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Synthesis and antitumor activity of some new 1,3,4-oxadiazole, pyrazole and pyrazolo[3,4-d]pyrimidine derivatives attached to 4-benzothiazol-2-yl phenyl moiety

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ABSTRACT

*Some 1,3,4-oxadiazole, pyrazole and pyrazolo[3,4-d]pyrimidine derivatives were synthesized starting from o-aminothiophenol **1** by reacting with different electrophilic and nucleophilic reagents. Some of the newly synthesized compounds have been evaluated for their potential cytotoxicity against breast cancer cell line (MCF7), compound **6c** exhibited the highest activity.*

Key words: O-Aminothiophenol, 4-benzothiazol-2-yl-phenyl, 1,3,4-oxadiazole, pyrazole, pyrazolo[3,4-d] pyrimidine, antibreast cancer activity.

INTRODUCTION

Compounds containing 1,3,4-oxadiazole nucleus are associated with diverse pharmacological activities which have made them important chemotherapeutic agents(1-3). Many of these compounds have been reported to posses analgesic, diuretic, antihypertensive, anti-inflammatory(4), anticonvulsive(5), antibacterial and antifungal(6) properties. Some derivatives exhibit antiviral activities against RNA viruses and are able to inhibit HIV replication(7). In addition, some derivatives are active against hepatitis B(8) and HIV-1(9) viruses. Also pyrazole derivatives attract organic chemists very much due to their biological and chemotherapeutic importance. They are known to exhibit biological activities such as antitumor(10-12), antileukemic(13), anti-inflammatory(14,15), analgesic(16), anticoagulant(17) and antimicrobial(18-20). In addition, pyrazolo[3,4-d]pyrimidines were identified as a general class of adenosine receptors(7). There is no much difference in the basic structures of pyrazolo pyrimidines and purines. Also, pyrazolo[3,4-d]pyrimidines have antimicrobial and anti-inflammatory activities(21).

The small and simple benzothiazole moiety possess interesting biological and industrial activities(22). Many of these compounds showed very intensive antitumor(23-31), antiviral(32-34), antibacterial(35-37), anti-inflammatory, antipyretic(38.39), analgesic(40,41), antioxidant(42), anticonvulsive(43,44) and antidepressant(45) activities. Among the most

efficient compounds reported(22) are riluzole(46), sulfathiazole, 2-mercapto benzothiazole and 4-fluoro-2-(4-amino-3-methylphenyl)benzothiazole which revealed neuroprotective, anticonvulsive, antiallergic and antibreast cancer activities(24), respectively.

Based on the above observations, we report here the synthesis and antibreast cancer activity of some substituted 1,3,4-oxadiazole and pyrazole derivatives attached to 4-benzothiazol-2-yl phenyl starting from o-aminothio phenol.

MATERIALS AND METHODS

Melting points (˚C) were taken in open capillary tubes using silicon oil on Gallen Kamp apparatus. ^{1}H-NMR Spectra were measured in d_6-DMSO on JEOL-270 Spectrometer with Me_4Si as an internal standard. Mass Spectra were obtained with a Schimadzu GCS-QP1000EX Spectrometer at 70 eV. The IR Spectra were recorded with a Philips Infra cord Spectrophotometer Model PU 9712 in KBr discs. Elemental analysis was performed at the Micro analytical Laboratory of the National Research Center. The antitumor activity of the synthesized compounds was carried out at the National Cancer Institute, Cairo, Egypt.

2-(4-Cyanophenyl)benzo[d]thiazole (2):
4-Cyanobenzaldehye (1.05g, 0.21 mol) and o-aminothiophenol **1** (1 ml, 0.21 mol) were dissolved in ethanol. This mixture was refluxed for 5h. and cooled to room temperature. Then, water was added slowly to the mixture with stirring. The suspension was maintained at -5ºC overnight. The product was washed repeatedly with ethanol-water (1:1) mixture and then recrystallized from acetone. Yield = 1.2g (64%), m.p. = 150-2˚C. Analysis for $C_{14}H_8N_2S$ (236.2): Calcd.: C, 69.0; H, 4.5; N, 12.4; S, 14.2; Fd.: C, 69.2; H, 4.6; N, 12.5; S, 14.3.

4-(Benzo[d]thiazol-2-yl)benzoic acid (3):
A mixture of **2** (2g, 0.01 mol) and 30ml 70% sulfuric acid was stirred in 100 ml three-necked flask at 140ºC for 5h, then suspended in 150 ml water and the resulting precipitate was filtered off. Recrystallization from diluted ethanol afforded white crystals. Yield = 0.8g (74%), m.p. = 250-3˚C. Analysis for $C_{14}H_9NO_2S$ (255.3): Calcd.: C, 65.9; H, 3.6; N, 5.5; S, 12.6; Fd.: C, 66.0; H, 3.6; N, 5.6; S, 12.8. MS: m/z (%):256 (M^++1, 19); 255 (M^+, 100); 238 (12.2); 209 (14.4); 198 (2); 183 (2.5).

Ethyl 4-(benzo[d]thiazol-2-yl)benzoate (4):
To a solution of compound **3** (2g; 0.073 mol) in absolute ethanol, few drops of concentrated sulfuric acid were added and the mixture was refluxed for 4h. The crude product was filtered, air-dried and crystallized from ethanol. Yield = 2.1g (94%), m.p. = 102-6˚C. Analysis for $C_{16}H_{13}NO_2S$ (283.3): Calcd.: C, 67.8; H, 4.6; N, 4.9; S, 11.3; Fd.: C, 68.0; H, 4.6; N, 5.0; S, 11.5. MS: m/z (%): 285 (M^++2, 25); 283 (M^+, 5); 257 (10); 239 (16); 211 (15); 178 (7). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.32 (t, 3H, C<u>H</u>$_3$); 4.32 (q, 2H, C<u>H</u>$_2$CH$_3$); 7.48-8.22 (m, 8H, Ar-H).

4-(Benzo[d]thiazol-2-yl)benzohydrazide (5):
To a solution of ester compound **4** (1g; 0.033 mol) in ethanol, hydrazine hydrate (98%; 2 ml) was added and heated for 5h on a water-bath. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from ethanol. Yield = 0.8g (84%), m.p. = 234-8˚C. Analysis for $C_{14}H_{11}N_3OS$ (269.3): Calcd.: C, 62.4; H, 4.1; N, 15.6; S, 11.9; Fd.: C, 62.5; H, 4.2; N, 15.7; S, 12.0. IR (cm^{-1}): 3753 (NH), 3414 (NH$_2$), 1684 (CO). MS: m/z (%): 270 (M^++1, 6); 269 (M^+, 16); 238 (100); 210 (65); 139(12). ^{1}H-NMR: δ, ppm (DMSO-d_6); 7.50-8.24 (m, 8H, Ar-H)**;** 9.96 (s, 2H, N<u>H</u>$_2$); 10.45 (s, 1H, N<u>H</u>).

General procedure for the preparation of 2-[4-(5-(un)substituted-phenyl-1,3,4-oxadiazol-2-yl)phenyl]benzo[d]thiazole (6a,c), 4-{5-[4-(benzo[d] thiazol-2-yl)phenyl]- 1,3,4-oxadiazol-2-yl}-benzenamine (6b)

A mixture of hydrazide **5** (1g, 0.01 mol), appropriate aromatic acid (benzoic / 4-amino benzoic or 4-nitro benzoic acid) (0.02 mol) and phosphoryl chloride (10 ml) was refluxed on a water-bath for 6-8h. After cooling to room temperature, it was poured onto crushed ice with stirring. The solid thus obtained was filtered, washed with water and crystallized from ethanol.

2-[4-(5-Phenyl-1,3,4-oxadiazol-2-yl)phenyl]benzo[d]thiazole (6a)

Yield = 0.7g (67%), m.p. = 220-3˚C. Analysis for $C_{21}H_{13}N_3OS$ (355.4)): Calcd.: C, 71.0; H, 3.7; N, 11.8; S, 9.0; Fd.: C, 71.1; H, 3.8; N, 12.0; S, 9.1. MS: m/z (%): 357 (M^++2, 3); 355 (M^+, 2); 326 (4); 315 (4); 298 (4); 283 (2); 256 (15); 239 (6); 211 (7); 197 (6); 183 (7). ^{1}H-NMR: δ, ppm (DMSO-d_6); 7.32-8.34 (m, 13H, Ar-H).

4-{5-[4-(Benzo[d]thiazol-2-yl)phenyl]- 1,3,4-oxadiazol-2-yl}-benzenamine (6b)

Yield = 0.7g (64%), m.p. = above 300˚C. Analysis for $C_{21}H_{14}N_4OS$(370.4): Calcd.: C, 68.1; H, 3.8; N, 15.1; S, 8.7; Fd.: C, 68.3; H, 3.5; N, 15.3; S, 8.9. IR (cm^{-1}): 3340 (NH_2). ^{1}H-NMR: δ, ppm (DMSO-d_6); 6.70 (s, 2H, N<u>H</u>$_2$); 7.59-8.34 (m, 12H, Ar-H).

2-{4-[5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl]phenyl}benzo[d]thiazole (6c)

Yield = 0.8g (66%), m.p. = 202-5˚C. Analysis for $C_{21}H_{12}N_4O_3S$(400.4): Calcd.: C, 63.0; H, 3.0; N, 14.0; S, 8.0; Fd.: C, 63.2; H, 2.7; N, 14.2; S, 8.0. IR (cm^{-1}): absence of NH and CO bands. ^{1}H-NMR: δ, ppm (DMSO-d_6); 7.80-8.42 (m, 12H, Ar-H).

5[4-(Benzo[d]thiazol-2-yl)phenyl]-1,3,4-oxadiazole-2(3H)-thione (7):

To a mixture consisting of the hydrazide compound **5** (1g; 0.017 mol), potassium hydroxide aqueous solution (0.2g/20 ml) in ethanol, carbon disulphide (3.5 ml) was gently added and the mixture was heated under reflux till no odor of H_2S is detected (12 h.). The reaction mixture was then poured on ice-water and rendered acidic with 2N hydrochloric acid. The precipitated solid was filtered, washed with water, dried and crystallized from DMF/ethanol. Yield = 1.1g (95%); m.p. = 147˚-9˚C. Analysis for $C_{15}H_9N_3OS_2$ (311.4): Calcd.: C, 57.9; H, 2.9; N, 13.5; S, 20.6; Fd.: C, 57.8; H, 3.0; N, 13.6; S, 20.6. IR (cm^{-1}): 3423 (NH), 1487 (C=S). MS: m/z (%): 329 (M^+, 60); 297 (10); 214 (70); 199 (80), 184 (27); 166 (100). ^{1}H-NMR: δ, ppm (DMSO-d_6); 8.05 (s, 1H, N<u>H</u>); 7.96-8.21 (m, 8H, Ar-H). ^{13}C-NMR δ, ppm (DMSO-d_6): showed the presence of 15 signals correspond to the 15 different carbon groups, signals appeared at δ 122.49-130.70 (Ar-8<u>C</u>H), 134.72 (<u>C</u>=C), 137.07 (<u>C</u>S), 152.99 (<u>C</u>=N), 154.38 (<u>C</u>-N), 162.96 (<u>C</u>=S), 165.70 (<u>C</u>=C) and 165.78 (<u>C</u>=N).

General procedure for the preparation of 5-substituted-2[4-(1,3,4-oxadiazol -2-yl) phenyl]benzo[d]thiazole (8a-e):

A mixture of the compound **7** (1g; 0.006 mol), methyliodide / methylchloro formate / methyl-2-bromoacetate / ethyl-2-bromoacetate or ethyl-2-bromo propanoate (0.006 mol), anhydrous sodium carbonate (4g.) and acetone (30 ml) was heated under reflux for 8h. Most of the alcohol was distilled off, the residue was diluted with water and the obtained product was collected.

2-{4-[5-(Methyl thio)-1,3,4-oxadiazol-2-yl]phenyl}benzo[d]thiazole (8a):

Yield = 0.9g (86%); m.p. = 115-7˚C. Analysis for $C_{16}H_{11}N_3OS_2$ (325.4): Calcd.: C, 59.1; H, 3.4; N, 12.9; S, 19.7; Fd.: C, 59.3; H, 3.5; N, 12.8; S, 19.4. MS: m/z (%): 325 (M^+, 2); 323 (M^+-2, 51); 309 (49); 295 (55); 283 (100); 269 (100); 238 (100); 210 (52); 183 (9). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.90 (s, 3H, C<u>H</u>$_3$); 7.51-8.27 (m, 8H, Ar-H).

5-[4-(Benzo[d]thiazol-2-yl) phenyl-1,3,4-oxadiazol-2-yl-O-methyl carbono thioate (8b):
Yield = 1.0g (84%); m.p. = 180-3˚C. Analysis for $C_{17}H_{11}N_3O_3S_2$ (369.4): Calcd.: C, 55.3; H, 3.0; N, 11.4; S, 17.4; Fd.: C, 55.5; H, 3.1; N, 11.7; S, 17.5. IR (cm^{-1}): 1769 (COO). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.01 (s, 3H, C<u>H</u>$_3$); 7.51-8.34 (m, 8H, Ar-H).

Methyl-2-{5-[4-(benzo[d]thiazol-2-yl) phenyl]-1,3,4-oxadiazol-2-yl thio} acetate (8c):
Yield = 1.1g (89%); m.p. = 162-5˚C. Analysis for $C_{18}H_{13}N_3O_3S_2$ (383.4): Calcd.: C, 56.4; H, 3.4; N, 11.0; S, 16.7; Fd.: C, 56.5; H, 3.5; N, 11.2; S, 16.6. IR (cm^{-1}): 1738 (COO). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.66 (s, 3H, C<u>H</u>$_3$); 4.33 (s, 2H, C<u>H</u>$_2$); 7.48-8.32 (m, 8H, Ar-H).

Ethyl-2-{5-[4-(benzo[d]thiazol-2-yl)phenyl]-1,3,4-oxadiazol-2-yl thio} acetate (8d):
Yield = 1.0g (78%); m.p. = 92-5˚C. Analysis for $C_{19}H_{15}N_3O_3S_2$ (397.5): Calcd.: C, 57.4; H, 3.8; N, 10.6; S, 16.1; Fd.: C, 57.5; H, 3.9; N, 10.9; S, 16.3. IR (cm^{-1}): 1724 (COO). MS: m/z (%): 399 (M^++2, 5); 398 (M^++1, 7); 397 (M^+, 28); 381 (42); 341 (6); 308 (4); 293 (3); 278 (5); 264 (14), 238 (100); 210 (20). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.18 (t, 3H, C<u>H</u>$_3$); 4.16 (q, 2H, C<u>H</u>$_2$); 4.79 (s, 2H, C<u>H</u>$_2$); 7.51-8.33 (m, 8H, Ar-H).

Ethyl-2-{5-[4-(benzo[d]thiazol-2-yl)phenyl]-1,3,4-oxadiazol-2-yl thio} propanoate (8e):
Yield = 1.1g (83%); m.p. = 96-9˚C. Analysis for $C_{20}H_{17}N_3O_3S_2$ (411.5): Calcd.: C, 58.4; H, 4.2; N, 10.2; S, 15.6; Fd.: C, 58.5; H, 4.3; N, 10.0; S, 15.7. ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.15 (t, 3H, C<u>H</u>$_3$); 1.61 (d, 3H, C<u>H</u>$_3$); 4.13 (q, 2H, C<u>H</u>$_2$); 4.59 (q, 1H, C<u>H</u>); 7.51-8.33 (m, 8H, Ar-H).

General procedure for preparation of 5-[4-(benzo[d]thiazol-2-yl)phenyl]-3-substituted-methyl-1,3,4-oxadiazol-2(3H)-thione (9a-b):
To a solution of compound **7** (2g; 0.006 mol) in absolute ethanol (60 ml), 40% formaldehyde solution (0.18 ml; 0.006 mol) was added and the reaction mixture was heated on a steam-bath till a clear solution was obtained. Dimethylamine or morpholine (0.006 mol) was added followed by few drops of hydrochloric acid. The mixture was heated for further 2h., and then left at room temperature overnight. The solvent was removed by distillation and the residue was neutralized with sodium carbonate solution. The formed solid was collected and recrystallized from ethanol.

5-[4-(Benzo[d]thiazol-2-yl) phenyl]-3-[(dimethylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (9a):
Yield = 0.8g (68%); m.p. = 130-3˚C. Analysis for $C_{18}H_{16}N_4OS_2$ (368.5): Calcd.: C, 58.7; H, 4.4; N, 15.2; S, 17.4; Fd.: C, 58.5; H, 4.3; N, 15.2; S, 17.4. IR (cm^{-1}): 1413 (CS), 1277 [$N(CH_2)_3$]. ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.16 (s, 6H, 2C<u>H</u>$_3$); 5.47 (s, 2H, C<u>H</u>$_2$); 7.46-8.31 (m, 8H, Ar-H).

5-[4-(Benzo[d]thiazol-2-yl) phenyl]-3-[(morpholino methyl)-1,3,4-oxadiazol-2(3H)-thione (9b):
Yield = 0.9g (68%); m.p. = 180-3˚C. Analysis for $C_{20}H_{18}N_4O_2S_2$ (410.1): Calcd.: C, 58.5; H, 4.4; N, 13.7; S, 15.6; Fd.: C, 58.3; H, 4.5; N, 13.6; S, 15.9. ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.67 (t, 4H, 2C<u>H</u>$_2$); 4.33 (t, 4H, 2C<u>H</u>$_2$); 5.42 (s, 2H, C<u>H</u>$_2$); 7.49-8.29 (m, 8H, Ar-H).

5-Amino-2-(4-benzothiazol-2-yl-benzoyl)-1,2-dihydro-pyrazol-3-one (10):
To a solution of compound **5** (1g, 0.002 mol) in 25 ml absolute ethanol, ethylcyanoacetate (0.42 ml, 0.002 mol) was added. The reaction mixture was heated under reflux for 4h. until all starting material had disappeared as indicated by TLC and then left to cool at room temperature. The solution was concentrated under vacuum, whereby the resulted product was treated with ice/water mixture and neutralized with dilute hydrochloric acid. The formed solid product was filtered off, washed several times with water, dried and crystallized from methanol. Yield = 0.8g (74%); m.p. = 240-5˚C. Analysis for $C_{17}H_{12}N_4O_2S$ (336.4): Calcd.: C, 60.7; H, 3.6; N, 16.7; S, 9.5; Fd.: C, 60.8; H, 3.7; N, 16.6; S, 9.6. IR (cm^{-1}): 3421-3200 (NH, NH_2), 1672-1645 (CO). MS: m/z (%): 336 (M^+, 10); 323 (10); 309 (15); 295 (10); 281 (20); 267 (11); 253 (15); 239 (30); 211

(35); 197 (21), 183 (25); 166 (100). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.49 (s, 2H, N$\underline{H}_2$); 4.66 (s, 1H, C$\underline{H}$); 7.48-8.28 (m, 8H, Ar-H); 8.59 (s, 1H, N$\underline{H}$).

General procedure for preparation of 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-substituted (11a-b):

To a solution of hydrazide **5** (1g, 0.01 mol) in 30 ml ethanol, ethoxy methylene malononitrile or ethyl (ethoxy methylene) cyano acetate (0.01 mol) was added. The reaction mixture was refluxed for 6-8h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The solid product was recrystallized from ethanol.

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-carbonitrile (11a):

Yield = 0.7g (55%); m.p. = 266-8˚C. Analysis for $C_{18}H_{11}N_5OS$ (345.4): Calcd.: C, 62.6; H, 3.2; N, 20.3; S, 9.3; Fd.: C, 62.5; H, 3.3; N, 20.2; S, 9.4. IR (cm^{-1}): 3424 (NH_2), 2217 (CN), 1677 (CO). MS: m/z (%): 345 (M^+, 57); 344 (M^+-1, 70); 334 (65); 323 (74); 314 (62); 306 (54); 294 (73); 287 (68); 278 (73); 257 (55); 237 (100). ^{1}H-NMR: δ, ppm (DMSO-d_6); 7.48 (s, 1H, C$\underline{H}$); 7.49-8.24 (m, 8H, Ar-H); 10.76 (s, 2H, N$\underline{H}_2$).

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-carboxylic acid ethyl ester (11b):

Yield = 0.9g (62%); m.p. = above 300˚C. Analysis for $C_{20}H_{16}N_4O_3S$ (392.4): Calcd.: C, 61.2; H, 4.1; N, 14.3; S, 8.2; Fd.: C, 61.3; H, 4.5; N, 14.6; S, 8.4. IR (cm^{-1}): 3224 (NH_2), 1677-1652 (CO). MS: m/z (%): 392 (M^+, 57); 365 (4); 338 (5); 312 (11); 279 (18); 255 (46); 238 (47); 210 (73); 188 (42). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.19 (t, 3H, C$\underline{H}_3$); 3.30 (q, 2H, C$\underline{H}_2$); 7.48 (s, 1H, C$\underline{H}$); 7.50-8.25 (m, 8H, Ar-H); 10.77 (s, 2H, N$\underline{H}_2$).

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2H-pyrazol-3-yl]-formimidic acid ethyl ester (12):

To a mixture of triethylorthoformate (0.21 ml, 0.01 mol) and acetic anhydride, compound **11a** (0.5g, 0.01 mol) was added and the reaction mixture was refluxed for 5h. The solvent was removed under reduced pressure and the separated solid was recrystallized from ethanol to afford **12** as a yellow powder. Yield = 0.35g (60%); m.p. = 273-5˚C. Analysis for $C_{21}H_{15}N_5O_2S$ (401.4): Calcd.: C, 62.8; H, 3.8; N, 17.5; S, 8.0; Fd.: C, 62.9; H, 3.7; N, 17.6; S, 8.2. IR (cm^{-1}): 2210 (CN), 1616 (CO), 1107 (OC_2H_5). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.86 (t, 3H, C$\underline{H}_3$); 4.35 (q, 2H, C$\underline{H}_2$); 7.50 (s, 1H, C$\underline{H}$-OC_2H_5); 7.56 (s, 1H, N=CH), 7.60-8.23 (m, 8H, Ar-H).

N-[2-(4-Benzothiazol-2-ylbenzoyl)-4-cyano-2H-pyrazol-3-yl]-2-cyano acetamide (13):

To a solution of compound **11a** (0.5g, 0.01 mol) in DMF (20 ml), ethylcyanoacetate (0.16 ml, 0.01 mol) was added. The reaction mixture was heated under reflux for 5h. The solid product formed upon pouring onto ice/water mixture was collected by filtration and crystallized from 1,4-dioxane. Yield = 0.4g (67%); m.p. = 205-8˚C. Analysis for $C_{21}H_{12}N_6O_2S$ (412.4): Calcd.: C, 61.2; H, 2.9; N, 20.4; S, 7.8; Fd.: C, 61.3; H, 3.3; N, 20.6; S, 7.6. IR (cm^{-1}): 3224 (NH), 2217 (CN), 1686-1644 (CO). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.94 (s, 2H, C$\underline{H}_2$); 7.51 (s, 1H, N=C$\underline{H}$); 7.51-8.28 (m, 8H, Ar-H); 9.98 (s, 1H, NH).

(5-Amino-4-imino-4,5-dihydro-pyrazol-[3,4-d]-pyrimidin-1-yl)-(4-benzo thiazol-2-yl-phenyl)-metha -none (14):

To a solution of compound **12** (0.5g; 0.01mol) in ethanol, hydrazine hydrate (98%; 2 ml) was added and heated for 5h on a water-bath. The reaction mixture was cooled. The precipitate was filtered, washed with water and dried. It was recrystallized from ethanol. Yield = 0.3g (62%); m.p. = 190-5˚C. Analysis for $C_{19}H_{13}N_7OS$ (387.4): Calcd.: C, 58.9; H, 3.4; N, 25.3; S, 8.3; Fd.: C, 58.9; H, 3.7; N, 25.4; S, 8.2. IR (cm^{-1}): 3752-3218 (NH, NH_2), 1644 (CO). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.30 (s, 2H, N$\underline{H}_2$); 7.03 (s, 1H, C$\underline{H}$-NNH_2); 7.19 (s, 1H, N=CH); 7.37-8.19 (m, 8H, Ar-H); 9.98 (s, 1H, NH).

(4-Amino-5-ethynyl-6-hydroxy-pyrazol[3,4-b]pyridine-1-yl)-(4-benzo thiazol -2-yl-phenyl)-metha -none (15):

A solution of compound **13** (0.5g, 0.01 mol) in 1,4-dioxane (20 ml) containing triethylamine (2 ml) was heated under reflux for 5h. The solid product formed upon pouring onto ice/water was collected by filtration and crystallized from 1,4-dioxane. Yield = 0.4g (67%); m.p. = 205-8˚C. Analysis for $C_{21}H_{12}N_6O_2S$ (412.4): Calcd.: C, 61.2; H, 2.9; N, 20.4; S, 7.8; Fd.: C, 61.3; H, 3.3; N, 20.6; S, 7.6. IR (cm^{-1}): 3424-3204 (OH, NH_2), 2219 (CN), 1690 (CO). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.93 (s, 1H, OH); 7.50 (s, 1H, N=CH); 7.60-8.48 (m, 8H, Ar-H); 8.87 (s, 1H, NH_2).

(4-Benzothiazol-2-yl-phenyl)-(4,6-dithioxo-4,5,6,7-tetrahydro-pyrazolo [3,4-d]pyrimidin-1-yl)-methanone (16):

To a mixture consisting of compound **11a** (0.5g; 0.017 mol), potassium hydroxide aqueous solution (0.2g/20 ml) in ethanol, carbon disulphide (3.5 ml) was gently added and the mixture was heated under reflux till no odor of H_2S is detected (12 h.). The reaction mixture was then poured on ice-water and rendered acidic with 2N hydrochloric acid. The precipitated solid was filtered, washed with water, dried and crystallized from DMF/ethanol. Yield = 0.4g (66%); m.p. = 190-5˚C. Analysis for $C_{19}H_{11}N_5OS_3$ (421.5): Calcd.: C, 54.1; H, 2.6; N, 16.6; S, 22.8; Fd.: C, 54.2; H, 2.7; N, 16.4; S, 22.7. IR (cm^{-1}): 3560-3402 (NH), 1677 (CO), 1403-1378 (CS). MS: m/z (%): 421 (M^+, 1); 420(M^+-1, 1); 406 (1); 394 (2); 379 (3); 368 (100); 352 (9); 337 (9); 323 (100); 309 (10); 295 (12); 281 (10); 267 (15); 211 (27); 183 (33). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.49 (s, 1H, NH); 7.51-8.29 (m, 8H, Ar-H); 10.80 (s, 1H, NH).

1-(4-Benzothiazol-2-yl-benzoyl)-5-(4-chlorophenyl)-4-imino-1,4,5,7-tetra hydro-pyrazolo[3,4-d] pyri -midin-6-one (17):

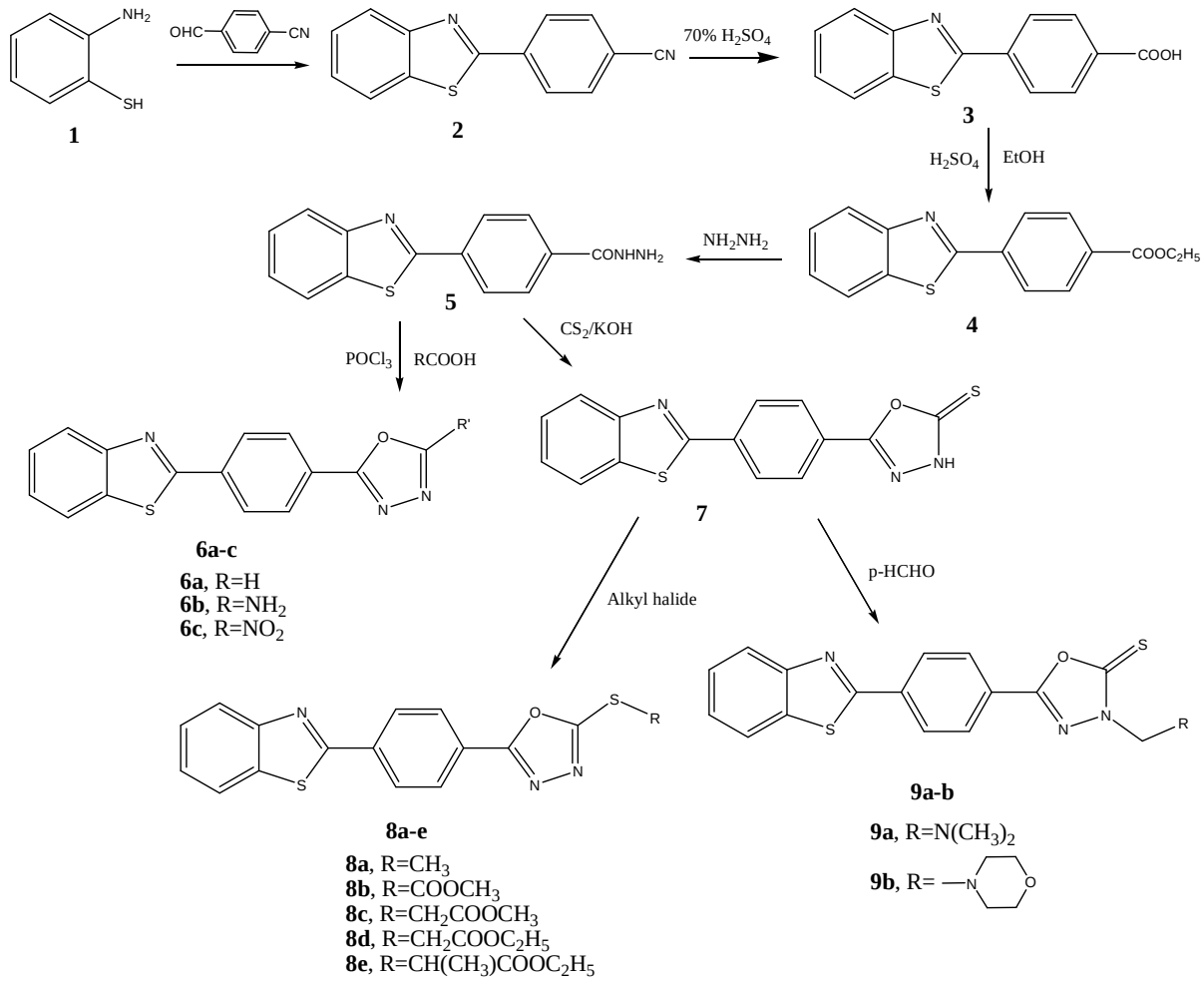
A solution of compound **11a** (0.5g, 0.01 mol) and 4-chlorophenyl isocyanate (0.44 ml, 0.01 mol) in 20 ml ethanol was heated at reflux temperature for 6h. A solid product was obtained after cooling to room temperature, which was filtered off and recrystallized from DMF. Yield = 0.4g (55%); m.p. = above 300˚C. Analysis for $C_{25}H_{15}ClN_6O_2S$ (498.9): Calcd.: C, 60.2; H, 3.0; N, 16.8; S, 6.4; Fd.: C, 60.3; H, 3.3; N, 16.6; S, 6.6. IR (cm^{-1}): 3743-3223 (NH), 1677-1652 (CO). MS: m/z (%): 498 (M^+, 11); 435(9); 407 (9); 393 (19); 375 (10); 354 (13); 323 (12); 297 (13); 278 (12); 252 (10); 235 (17); 211 (21); 197 (15). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.10 (s, 1H, NH); 7.60 (s, 1H, CH); 7.33-8.29 (m, 12H, Ar-H); 10.80 (s, 1H, NH).

RESULTS AND DISCUSSION

Chemistry:

2-(4-Cyanophenyl)benzo[d]thiazole **2** was prepared from reacting o-aminothiophenol **1** with 4-cyanobenzaldehyde in absolute ethanol according to reported procedure[23]. Acid oxidation of carbonitrile group by stirring with 70% sulfuric acid to give 4-(benzo[d]thiazol-2-yl) benzoic acid **3** followed by esterification and reacting with hydrazine hydrate to form the corresponding 4-(benzo[d]thiazol-2-yl)benzohydrazide **5**. Reacting **5** with the appropriate aromatic acid (benzoic / 4-amino benzoic or 4-nitro benzoic acid) in the presence of phosphoryl chloride to give the corresponding 5-substituted-2[4-(1,3,4-oxadiazol-2-yl)phenyl]benzo[d]thiazole **6a-c**. Also, refluxing benzohydrazide 5 with carbon disulphide in alkaline medium, yielded 5[4-(benzo[d]thiazol-2-yl)phenyl]-1,3,4-oxadiazole-2(3H)-thione **7**. Condensing compound **7** with the required halide (methyliodide / methylchloroformate / methyl-2-bromoacetate / ethyl-2-bromoacetate or ethyl-2-bromopropanoate) in the presence of sodium carbonate as acid halide abstract and acetone as solvent. The products **8a-e** was obtained mostly in pure state. Refluxing compound **7** with formaldehyde and dimethylamine or morpholine gave the Mannich bases 5-[4-(benzo[d]thiazol-2-yl)phenyl]-3-[(dimethylamino)methyl]-1,3,4-oxadiazol -2(3H)-thione **9a**

and 5-[4-(benzo[d]thiazol-2-yl)phenyl]-3-[(morpholino methyl)-1,3,4-oxadiazol-2(3H)-thione **9b**, respectively (***Scheme I***).



1 **2** **3** **4** **5** **7**

6a-c

6a, R=H
6b, R=NH_2
6c, R=NO_2

8a-e

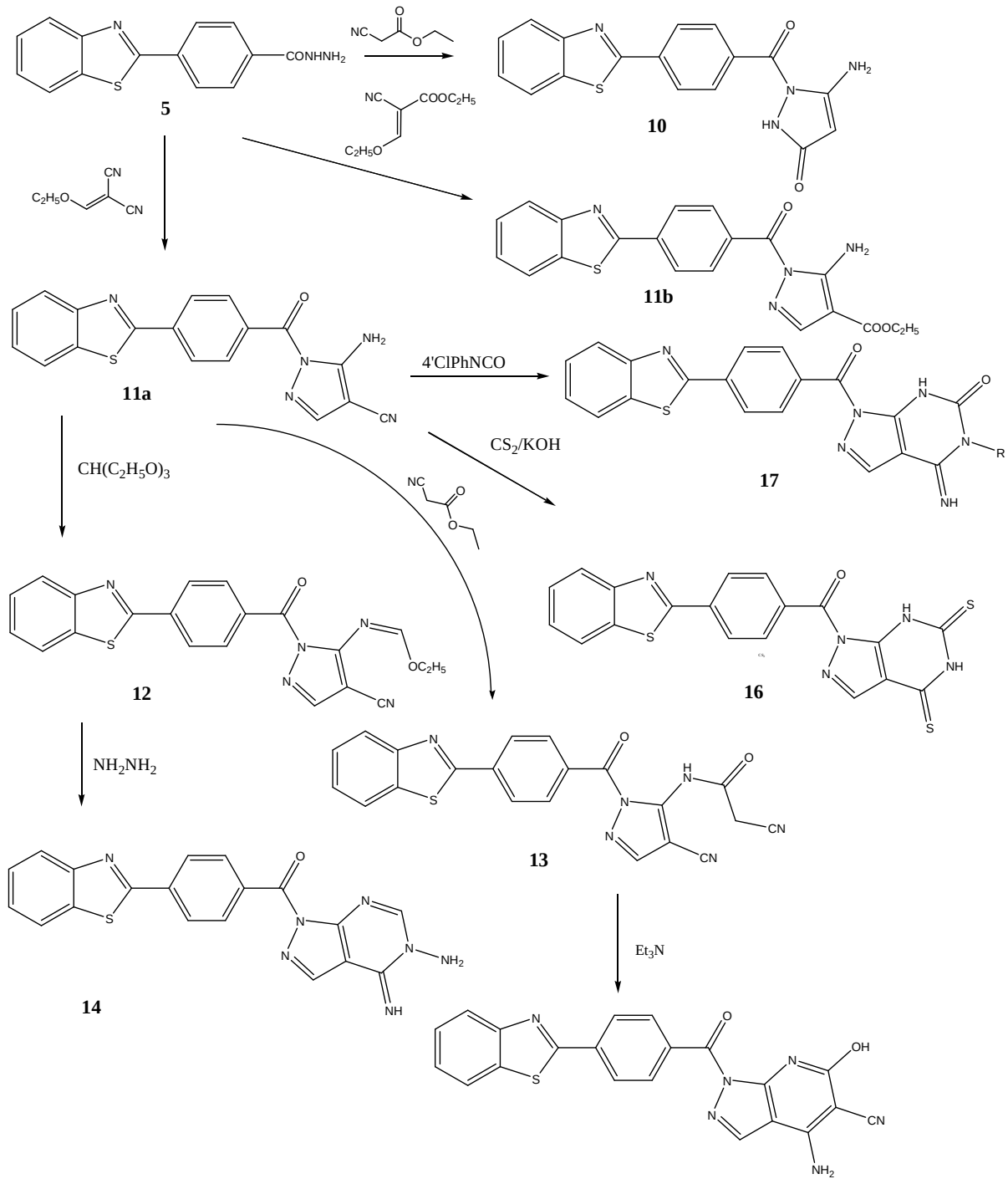
8a, R=CH_3
8b, R=$COOCH_3$
8c, R=CH_2COOCH_3
8d, R=$CH_2COOC_2H_5$
8e, R=$CH(CH_3)COOC_2H_5$

9a-b

9a, R=$N(CH_3)_2$
9b, R= —N O

Scheme I

The reaction of compound **7** with ethylcyanoacetate at reflux temperature afforded 5-amino-2-(4-benzothiazol-2-yl-benzoyl)-1,2-dihydro-pyrazol-3-one **10**. In addition, refluxing compound **7** with ethoxy methylene malononitrile or ethyl (ethoxy methylene)cyano acetate in ethanol, yielded the corresponding 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-carbonitrile **11a** and 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-carboxylic acid ethyl ester **11b**, respectively. Furthermore, preparing some pyrazole derivatives were shown in ***Scheme II***. Refluxing **11a** with triethylorthoformate and acetic anhydride followed by hydrazine hydrate gave the corresponding (5-amino-4-imino-4,5-dihydro-pyrazol-[3,4-d]-pyrimidin-1-yl)-(4-benzothiazol-2-ylphenyl) -methanone **14**.



Scheme II

In addition, refluxing **11a** with ethylcyanoacetate followed by refluxing with triethylamine in 1,4-dioxane afforded (4-amino-5-ethynyl-6-hydroxy-pyrazol [3,4-b]pyridine-1-yl)-(4-benzothiazol-2-yl-phenyl)-methanone **15**. Also, heating compound **11a** with carbon disulfide at reflux temperature in the presence of potassium hydroxide solution gave the following product (4-benzothiazol-2-yl-phenyl)-(4,6-dithioxo-4,5,6,7-tetrahydro-pyrazolo[3,4-d] pyrimidin-1-yl)-methanone **16**. Compound **17** was synthesized via reaction of compound **11a** with 4-chlorophenyl isocyanate in ethanol, heated at reflux temperature (***Scheme II***).

In *Vitro* Antitumor Activity:

Measurement of potential cytotoxicty by SRB assay:

Some of the newly synthesized compounds have been evaluated for their potential cytotoxicity testing against breast cancer (MCF7) using the method of Skehan et al.[47]. Cells were plated in 96-multiwell plate (10^4 cell/well) for 24hrs before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentration of the compound 0, 5, 12.5, 25 and 50 µg/ml were added to the cell monolayer triplicate wells were prepared for each individual dose and left to attach to the plates for 24 hrs. After 24 hrs, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200 µl volume/well using fresh medium and incubation was continued for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used. Following 24, 48 and 72 hrs treatment, the cells were fixed with 50 µl cold 50 % trichloroacetic acid for 1 hr at 4 ^{0}C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50 µl 0.4 % SRB dissolved in 1 % acetic acid. The wells were then washed 4 times with 1 % acetic acid.The plates were air-dried and the dye was solubilized with 100 µl/well of 10 mM tris base (ph 10.5) for 5 min on a shaker at 1600rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564nm with an ELISA micro plate reader. The mean background absorbances was automatically subtracted and mean values of each drug concentration was calculated. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line for the specified compound. The IC_{50} percent control of infected and uninfected response values were calculated for the various active compounds were reported in Table 1.

Table 1: IC_{50} (µg/ml) of some selected new compounds against Breast cancer cell line (MCF7)

Compound	IC_{50} (µg/ml)
DOX	4.5
5	12
6c	5.52
7	>50
8d	11
9b	13.2
11a	10.7
13	19.5
14	20.9
16	18
17	21.2

Doxirubsin (DOX) was used as positive standard. Compounds having IC_{50} less than 12 are considered potentially active and exposed to further in *vivo* studies.

The results obtained in table 1 show that compounds **6c**, **11a** and **8d** possess high significant effect against breast cancer cell line (MCF7) and this is might be due to the presence of nitro group attached to 1,3,4-oxadiazole in compound **6c**. The other compounds show less effect against breast cancer cell line.

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