivatives for the management of breast cancer

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Abstract

It has been seen that Cancer is perhaps the most threatening diseases which cause major death in all over the globe. There are various types of cancer, such as lung cancer, breast, prostate, liver, skin, stomach and many more which may lead in the major death. Pyrimidine moiety is one of the important heterocyclic nucleuses having a wide scope of biological activity. A series of 4,6-disubstitutedpyrimidine-2-ones (4a-f) and 4,6-disubstitutedpyrimidine-2-thiones (5a-f) derivatives were synthesized using benzthiol, fluorobenzaldeyde and substituted acetophenones. Above derivatives utilized to discover dynamic derivatives and two arrangement of such dynamic compounds for example 4,6-disubstitutedpyrimidine-2-thiones and 4,6-disubstitutedpyrimidine-2-ones. The entire novel derivatives were analyzed and characterized by TLC, melting point analysis and spectral data studied by using FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The entire synthesized derivatives were screened against human breast tumor cell line (MCF7) for their in vitro anticancer activity by MTT assay method. The greater part of the prepared compounds displayed breast anti-cancer activity contrasted with Doxorubicin as a kind of reference medication. Compounds 4c, 4d 4f, 5b, 5c and 5f were showing significant cytotoxic potential properties with the IC_{50} concentration at 13.09µg/ml, 8.53 µg/ml, 11.32 µg/ml, 10.85µg/ml, 9.74 µg/ml and 14.87 µg/ml respectively against breast cancer cell line compared to the standard drug Doxorubicin which was showing the IC_{50} value at 7.51 µg/ml used for the study. Among all the synthesized compounds, 4d and 5c were found as most potent anticancer agent due to its low IC_{50} value on MCF7 cells.

Keywords: Pyrimidine, 4,6-disubstitutedpyrimidine, Breast Cancer Cell Line (MCF7), MTT assay, Anticancer activity

INTRODUCTION

It has been seen that Cancer is perhaps the most threatening diseases which cause major death in all over the globe. The major death caused by various types of cancers such as lung cancer, breast, prostate, liver, skin, stomach and many more which may lead in the major death. By collecting all the data about causing of different types of cancers it has been required to develop a new lead molecule to search and synthesize a novel compound and act as strong anticancer activity to manage cancer. Pyrimidine moiety is one of the important heterocyclic nucleuses having a wide scope of biological activity. During our consistence investigations focused on the disclosure of new heterocycles supplied with antitumour activity, we have given an account of the amalgamation and antitumor activities of a progression of heterocyclic derivatives[1-6]. Pyrimidine has expanded broad thought taking into account its variety in natural action and vast applications in the field of pharmaceuticals[8,9]. Pyrimidine nucleus having an extensive biological activity such as antimicrobial[10,11], anticancer[12] anti-inflammatory[13], analgesic[13], antibacterial[15], anti-fungal[16], anthelmintic activity[17], antitubercular activity[18], anticonvulsant activity[19] and anti-oxidant activity[20].

Due to striking pharmacological movement of pyrimidine derivatives, it has been centered around these activities. Pyrimidine nucleus is extremely consistence and has enlivened to utilize these consistence areas in bioactive moieties to get ready new compounds having biological exercises. Pyrimidines include an indisputable and uncommon spot in our life. This pyrimidne nucleus has extraordinary activity due to the phenomenal biological and medicinal importance. Distinctive designed viewpoint show that pyrimidine derivatives are easy to plan and it can deliver tremendous biological activity. It was empowered by the different biological activity of pyrimidine derivatives, it was picked to set up another series of pyrimidine derivatives[21].

MATERIALS AND METHODS

The research facility grade chemical substances and reagents were utilized to prepare all the described derivatives. The melting points were analyzed using melting point apparatus. The IR spectra of synthesized derivatives with KBr in the form of pellet were recorded on a FTIR-8400S spectrophotometer (SHIMADZU), proton NMR (^{1}H NMR), carbon thirteen NMR (^{13}C NMR) spectra were observed and recorded by using TMS as internal standard, DMSO as solvent on Bruker Advance-(400 MHZ, FT NMR) spectrophotometer. Mass spectra were recorded the spectra by using electron spray ionization (ESI) technique on Water UPLC-TQD spectrometer and types of elements were analyzed of the synthesized compounds by using Elemental Vario EL III, Carlo-Erba 1108. The purity of the synthesized product was monitored and performed by using TLC. The anticancer activity was performed by using cell culture medium DMEM high glucose media (Cat No: 2120785, Gibco), 96-well plate for culturing the cells (from Corning USA), MTT reagent (4060 Himedia) and adjustable multichannel pipettes and pipettor (Benchtop USA).

GENERAL PROCEDURE

Procedure for the Preparation of 4-(phenylthio)benzaldehyde:

A mixture of 4-fluorobenzaldehyde (0.01 mol) and thiophenol (0.01 mol) in dimethylsulfoxide (DMSO) (20 ml) was added. The mixture was heated in the presence of anhydrous potassium carbonate and refluxed for 1 hrs. It was allowed to cool and transferred into grounded ice. The crystals of product were obtained, filtered and recrystalized from acetic acid and water (1:2) as colorless crystals[20].

Preparation of (*E*)-1-(substitutedphenyl)-3-(4-(phenylthio)phenyl)prop-2-en-1-one(3a-f)[21,22]

A mixture of 4-(Phenylthio)benzaldehyde (0.01mol) and substituted acetophenone (0.01 mol) was mixed with ethanolic solution of 40% NaOH, stirred vigorously and continued for 8 hrs. The mixture was kept in refrigerator for a night. After filtration the solid product was obtained, washed with chilled aqueous solvent. Pure crystals were obtained after recrystallization from ethanol.

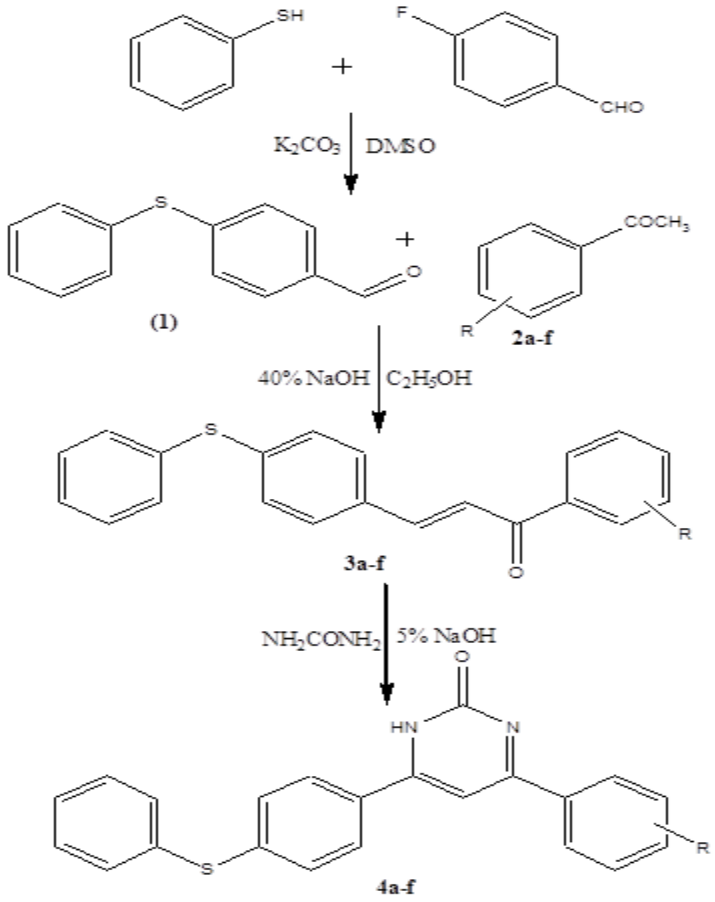
Preparation of 4-(substitutedphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-one (4a-f)

(*E*)-1-(substitutedphenyl)-3-(4-(phenylthio)phenyl)prop-2-en-1-one (3a-i) (2.26g, 0.1 mol) and urea (0.062g, 0.01 mol) was mixed to dissolve in 15ml of 5% solution of sodium hydroxide in ethanol. The mixture was refluxed for 7hr, cooled and transferred into chilled water. The resultant solid product which was obtained as reddish brown color, was filtered, washed with water. The reddish brown colored crystals of pure product were obtained after recrystallization from ethanol.

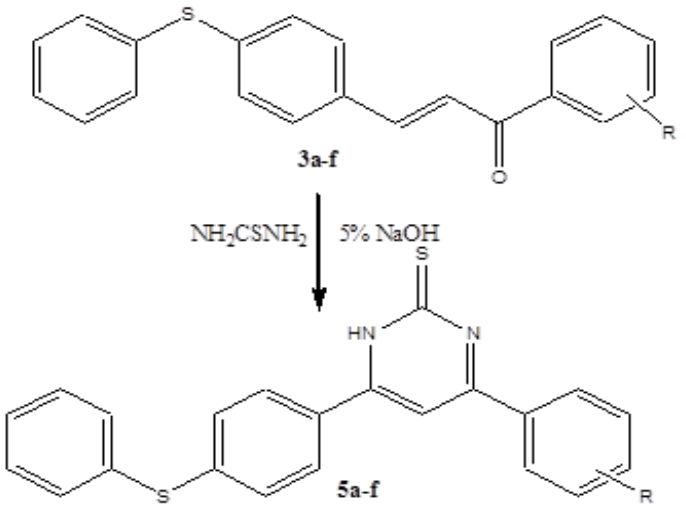
Preparation of 4-(substitutedphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5a-f)

(*E*)-1-(substitutedphenyl)-3-(4-(phenylthio)phenyl)prop-2-en-1-one (3a-g) (2.26g, 0.1 mol) and thiourea (0.064g, 0.01 mol) were mixed and dissolved in 15ml of 5% solution of sodium hydroxide in ethanol. The mixture was refluxed for 7hr, cooled and transferred into chilled water. The resultant solid product which was obtained as reddish brown color, was filtered, washed with water. The reddish brown colored crystals of pure product were obtained after recrystallization from ethanol.

Scheme-A



Scheme-B



4a-f : R=2-Br, 2,4-Cl, 2-OH, 2-OCH_3, 2-NO_2, 2-NH_2
5a-f : R=2-Cl, 2,4,5-OH, 2,4,5-OCH_3, 2-NO_2, 2-NH_2, 2,4-NH_2

4-(2-bromophenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4a)

IR (KBr, ν, cm^{-1}): 3328.42 (N-H aromatic), 3032.64 (C-H aromatic), 1687.67 (C=O), 1652.31 (C=N) 1571.51 (C=C), 1456.78 (C-N stretching), 1226.36 (C-O), 696.64 (disubstituted benzene deformation), 652.88 (C-S), 556.98 (C-Br); ^{1}H NMR (400 MHz) DMSO-d_6 δ_{ppm}: 9.340 (s, 1H, N-H, D_2O exchangeable), 3.314 (s, 1H, S-H), 6.858-7.996 (m, 14H, Ar-H); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 164.7, 163.3, 156.6, 138.1, 135.7, 134.2, 131.8, 129.6, 127.5, 121.1, 118.9, 116.4, 106.5; EIMS (m/z): $[M]^+$ 434.13, $[M+2]^+$ 436.15; Fragments: 280.06, 249.78, 172.27, 110.24, 96.15, 68.32; Elemental Analysis: Calcd for $C_{22}H_{15}BrN_2OS$: C, 60.71; H, 3.48; N, 6.42; Found: C, 60.73; H, 3.46; N, 6.44.

4-(2,4-dichlorophenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4b)

IR (KBr, ν, cm^{-1}): 3327.15 (N-H aromatic), 3024.67 (C-H aromatic), 1686.92 (C=O), 1652.43 (C=N) 1577.11 (C=C), 1458.14 (C-N), 1226.74 (C-O), 7144.43 (C-S), 696.51 (disubstituted benzene deformation), 659.42 (C-Cl); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.191 (s, 1H, N-H, D_2O exchangeable), 6.881-7.981 (m, 13H, Ar-H); ^{13}C NMR (400 MHz) DMSO-d_6 δ_{ppm}: 164.3, 163.4, 156.9, 138.2, 135.8, 134.7, 131.3, 129.8, 127.4, 118.1, 116.5, 106.8; EIMS (m/z): $[M]^+$ 424.16, $[M+2]^+$ 426.15, $[M+4]^+$ 428.24; Fragments: 280.26, 239.81, 172.26, 110.11, 96.17, 68.38; Elemental Analysis: Calcd for $C_{22}H_{14}Cl_2N_2OS$: C, 62.13; H, 3.36; N, 6.61; Found: C, 62.15; H, 3.34; N, 6.59.

4-(2-hydroxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(1*H*)-one (4c)

IR (KBr, ν, cm^{-1}): 3482.89 (O-H aromtic), 3328.12 (N-H aromatic), 3028.46 (C-H aromatic), 1685.92 (C=O), 1651.11 (C=N) 1572.08 (C=C), 1458.99 (C-N), 1225.98 (C-O), 695.96 (disubstituted benzene deformation), 658.69 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.210 (s, 1H, N-H, D_2O exchangeable), 6.898-7.969 (m, 14H, Ar-H), 6.410 (s, 1H, O-H, D_2O exchangeable); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 164.6, 163.2, 161.8, 156.3, 138.7, 135.1, 134.8, 131.1, 129.8, 127.3, 123.5, 121.7, 118.1, 116.8, 106.5; EIMS (m/z): $[M]^+$ 372.09, $[M+1]^+$ 373.18; Fragments: 280.11, 188.13, 172.16, 110.12, 96.10, 68.01; Elemental Analysis: Calcd for $C_{22}H_{16}N_2O_2S$: C, 70.94; H, 4.33; N, 7.52; Found: C, 70.95; H, 4.33; N, 7.51.

4-(2-methoxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4d)

IR (KBr, ν, cm^{-1}): 3328.96 (N-H aromatic), 3025.68 (C-H aromatic), 1686.89 (C=O), 1651.33 (C=N) 1573.10 (C=C), 1458.10 (C-N), 1225.89 (C-O), 1152.65 (C-O-C), 697.01 (disubstituted benzene deformation), 636.72 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.156 (s, 1H, N-H, D_2O exchangeable), 6.926-7.579 (m, 14H, Ar-H), 3.956 (s, 3H, OCH_3); ^{13}CNMR (400 MHz, DMSO-d_6) δ_{ppm}: 164.9, 163.2, 160.8, 156.1, 138.3, 134.2, 132.8, 130.6, 129.3, 127.1, 123.6, 121.7, 116.1, 106.9, 55.3; EIMS (m/z): $[M]^+$ 386.31, [M+1] 387.06; Fragments: 280.09, 202.01, 172.07, 110.10, 96.09, 68.11; Elemental analysis: Calcd for $C_{23}H_{18}N_2O_2S$: C, 71.51; H, 4.61; N, 7.25; Found: C, 71.54; H, 4.73; N, 7.29.

4-(2-nitrophenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4e)

IR (KBr, ν, cm^{-1}): 3328.18 (N-H aromatic), 3027.10 (C-H aromatic), 1686.02 (C=O), 1655.21 (C=N) 1572.05 (C=C), 1490.94 (N-O), 1351.43 (N=O), 858.71 (C-N), 696.11 (disubstituted benzene deformation), 635.17 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.167 (s, 1H, N-H, D_2O exchangeable), 7.152-8.582 (m, 14H, Ar-H); ^{13}CNMR (400 MHz), DMSO-d_6 δ_{ppm}: 164.2, 163.5, 156.8, 148.7, 135.8, 134.2, 132.1, 131.4, 129.7, 127.2, 123.8, 121.5, 106.9; EIMS (m/z): $[M]^+$ 401.12, [M+1] 402.21; Fragments: 280.11, 217.26, 172.16,

110.19, 96.03, 68.08; Elemental analysis: Calcd for $C_{22}H_{15}N_3O_3S$: C, 65.81; H, 3.76; N, 10.48; Found: C, 65.86; H, 3.77; N, 10.49.

4-(2-aminophenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4f)

IR (KBr, ν, cm^{-1}): 3330.89 (N-H aromatic), 3031.09 (C-H aromatic), 1686.04 (C=O), 1656.91 (C=N) 1566.98 (C=C), 1461.04 (C-N), 654.71 (C-S), 697.10 (disubstituted benzene deformation), 649.01 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.171 (s, 1H, N-H, D_2O exchangeable), δ 4.682 (s, 2H, N-H, D_2O exchangeable), 6.272-7.582 (m, 14H, Ar-H); ^{13}CNMR (400 MHz), DMSO-d_6 δ_{ppm}: 164.8, 163.1, 156.6, 150.3, 135.8, 134.2, 131.7, 131.3, 129.5, 127.1, 123.8, 118.2, 117.4, 116.2, 106.1; EIMS (m/z): $[M]^+$ 371.02, $[M+1]^+$ 372.18; Fragments: 280.13, 187.11, 172.14, 110.01, 96.10, 68.09; Elemental analysis: Calcd for $C_{22}H_{17}N_3OS$: C, 71.13; H, 4.62; N, 11.32; Found: C, 71.17; H, 4.66; N, 11.34.

4-(2-chlorophenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5a)

IR (KBr, ν, cm^{-1}): 3327.98 (N-H aromatic), 3026.65 (C-H aromatic), 1651.09 (C=N) 1574.07 (C=C), 1456.10 (C-N), 1225.06 (C-O), 1218.03 (C=S), 694.10 (disubstituted benzene deformation), 656.78 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.312 (s, 1H, N-H, D_2O exchangeable), 6.885-7.991 (m, 14H, Ar-H); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 180.2, 176.6, 164.8, 135.2, 134.1, 134.6, 131.1, 131.3, 129.8, 104.3; EIMS (m/z): $[M]^+$ 406.89, $[M+2]^+$ 408.12; Fragments: 296.13, 222.09, 188.07, 112.11, 110.06, 68.10 Elemental Analysis: Calcd for $C_{22}H_{15}ClN_2S_2$: C, 64.93; H, 3.71; N, 6.89; Found: C, 64.97; H, 3.76; N, 6.77.

4-(2,4,5-trihydroxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5b)

IR (KBr, ν, cm^{-1}): 3483.93 (O-H aromatic), 3323.87 (N-H aromatic), 3025.67 (C-H aromatic), 1651.10 (C=N) 1576.18 (C=C), 1455.89 (C-N), 1225.10 (C-O), 1218.06 (C=S), 696.03 (disubstituted benzene deformation), 675.19 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 11.261 (s, 1H, 2-OH, D_2O exchangeable), 9.223 (s, 1H, N-H, D_2O exchangeable), δ 6.634 (s, 2H, 4-OH, 5-OH, D_2O exchangeable), 6.192-7.912 (m, 12H, Ar-H); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 180.7, 176.1, 164.7, 162.3, 162.8, 135.2, 134.6, 131.8, 129.1, 127.3, 111.6, 104.8, 103.4; EIMS (m/z): $[M]^+$ 404.10, $[M+1]^+$ 405.13; Fragments: 296.14, 220.34, 188.09, 112.03, 110.09, 68.10; Elemental analysis: Calcd for $C_{22}H_{16}N_2O_3S_2$: C, 62.88; H, 3.79; N, 6.62; Found: C, 62.89; H, 3.78; N, 6.60.

4-(2,4,5-trimethoxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5c)

IR (KBr, ν, cm^{-1}): 3329.76 (N-H aromatic), 3028.01 (C-H aromatic), 1652.12 (C=N) 1573.02 (C=C), 1455.09 (C-N), 1226.03 (C-O), 1218.47 (C=S), 1148.05 (C-O-C), 697.10 (disubstituted benzene deformation), 679.24 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.123 (s, 1H, N-H, D_2O exchangeable), 6.798-7.504 (m, 12H, Ar-H), 3.608-3.661 (s, 9H, OCH_3); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 181.2, 177.1, 164.8, 162.1, 136.5, 135.1, 131.8, 129.2, 121.7, 118.8, 109.1, 106.7, 104.8, 100.2, 56.1; EIMS (m/z): $[M]^+$ 432.09, $[M+1]^+$ 433.55; Fragments: 296.25, 248.10, 188.32, 112.10, 110.08, 68.10; Elemental analysis: Calcd for $C_{25}H_{22}N_2O_3S_2$: C, 64.91; H, 4.79; N, 6.06; Found: C, 64.90; H, 4.83; N, 6.12.

4-(2-nitrophenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5d)

IR (KBr, ν, cm^{-1}): 3329.23 (N-H aromatic), 3026.12 (C-H aromatic), 1654.34 (C=N) 1572.26 (C=C), 1486.68 (N-O), 1347.87 (N=O), 1218.63 (C=S), 853.08 (C-N), 697.04 (disubstituted benzene deformation), 634.68 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.387 (s, 1H, N-H, D_2O exchangeable), 6.598-8.602 (m, 14H, Ar-H); ^{13}CNMR (400 MHz), DMSO-d_6 δ_{ppm}: 180.6, 176.2, 164.8, 161.3, 148.5, 134.9, 134.2, 131.8, 131.4, 129.5, 127.8, 124.5, 123.2, 118.7, 116.8, 104.3; EIMS (m/z): $[M]^+$ 417.12, $[M+1]^+$ 418.52; Fragments:

296.09, 233.02, 188.12, 112.23, 110.43, 68.27; Elemental analysis: Calcd for $C_{22}H_{15}N_3O_2S_2$: C, 63.29; H, 3.62; N, 10.06; Found: C, 63.31; H, 3.66; N, 10.12.

4-(2-aminophenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5e)

IR (KBr, ν, cm^{-1}): 3334.05 (N-H aromatic), 3029.21 (C-H aromatic), 1665.03 (C=N) 1571.87 (C=C), 1459.10 (C-N), 1223.67 (C-O), 1215.86 (C=S), 695.16 (disubstituted benzene deformation), 678.02 (C-S); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.345 (s, 1H, N-H, D_2O exchangeable), 7.513 (s, 2H, N-H_2), 6.298-7.622 (m, 14H, Ar-H); ^{13}CNMR (400 MHz), DMSO-d_6 δ_{ppm}: 180.2, 176.7, 164.8, 161.4, 135.1, 134.7, 134.5, 131.7, 131.1, 129.8, 121.3, 118.9, 116.4, 104.1; EIMS (m/z): $[M]^+$ 387.09, $[M+1]^+$ 388.04; Fragments: 296.08, 203.02, 188.04, 112.08, 110.01, 68.12; Elemental analysis: Calcd for $C_{22}H_{17}N_3S_2$: C, 68.17; H, 4.44; N, 10.82; Found: C, 68.22; H, 4.45; N, 10.86.

4-(2,4-diaminophenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5f)

IR (KBr, ν, cm^{-1}): 3329.07 (N-H aromatic), 3025.93 (C-H aromatic), 1650.89 (C=N) 1574.12 (C=C), 1457.10 (C-N), 1225.04 (C-O), 1217.61 (C=S), 713.34 (C-S), 698.03 (disubstituted benzene deformation), 567.12 (C-Br); ^{1}H NMR (400 MHz), DMSO-d_6 δ_{ppm}: 9.164 (s, 1H, N-H, D_2O exchangeable), 7.512 (s, 4H, 2,4-NH_2) 6.870-7.989 (m, 13H, Ar-H), 4.854-5.161 (d, 4H, N-H_2, D_2O exchangeable); ^{13}C NMR (400 MHz), DMSO-d_6 δ_{ppm}: 180.5, 176.1, 164.7, 135.2, 134.8, 134.7, 131.3, 131.5, 129.4, 127.3, 123.9, 104.4; EIMS (m/z): $[M]^+$ 529.92, $[M+1]^+$ 530.92; Fragments: 345.90, 296.13, 188.10, 112.06, 110.01, 68.10; Elemental Analysis: Calcd for $C_{22}H_{18}N_4S_2$: C, 65.65; H, 4.53; N, 13.95; Found: C, 65.69; H, 4.52; N, 13.97.

ANTICANCER ACTIVITY

Cell Cultutre:

The MCF7 (Human Breast adeno-carcinoma cell line) were kept up with in Dulbecco's Modified Eagle Medium high glucose media supplemented with 10 % fetal bovine serum along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^{0}C temperature in the CO_2 incubator and sub-cultured for every 2days.

Antitumor Activity:

Seed 200µl, cell suspension in a 96-well plate at required cell density (20,000 cells for each well), in the absence of test specialists. Permitted the cells to develop for around 24 hrs. Fitting centralizations of the test specialist (Noticed in the outcomes -Spread sheet) were added. The plate was incubated for at 37°C for 24 hrs in a 5% CO_2 atmosphere. Completing the hatching duration, plates were takeout from hatchery, and eliminate spent media and MTT reagent was added to a final concentrtion of 0.5mg/mL of complete volume. Plate was wrapped with aluminum foil to maintain a strategic distance from presentation to the light. Plates were returned to the hatchery and brooded for 3 hrs. (Note: The duration of brooding was different for various cell lines. Inside one trial, during comparison brooding duration likely to be kept consistent). MTT reagent was taken out and afterward added 100µl of solubilization arrangement (DMSO). Delicate stirring in a gyratory shaker was improved dissolution. Sometimes, pipetting here and there might be needed to totally solubilize the MTT formazan crystals particularly in thick cultures. The absorbance were recorded through spectrophotometer or through ELISA recorder, recorded at 570nm and 630 nm as reference wavelength. The IC_{50} value was calculated by utilizing linear regression condition for example Y =Mx+C. Here, Y = 50, M and C values were obtained from the practicality graph.

RESULT AND DISCUSSION

4-(Phenylthio)benzaldehyde was synthesized from thiophenol and 4-fluorobenzaldehyde[20]. It was converted into (*E*)-1-(substitutedphenyl)-3-(4-(phenylthio)phenyl)prop-2-en-1-ones (3a-f) by the reaction with substituted acetophenones in the ethanolic solution of sodium hydroxide. The reaction of (*E*)-1-(substitutedphenyl)-3-(4-(phenylthio)phenyl)prop-2-en-1-one (3a-f) with the urea and thiourea took place separately in the ethanolic solution of sodium hydroxide and derivatives of 4-(substitutedphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-one (4a-f) and 4-(substitutedphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thiones (5a-f) were produced respectively.[21,22] The structure of all newly synthesized derivatives (4a-f) and (5a-f) were confirmed on the basis of TLC, melting point, FTIR, ^{1}H NMR, ^{13}C NMR, and Mass spectroscopy. The peaks of synthesized derivatives were observed through FTIR, the absorption bands of N-H (3420-3310 cm^{-1}), C=O (1730-1685 cm^{-1}), C=S (1252-1210 cm^{-1}) and C-S (700-600 cm^{-1}) were observed for both series of derivatives (4a-f) and (5a-f). The ^{1}H NMR and ^{13}C NMR spectra of both series of compounds (4a-f) and (5a-f) were consistently observed. The Mass spectra of the synthesized derivatives of (4a-f) and (5a-f) revealed the $[M+1]^+$ peak, $[M+2]^+$ isotopic peak of monochloro and monobromo containing derivatives and $[M+4]^+$ isotopic peak was observed of dichloro containing derivatives. All the newly synthesized derivatives gave satisfactory elemental analysis.

Biological Activity

3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) examination is a colorimetric test utilized for the assurance of cell multiplication and cytotoxicity, in view of decrease of the yellow shaded water dissolvable tetrazolium color MTT to formazan crystals. Mitochondrial lactate dehydrogenase delivered by live cells lessens MTT to insoluble formazan precious stones, which upon disintegration into a fitting dissolvable shows purple tone, the force of which is relative to the quantity of practical cells and can be estimated spectrophotometrically at 570nm[27,28]. The perceptions in Statistical information of cell cytotoxicity examination by MTT measure recommending us that against MCF7 cell lines. Test Compounds 4c, 4d, 4f, 5b, 5c and 5f showing significant cytotoxic potential properties with the IC_{50} concentration at 11.32µg/ml, 8.53 µg/ml, 13.09 µg/ml, 10.85µg/ml, 9.74 µg/ml and 14.87 µg/ml respectively against breast anticancer activity compared to the standard drug Doxorubicin which is showing the IC_{50} value at 7.49 µg/ml used for the study. Among all the synthesized compounds, 4d and 5c might be chosen as powerful anticancer agent due to its low IC_{50} value on MCF7 cells.

The synthesized derivatives 4d and 5c having dimethoxy and trimethoxy group respectively as electron donating showed highest anticancer activity. Electron donating group increases the electron cloud on the parent molecule resulting increasing the potency of the molecule towards anticancer activity.

Table I: Physical characterization of the prepared derivatives (4a-f) and (5a-f)

Compounds	R	M.P (°C)	R_f *value	Yield (%)
4a	2-Br	170-172	0.70	51
4b	2,4-Cl	145-147	0.66	55

4c	2-OH	135-137	0.74	46
4d	2-OCH_3	143-145	0.76	51
4e	2-NO_2	144-146	0.64	56
4f	2-NH_2	122-124	0.59	53
5a	2-Cl	118-120	0.72	51
5b	2,4,5-OH	124-126	0.69	68
5c	2,4,5-OCH_3	163-165	0.71	65
5d	2-NO_2	134-136	0.68	67
5e	2-NH_2	153-155	0.62	61
5f	2,4-NH_2	139-141	0.73	62

*Solvent system for 4(a-f) and 5(a-f) Benzene:ethyl acetate:formic acid (2:1:1)

Table 2: *In-vitro* anticancer screening of the synthesized novel derivatives against human breast cancer cell line (MCF-7)

Compound No.	IC_{50} Value (µg/mL)
4a	16.54
4b	11.29
4c	13.09
4d	8.53
4e	17.45
4f	11.32
5a	18.31
5b	10.85
5c	9.74
5d	18.69
5e	15.24
5f	14.87
Doxorubicin (Std.)	7.51

CONCLUSION: A series of newly synthesized derivatives of 4,6-disubstitutedpyrimidine-2-one and 4,6-disubstitutedpyrimidine-2-thione were screened against breast cancer cell line (MCF7), the synthesized compounds 4-(2,4-dimethoxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidin-2(*1H*)-one (4c) and 4-(2,4,5-

trimethoxyphenyl)-6-(4-(phenylthio)phenyl)pyrimidine-2(*1H*)-thione (5c) were revealed better anticancer activity when compared with the standard drug Doxorubicin. The better activity showed by newly synthesized 4c and 5c due to their electron donating nature. The study stated that pyrimidine is an important nucleus which produced a strong anticancer activity.

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