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Research Article

Synthesis and Biological Evaluation of Some New 1,3,4-Oxa, Thiadiazole and 1,2,4-Triazole Derivatives attached to Benzimidazole

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Abstract

Some of novel benzimidazole derivatives bearing different heterocyclic moieties such as 1,3,4-Oxadiazole ،1, 3,4 -Thiadiazole and 1,2,4- Triazole were prepared. The structure of the new compounds were elucidated by IR , ^{1}H,$^{13}CNMR$,MS spectra and elemental analysis .Some of the new compounds were evaluated for *in vitro* cytotoxic activity against breast carcinoma(MCF-7) and colon carcinoma (HCT-116) cell line. Some of the tested compounds exhibited moderate cytotoxic activity .The preliminary antimicrobial activity of all of the novel compounds were investigated against Gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*), Gram-negative bacteria (*Escherichia coli and Pseudomonas aerugnosa*) and fungi (*Candida albicans*)and the majority of the tested compounds exhibited promising antimicrobial activity.

Keywords: 1,3,4 Oxadiazole, 1,3,4 Thiadiazole, 1,2,4 Triazole, benzimidazole, cytotoxic activity, antimicrobial activity

1. Introduction

The study of recent literature reveals that the increasing incidence of infection caused by the rapid development of microbial resistance to most of the known antibiotics is a serious health problem. There are a number of factors responsible for mutations in the microbial genomes. As multi drug resistant microbial strains proliferate, the necessity for effective therapy has stimulated research on the design and synthesis of novel antimicrobial molecules. Also, the need for anticancer agents that selectively kill or inhibit the growth of neoplastic cells without affecting non-cancerous host tissues is high and persistent. While searching for compounds with potential antimicrobial and anticancer agents, we directed our attention to the benzimidazole and its derivatives, which are very useful intermediates in the development of molecules of pharmaceutical or biological interest such as, antimicrobial, antioxidant, antiviral, antihypertensive, antiprotozoal, anti-inflammatory and molluscicidal agents[1-9]. Furthermore, benzimidazoles showed anticancer activity against DNA topoisomerase I[10] and colon cancer cell lines[11]. Indeed, a number of important drugs used in different therapeutic areas contain the benzimidazole ring, as proton pump inhibitors (omeprazole), anthelmintic (Albendazole, mebendazole) antihypertensives (candesartan, telmisartan), antihistamines (astemizole), as well as several other kinds of still investigational therapeutic agents, including antitumors and antivirals[12-16]. Also, triazoles, oxa-, and thiadiazoles have been the subject of chemical and biological studies on account of their interesting pharmacological properties such as antimicrobial, antidepressant, anti-inflammatory, analgesic, and antitumor activities[17-22].

2. Material and Methods

2.1 Experimental

All melting points are uncorrected, that were determined using a Kofler block instrument. IR spectra were recorded with a Perkin-Elmer model 1720 FTIR (KBr), ^{1}H, $^{13}CNMR$ spectra were recorded with Bruker AC 300 FT NMR spectrometer at 300MHz with TMS as an internal standard. MS spectra were recorded with a Shimadzu QP-2010 Plus. The elemental analysis and the evaluation of cytotoxicity against (MCF7) cell line and (HCT-116) cell line were carried at The Regional Center for Mycology & Biotechnology, Al-Azhar University, Egypt. The antimicrobial evaluation was carried in the microbiology department, Faculty of pharmacy, Zagazig University, Zagazig, Egypt.

2-(1H-benzo [d] imidazol-1-yl) acetohydrazide **1** was prepared according to reported methods[23,24].

2-(2-(1H-benzo[d]imidazol-1-yl) acetyl)-N-aryl hydrazinecarbothioamide 2a-c
General procedure

To a mixture of the acetohydrazide **1** (1.9 g, 10 mmole) in absolute ethanol (50 ml)arylisothiocyanate (10 mmol), was added, and the reaction mixture was heated under reflux for 6 h (follow by TLC), then left to cool. The separated solid was collected by filtration, washed with ethanol and finally crystallized from ethanol to afford thiosemicarbazide **2a-c**

2-(2-(1H-benzo [d] imidazol-1-yl) acetyl) -N-phenylhydrazinecarbothioamide 2a

Yield(2.93g,90%),m.p168-170°C.IR(KBr,ύcm^{-1}):3251, 3143NH, 3062 (CH,aromatic), 2968(CH,aliphatic),1707 (C=O), 1616(C=N), 1531(C=C).^{1}H NMR (300 MHz, DMSO-d_6)δ ppm 5.06 (s,2H, N$\underline{CH_2}$), 7.16-7.68 (m, 9H,Ar-H,), 8.21 (s, 1H,C2 imidazole), 9.71 (s, 1H, NH, D_2Oexchangeable), 9.76 (s, 1H, NH, D_2Oexchangeable), 10.50 (s, 1H, NH, D_2O-exchangeable); MS :*m/z*(%) 325 (M^+).Microanalysis for $C_{16}H_{15}N_5S$(325).Calcd : %C ,59.06 ; H ,4.65 ; N,21.52
Found: %C, 59.41; H, 4.69 ; N ,21.67

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2-(2-(1H-benzo[d]imidazol-1-yl)acetyl)-N-(4-bromophenyl)hydrazinecarbo-thioamide 2b
Yield (3.84 g, 95 %), m.p. 177-180°C. IR (KBr, ύ cm^{-1}): 3233, 3170, NH, 3060 (CH aromatic), 2973(CH aliphatic), 1702 (C=O), 1630(C=N), 1568 (C=C),1490 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6): δppm 5.81 (s,2H, N$\underline{CH_2}$), 7.24-7.70 (m, 8H,Ar-H,), 8.20 (s, 1H,C2 imidazole), 9.77 (s, 1H, NH, D_2Oexchangeable), 10.65 (s, 1H, NH, D_2O-exchangeable), 11.17 (s, 1H, NH, D_2O-exchangeable) ; ^{13}CNMR(DMSO-d6)δppm: 39.90, 110.40 ,113.40 , 115.74,118.92, 119.26, 121.64, 122.03, 125.57 , 131.20 , 133.58 , 137.77 , 143.23, 144.11 , 155.60 , 159.97,165.50.
MS: *m/z*(%) 405 (3.2)M^++1, 404 (3.85)M^+,165(21.22),80(100). Microanalysis for $C_{16}H_{14}BrN_5OS$ (404.3). Calcd: %C, 47.53; H, 3.49; N, 17.32. Found%: C, 47.59; H, 3.51; N, 17.48.

2-(2-(1H-benzo[d]imidazol-1-yl) acetyl)-N-(4-methoxyphenyl) hydrazinecarbo-thioamide 2c
Yield (3.41 g, 96 %), m.p. 175-177 °C. IR (KBr, ύ cm^{-1}): 3181, 3101, NH, 3050 (CH aromatic), 2956(CH aliphatic), 1703(C=O), 1633(C=N), 1576(C=C),1468 (C-N) ; ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.68(s,3H, OCH_3), 5.81 (s,2H, N$\underline{CH_2}$), 7.21-7.70 (m, 8H,Ar-H,), 8.37 (s, 1H,C2 imidazole), 9.77 (s, 1H, NH, D_2Oexchangeable), 10.65 (s, 1H, NH, D_2O-exchangeable),11.17 (s, 1H, NH, D_2O-exchangeable); MS : *m/z*(%) 356(0.13) M^++1, 355(0.25)M^+. Microanalysis for $C_{17}H_{17}N_5O_2S$ (355.4). Calcd:% C, 57.45; H, 4.82; N, 19.70 . Found: % C, 57.53; H, 4.78; N, 19.83.

General procedure for the synthesis of 1,3,4-thiadiazole 3a-c
Each thiosemicarbazide **2a-c** (10 mmol) was added portion wise to 10 ml of conc. sulfuric acid with continuous stirring. The reaction mixture was stirred further for 4 h at room temperature. The reaction mixture was slowly poured into crushed ice with stirring and neutralized with ammonia. The mixture was allowed to stand overnight and the solid separated out was filtered and washed with cold water. The solid was dried and crystallized from a mixture of DMF and water (1:1) to furnish di-substituted 1,3,4-thiadiazole **3a-c**

5-((1H-benzo[d]imidazol-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine 3a: Yield (2.8 g, 68 %), m.p. > 300 °C. IR (KBr, ύ cm^{-1}): 3263 NH,3049(CH aromatic),2850(CH aliphatic) , 1600(C=N), 1506(C=C) ,1448(C-N) .
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm : 6.14 (s,2H, N$\underline{CH_2}$), 7.48-8.04 (m, 9H,Ar-H,), 9.75 (s, 1H,C2 imidazole), 10.56 (s, 1H, NH, D_2O-exchangeable); MS : *m/z*(%) 307 (2.3)M^+ , 118 (100),77(24),63(59.8).Microanalysis for $C_{16}H_{13}N_5S$ (307.4) .Calcd:% C, 62.52; H, 4.26; N, 22.78. Found: % C, 62.70; H, 4.32; N, 22.93.

5-((1H-benzo[d]imidazol-1-yl)methyl)-N-(4-bromophenyl)-1,3,4-thiadiazol-2-amine 3b
Yield (2.8 g, 73 %), m.p. 118-120 °C. IR (KBr, ύ cm^{-1}): 3239, NH,3034 (CH aromatic) ,2851(CH aliphatic) , 1611(C=N), 1546(C=C) ,1497(C-N) .
^{1}H NMR (300 MHz, DMSO-d_6) δppm: 6.03 (s,2H, N$\underline{CH_2}$), 7.44-7.87 (m, 8H,Ar-H,), 9.23 (s, 1H,C2 imidazole), 10.81 (s, 1H, NH, D_2O-exchangeable); MS : *m/z*(%) 387 (46.10)M^++1, 386(38.96) M^+,378(50.60).Microanalysis for $C_{16}H_{12}BrN_5S$ (386.3) Calcd :%C, 49.75; H, 3.13; N, 18.13; . Found: %C, 49.86; H, 3.15; N, 18.29.

5-((1H-benzo[d]imidazol-1-yl)methyl)-N-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine 3c
Yield (2.4 g, 71 %), m.p. 256-258 °C. IR (KBr, ύ cm^{-1}): 3241, NH,3042 (CH aromatic),2851(CH aliphatic) , 1610(C=N), 1549(C=C),1493 (C-N).
^{1}H NMR (300 MHz, DMSO-d_6) δppm: 3.68(s,3H, OCH_3), 6.07 (s,2H, N$\underline{CH_2}$), 6.91-7.88 (m, 8H,Ar-H,), 9.59 (s, 1H,C2 imidazole), 10.31 (s, 1H, NH, D_2O-exchangeable); MS : *m/z(%)* 338 (0.27)M^++1, 337(0.76) M^+,336 (0.59). Microanalysis for $C_{17}H_{15}N_5OS$ (337.4) Calcd: % C, 60.52; H, 4.48; N, 20.76. Found: % C, 60.67; H, 4.54; N, 20.98.

General procedure for the synthesis of 1,3,4-oxadiazol-2-amine 4a-c
To a suspension of the thiosemicarbazides **2a-c** (10 mmol) in ethanol 100 ml, (4N, sodium hydroxide solution) 10 ml was added with shaking. A solution of iodine and potassium iodide was added dropwise with stirring till the color of iodine persisted. The mixture was stirred for 5 h, then left to cool. The separated solid was filtered off, washed with water and crystallized from EtOH to give **4a-c**.

5-((1H-benzo [d] imidazol-1-yl) methyl) -N-phenyl-1,3,4-oxadiazol-2-amine 4a
Yield (2.24 g, 77 %), m.p. 245-247 °C. IR (KBr, ύ cm^{-1}): 3251 NH,3077(CH aromatic) ,2932(CH aliphatic) , 1626(C=N), 1570(C=C),1495 (C-N) ;^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:5.81 (s,2H, N$\underline{CH_2}$), 6.94-7.71 (m, 9H,Ar-H,), 8.41 (s, 1H,C2 imidazole), 10.46 (s, 1H, NH, D_2O-exchangeable); MS: *m/z*(%) 292 (38.5)M^++ 1 , 291 (50.8)M^+ .Microanalysis for $C_{16}H_{13}N_5O$ (291.3) Calcd: %C, 65.97; H, 4.50; N, 24.04. Found: %C, 66.13; H, 4.47; N, 24.17.

5-((1H-benzo[d]imidazol-1-yl) methyl)-N-(4-bromophenyl)-1,3,4-oxadiazol-2-amine 4b
Yield (2.81 g, 76 %), m.p. 118-120 °C. IR (KBr, ύ cm^{-1}): 3252, NH, 3050 (CH aromatic), 2975(CH aliphatic), 1632(C=N), 1571(C=C), 1491 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 5.81 (s,2H, N$\underline{CH_2}$), 7.24-7.70 (m, 8H,Ar-H,), 8.36 (s, 1H,C2 imidazole), 10.64 (s, 1H, NH, D_2O-exchangeable); MS :*m/z(%)* 370 (20.36)M^+. Microanalysis for $C_{16}H_{12}BrN_5O$ (370.2). Calcd.:% C, 51.91; H, 3.27; N, 18.92; . Found: % C, 52.08; H, 3.24; N, 19.07

5-((1H-benzo[d]imidazol-1-yl) methyl)-N-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine 4c
Yield (2.41 g, 75 %), m.p. 214-216 °C. IR ύ cm^{-1}): (KBr, 3264, NH, 3051(CH aromatic), 2947 (CH aliphatic), 1636(C=N), 1579(C=C), 1463 (C-N); ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm :3.70(s,3H, OCH_3), 5.78 (s,2H, N$\underline{CH_2}$), 6.87-7.70 (m, 8H,Ar-H,), 8.36 (s, 1H,C2 imidazole), 10.22 (s, 1H, NH, D_2O-exchangeable); MS :*m/z*(%) 321 (100)M^+, 320 (14.57).Microanalysis for $C_{17}H_{15}N_5O_2$ (321.3)Calcd : % C, 63.54; H, 4.71; N, 21.79 . Found : %C, 63.68 ; H, 4.78; N, 22.03.

General procedure for the synthesis of 1,2,4-triazole-3-thiol 5a-c
To a suspension of the thiosemicarbazides **2a-c** (10 mmol) in ethanol(15ml), 10ml of 2 N NaOH was added. The mixture was refluxed for 4 h, then left to cool. The reaction mixture was acidified with concentrated hydrochloric acid .The separated solid was filtered off, washed with water and crystallized from ethanol

5-((1H-benzo[d]imidazol-1-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol 5a
Yield (2.7 g, 89%), m.p. 243-245 °C. IR (KBr, ύ cm^{-1}): 3145 NH, 3051(CH aromatic) ,2935(CH aliphatic), 2746 (SH), 1614(C=N), 1579 (C=C), 1462(C-N).

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 5.48 (s, 2H, N$\underline{CH_2}$), 7.19-7.62 (m, 9H, Ar-H), 7.68 (s, 1H, C2 imidazole), 13.98 (s, 1H, NH ,D_2O-exchangeable).MS : *m/z(%)* 307 (100) M^+,306(67.5),118(76).Microanalysis for $C_{16}H_{13}N_5S$(307)
Calcd:%C ,62.52 ; H ,4.26 ;N ,22.78 . Found ; %C ,62.68 ; H,3.41; N ,22.94

5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-bromophenyl)-4H-1,2,4-triazole-3-thiol 5b
Yield (2.97 g, 77 %), m. p. 172-174 °C. (KBr, ύ cm^{-1}): 3439 NH, 3060(CH aromatic),2926(CH aliphatic), , 1633(C=N), 1484 (C=C) , 1426 (C-N).
^{1}H NMR (300 MHz, DMSO-d_6)δppm :5.48 (s,2H, N$\underline{CH_2}$), 7.18-7.71 (m, 8H,Ar-H,), 7.78 (s, 1H,C2 imidazole), 13.96 (s, 1H, NH, D_2O-exchangeable); MS : *m/z(%)* 387(97.25)M^++1, 386(38.34)M^+,385(93.02%).Microanalysis for $C_{16}H_{12}BrN_5S$ (386.3).Calcd : % C, 49.75; H, 3.13; N, 18.13. Found:% C, 49.83; H, 3.16; N, 18.20.

5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol 5c
Yield (2.80 g, 83 %), m.p. 261-263 °C. (KBr, ύ cm^{-1}): 3210 NH, 3057(CH aromatic) ,2995(CH aliphatic), 1607(C=N), 1552(C=C) , 1455 (C-N).^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.81(s,3H, OCH_3), 5.84 (s,2H, N$\underline{CH_2}$), 6.80-7.71 (m, 8H,Ar-H,), 8.76 (s, 1H,C2 imidazole), 13.98 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR(DMSO-d6) ,δppm 40.22, 55.31 , 110.69 , 113.87 , 119.19, 121.47 , 122.14 , 128.51 , 133.45, 143.07, 146.25 , 158.54 .
MS:*m/z*(%)338,(7.3)M^++1 ,337(7.87)M^+, 118(100).Microanalysis for $C_{17}H_{15}N_5OS$ (337.4) Calcd;% C, 60.52; H, 4.48; N, 20.76 . Found : % C, 60.64; H, 4.51; N, 20.91.

General procedure of Preparation of compounds 6-17.
To a solution of **5a-c** of (10 mmol) and 0.4 g NaOH (10 mmol) in a mixture of H_2O (25 ml) and ethanol (10 ml), the alkyl halide (methyl iodide and ethyl iodide) and aralkylhalide(2-chloro-N-phenylacetamide,and2-chloro-N-(4- methoxyphenyl)acetamide) (10 mmol) was added. The solution was stirred at 50-60 °C for 1-3 h. The resulting precipitate was filtered off and recrystallized from ethanol.

1-{(5-(methylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 6
Yield (2.66 g, 83 %), m.p. 154-156 °C. IR (KBr, ύ cm^{-1}): 3080 (CH aromatic) ,2950(CH aliphtic) ,1625(C=N), 1523(C=C) , 1496(C-N). ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm : 2.54 (s, 3H, SCH_3), 5.58 (s, 2H, N$\underline{CH_2}$), 7.16-7.57 (m, 9H, Ar-H), 7.59 (s, 1H, C2 imidazole).Microanalysis for $C_{17}H_{15}N_5S$ (321.4) Calcd:% C, 63.53; H, 4.70; N, 21.79 . Found: %C, 63.59; H, 4.67; N, 21.93 .

1-{(5-(ethylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 7
Yield (2.85 g, 85 %), m.p. 85-87 °C. IR (KBr, ύ cm^{-1}): 3095 (CH aromatic) ,2976 (CH aliphatic) ,1616(C=N), 1521 (C=C) , 1460 (C-N). ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 1.21-1.24 (t, 3H, $\underline{CH_3}$), 3.02-3.06 (q, 2H, $\underline{CH_2}$), 5.59 (s, 2H, N$\underline{CH_2}$), 7.16-7.57 (m, 9H, Ar-H), 7.61 (s, 1H,C2 imidazole); MS : *m/z* (%)336 (18) M^++1,217(78.8), 77(100).Microanalysis for $C_{18}H_{17}N_5S$ (335.4) Calcd: % C, 64.45; H, 5.11; N, 20.88 . Found : % C, 64.58; H, 5.14; N, 21.02.

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 8
Yield (3.21 g, 73 %), m.p. 191-193 °C. IR (KBr, ύ cm^{-1}): 3254 (NH),3037(CH aromatic) ,2950 (CH aliphatic) ,1682(C=O) ,1609(C=N), 1555(C=C) , 1494(C-N).^{1}HNMR (300 MHz, DMSO-d_6) δppm: 4.12 (s, 2H, S$\underline{CH_2}$), 5.60 (s, 2H, N$\underline{CH_2}$), 7.09-7.59 (m, 14H, Ar-H), 7.62 (s,1H,C2imidazole),10.37(s,1H,NH,D_2O-exchangeable).^{13}CNMR(DMSO-d6),δppm:36.90,110.42,
119.05,119.35,120.73,121.69,122.464,128.694, 129.92, 138.80, 143.07 , 143.67 , 151.16, 151.38, 165.34.
MS: *m/z(%)* 442(1.57), M^++2, 440 (6.61), M^+ , 131(76), 91(100%). Microanalysis for $C_{24}H_{20}N_6OS$ (440.5) Calcd.:% C, 65.44; H, 4.58; N, 19.08. Found: %C, 65.70; H, 4.63; N, 19.20.

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)-N-(4-methoxyphenyl)Acetamide 9
Yield (3.14 g, 67 %), m.p. 193-195 °C. IR (KBr, ύ cm^{-1}): 3249 (NH),3057(CH aromatic) ,2939(CH aliphtic) ,1675(C=O) ,1612(C=N), 1553(C=C) , 1449(C-N).
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm :3.70 (s, 3H, OCH_3), 4.09 (s, 2H, S$\underline{CH_2}$), 5.60 (s, 2H, N$\underline{CH_2}$), 6.85-7.55 (m, 13H, Ar-H), 7.62 (s, 1H,C2 imidazole), 10.17 (s, 1H, NH, D_2O-exchangeable); MS: *m/z* (%) 472 (3.21) M^++2, 470(21.03)M^+, 348(100), 131(80.97). 91(63.29). Microanalysis for $C_{24}H_{20}N_6OS$ (470.5) Calcd.: %C, 63.81; H, 4.70; N, 17.86 . Found: %C, 63.90; H, 4.77; N, 17.98.

1-{(4-(4-bromophenyl)-5-(methylthio)-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 10
Yield (2.92 g,73%), m.p. 112-114 °C. IR (KBr, ύ cm^{-1}): 3053(CH aromatic),2971 (CH aliphtic) ,1617(C=N), 1489(C=C) . ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 2.54 (s, 3H, SCH_3), 5.59 (s, 2H, N$\underline{CH_2}$), 7.16-7.67 (m, 8H, Ar-H), 7.71 (s, 1H,C2 imidazole); MS : *m/z*(%) 402(7.26) M^++2, ,400(14.42) M^+ ,284 (38.35), 203(54.06), 131(50.32) .Microanalysis for $C_{17}H_{14}BrN_5S$ (400.3).Calcd: %C,51.01; H, 3.53; N, 17.50.Found: %C, C,51.09; H, 3.59; N, 17.67 .

1-{(4-(4-bromophenyl)-5-(ethylthio)-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 11
Yield (3.10 g, 74 %), m.p. 149-151 °C. IR (KBr, ύ cm^{-1}): 3055(CH aromatic), 2967(CH aliphatic) ,1615(C=N), 1487(C=C).
^{1}H NMR (300 MHz, DMSO-d_6): δppm 1.22-1.27 (t, 3H, $\underline{CH_3}$), 3.04-3..09 (q, 2H, $\underline{CH_2}$), 5.59 (s, 2H, N$\underline{CH_2}$), 7.16-7.70 (m, 8H, Ar-H), 7.72 (s, 1H,C2 imidazole); MS : *m/z*(%) 416(18.02)M^++2, 414(34.53) M^+,415(69.84),298(100).Microanalysis for $C_{18}H_{16}BrN_5S$ (414.3). Calcd.: %C, 52.18; H, 3.89; N, 16.90 .Found: %C, 52.26; H, 3.93; N, 17.03.

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-bromophenyl)-4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 12
Yield (3.25 g, 63 %), m.p. 164-166 °C. IR (KBr, ύ cm^{-1}): 3255 (NH),3060 (CH aromatic) ,2927 (CH aliphatic) ,1678 (C=O) 1604(C=N), 1552(C=C), 1491(C-N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm :4.10 (s, 2H, S$\underline{CH_2}$), 5.61 (s, 2H, N$\underline{CH_2}$), 7.08-7.67 (m, 13H, Ar-H), 7.73 (s, 1H,C2imidazole), 10.25 (s, 1H, NH, D_2O-exchangeable); MS: *m/z*(%) 520 (60.64)M^++ 1, 519(91.49) M^+. Microanalysis for $C_{24}H_{19}BrN_6OS$ (519.4). Calcd: %C, 55.50; H, 3.69; N, 16.27. Found: %C, 55.62; H, 3.72; N, 16.27 .

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-bromophenyl)-4H-1,2,4-triazol-3-ylthio)-N-(4-methoxyphenyl)Acetamide 13
Yield (4.5 g, 82 %), m.p. 152-154 °C. IR (KBr, ύ cm^{-1}): 3248 (NH),3050(CH aromatic) ,2928(CH aliphtic) ,1674(C=O) ,1608(C=N), 1558(C=C) , 1447(C-N) . ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.68 (s, 3H, OCH_3), 4.06 (s, 2H, S$\underline{CH_2}$), 5.61 (s, 2H, N$\underline{CH_2}$), 6.82-7.70 (m,

12H, Ar-H), 8.05 (s, 1H,C2 imidazole), 10.15 (s, 1H, NH, D_2O-exchangeable); MS :*m/z* (%) 551(10.16) M^++2, , 549 (39.11) M^+, 543 (46.93).Microanalysis for $C_{25}H_{21}BrN_6O_2S$ (549.4). Calcd: %C, 54.65; H, 3.85; N, 15.30. Found: %C, 54.71; H, 3.84; N, 15.47 .

1-{(4-(4-methoxyphenyl)-5-(methylthio)-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 14
Yield (2.31 g, 66 %), m.p. 130-132 °C. I (KBr, ύ cm^{-1}): 3093 (CH aromatic),2998 (CH aliphatic) ,1608(C=N), 1509(C=C) , 1454(C-N). ^{1}H NMR (300 MHz, DMSO-d_6)δ ppm: 2.13 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 5.54 (s, 2H, $N\underline{CH_2}$), 7.01-7.61 (m, 8H, Ar-H), 7.65 (s, 1H,C2 imidazole); MS :*m/z* (%) 352(17.34) M^++ 1, 351 (13.06) M^+, 80 (100).Microanalysis for $C_{18}H_{17}N_5OS$ (351.4) .Calcd.: %C, 61.52; H, 4.88; N, 19.93. Found: %C, 61.74; H, 4.93; N, 20.06 .

1-{(5-(ethylthio)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-yl)methyl}-1H-benzo[d]imidazole 15
Yield (2.71 g, 74 %), m.p. 150-152 °C. IR (KBr, ύ cm^{-1}): 3064(CH aromatic), 2968 (CH aliphatic) ,1612(C=N), 1511(C=C) , 1452(C-N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm :1.22-1.27 (t, 3H, $\underline{CH_3}$), 3.01-3.09 (q, 2H, $\underline{CH_2}$), 3.81 (s, 3H, OCH_3), 5.54 (s, 2H, $N\underline{CH_2}$), 7.00-7.61 (m, 8H, Ar-H), 7.65 (s, 1H,C2 imidazole); MS: *m*/z(%) 366(7.41) M^++1,365(66.67) M^+ , 325(93.83) ,299 (90.12),109(100). Microanalysisfor $C_{19}H_{19}N_5OS$ (365.5).Calcd.:% C, 62.44; H, 5.24; N, 19.16. Found: %C, 62.58; H, 5.28; N, 19.28 .

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio)-N-phenylacetamide 16
Yield (3.29 g,70 %), m.p.107-109°C.IR (KBr, ύ cm^{-1}): 3260 (NH),3058(CH aromatic) ,2970(CH aliphatic) ,1682(C=O) , 1604(C=N), 1551(C=C), 1448(C-N).
^{1}H NMR (300 MHz, DMSO-d_6)δ ppm :3.81 (s, 3H, OCH_3), 4.10 (s, 2H, $S\underline{CH_2}$), 5.56 (s, 2H, $N\underline{CH_2}$), 7.01-7.61 (m, 13H, Ar-H), 7.68 (s, 1H,C2 imidazole), 10.28 (s, 1H, NH, D_2O-exchangeable); MS : *m/z* (%) 471(44.63) M^++1,470 (15.70) M^+, 438(61.16), 382(69.42) ,327(65.29). Microanalysis for $C_{25}H_{22}N_6O_2S$ (470.5)..Calcd.: %C, 63.81; H, 4.71; N, 17.86. Found: %C, 63.89; H, 4.69; N, 18.02.

2-(5-((1H-benzo[d]imidazol-1-yl)methyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio)-N-(4-methoxyphenyl)Acetamide 17
Yield (3.9 g, 78 %), m.p. 110-112 °C. IR (KBr, ύ cm^{-1}): 3255 (NH),3055(CH aromatic) ,2930(CH aliphatic) ,1673(C=O),1609(C=N), 1554 (C=C), 1452(C-N).
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm :3.67-3.71 (s, 6H, $2OCH_3$), 4.07 (s, 2H, $S\underline{CH_2}$), 5.56 (s, 2H, $N\underline{CH_2}$), 6.85-7.61 (m, 12H, Ar-H), 7.67 (s, 1H,C2 imidazole), 10.15 (s, 1H, NH, D_2O-exchangeable);
MS:*m/z*(%) 501(42.3) M^++1, 500(53.62)M^+ ,339(66.67) .Microanalysis for $C_{26}H_{24}N_6O_3S$ (500.6) .Calcd.: %C, 62.38; H, 4.83; N, 16.79. Found: %C, 62.44; H, 4.90; N, 16.93 .

2.2 Biological Screening
2.2.1 Antimicrobial activity test

All the newly synthesized compounds were evaluated for *in vitro* antimicrobial activity against Gram positive bacteria such as Staphylococcus aureues and Staphylococcus epidermidis,Gram negative bacteria as Pseudomonas aeruginosa and Escherichia coli and Fungi such as Candida albicans at concentration 50 mg/ml by agar well diffusion method[25,26] as modified from NCCLS. Mueller-Hinton agar plates were surface-inoculated with the tested strains suspensions adjusted to match 0.5 McFarland standard and the inocula were spread over the surfaces of the plates using sterile cotton swabs. After drying of the plates, cups (10 mm diameter) were punched in the agar and 100 µl of the samples in DMF or the antimicrobial agents were added into the wells. The plates were incubated at 37ºC for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The test was repeated three times and the mean inhibition zones were calculated. A total of five standard microbial strains were used in this study obtained from the Egyptian Pharmaceutical Industries Company (EPICO).

DMF used as solvent control, nutrient agar was employed as culture media. The activity was compared with Cefotaxim as positive control for bacteria and Nystatin for fungi .The results were represented in Table 1

2.2.3 The anticancer activity (*In vitro* Antitumor Activity)[27-29]

The cell lines were grown as monolayers in growth medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate (Falcon, NJ, USA) incubated for 24h at 37ºC in a humidified incubator with 5%CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of tested compound in fresh maintenance medium and incubated at 37ºC. A control of untreated cells was made in the absence of the tested compound. Three wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested compounds and ODc is the mean optical density of untreated cells.

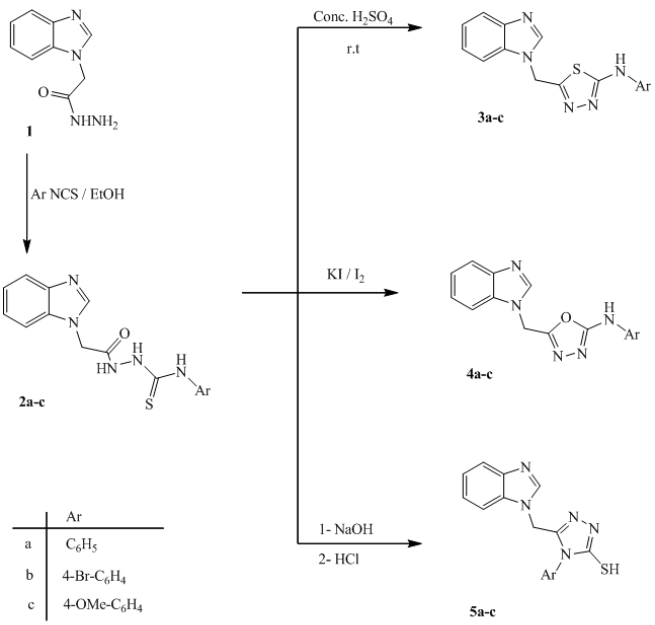
The cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO2 and were subcultured two to three times a week.

3. Results and discussion

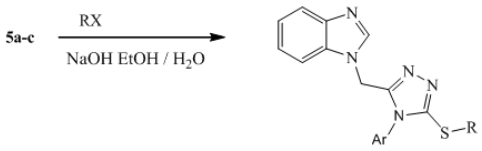
3.1 Chemistry

The synthesis of new 1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles was performed in several steps shown in scheme **1**. The required 2-(1H-benzo [d] imidazol-1-yl) acetohydrazide **1** was prepared according to reported methods[23,24]. The treatment of acetohydrazide **1** with arylisothiocyanate in ethanol at refluxed temperature gives the corresponding thiosemicarbazide derivatives **2a-c** the key intermediate for the synthesis of 1,3,4-thiadiazoles, oxadiazoles and 1,2,4-triazoles.

The treatment of thiosemicarbazide derivatives with conc. sulfuric acid undergo intramolecular dehydration forming 1,3,4-thiadiazole derivatives **3a-c**. While 1,3,4-oxadiazole derivatives **4a-c** were synthesized by treatment of **2a-c** with KI/I_2 as an oxidizing agent. Moreover the reaction of the thiosemicarbazide derivatives with sodium hydroxide in ethanol under reflux followed by acidification with hydrochloric acid give the corresponding **5a-c**. Their structures were established by ^{1}H NMR spectra shown a single signal at 13.98 ppm. due to the resonance of NH proton. In the IR spectra of compounds **5a-c** the absence of absorptions due carbonyl group. Furthermore the treatment of compounds **5a-c** with methyl iodide, ethyl iodide, 2-chloro-N-phenyl- acetamide, and 2-chloro-N-(4-methoxyphenyl) acetamide respectively in the presence of alkaline medium give S-substituted 1,2,4-triazole **6-17** scheme 2.



Scheme 1



	Ar	R	X
6	C_6H_5	$-CH_3$	I
7	C_6H_5	$-CH_2CH_3$	I
8	C_6H_5	$C_6H_5NHCOCH_2-$	Cl
9	C_6H_5	4-MeO-$C_6H_4NHCOCH_2-$	Cl
10	4-Br-C_6H_4	$-CH_3$	I
11	4-Br-C_6H_4	$-CH_2CH_3$	I
12	4-Br-C_6H_4	$C_6H_5NHCOCH_2-$	Cl
13	4-Br-C_6H_4	4-MeO-$C_6H_4NHCOCH_2-$	Cl
14	4-MeO-C_6H_4	$-CH_3$	I
15	4-MeO-C_6H_4	$-CH_2CH_3$	I
16	4-MeO-C_6H_4	$C_6H_5NHCOCH_2-$	Cl
17	4-MeO-C_6H_4	4-MeO-$C_6H_4NHCOCH_2-$	Cl

Scheme 2

3.2 Biological Screening

3.2.1 Antimicrobial activity evaluation

The new synthesized compounds have been investigated against microorganisms representing Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*), Gram-negative (*Escherichia coli* and *Pseudomonas aerugnosa)* and fungi (*Candida albicans*). The results obtained in table **1** showed that all the tested compounds show high inhibitory activity against fungi. Moreover compounds **3b, 4b, c, 5b, c, 7** show high inhibitory activity against gram- positive and gram negative bacteria. Furthermore, compounds **2b, c, 6** have high inhibitory activity against gram positive, but have moderate inhibitory activity against gram- negative bacteria. In addition compounds **3a, c, 6, 12, 14, 17** have moderate inhibitory activity against gram-positive bacteria but high inhibitory activity against gram negative bacteria .Compounds **4a, 13**have moderate inhibitory activity against gram positive and gram negative bacteria. Finally compound **15** shows weak activity against gram positive, but has moderate activity against gram negative bacteria but while compounds **8, 9, 11** have weak inhibitory activity against gram positive and gram negative bacteria.

Table 1: Diameter (mm) of inhibition zones against the corresponding standard strains of different microorganisms

Tested Samples	Gm +ve bacteria		Gm -ve bacteria		Fungi
	Staphylococcus aureus **ATCC 6538**	***Staphylococcus epidermidis*** **ATCC 12228**	***Pseudomonas aeruginosa*** **ATCC 9027**	***Escherichia coli*** **ATCC 10536**	***Candida albicans*** **ATCC 10231**
2b	25	24	24	25	27
2c	25	24	22	24	30
3a	22	21	25	26	30
3b	27	26	28	28	28
3c	21	20	28	27	26
4a	24	22	22	24	26
4b	27	25	26	27	30
4c	28	26	26	27	30
5b	26	24	25	27	30
5c	25	24	25	26	30
6	24	23	25	26	28
7	27	25	26	28	31
8	18	17	21	22	30
9	19	19	20	21	23
11	20	18	17	19	28
12	24	23	26	27	30
13	23	22	23	23	28
14	24	23	25	27	29
15	20	19	25	25	25
16	25	24	23	24	27
17	23	22	25	27	31
Cefotaxime	30	28	30	35	-
Nystatin	-	-	-	-	24
DMF	-	-	-	-	-

3.2.2 The anticancer activity (*In vitro* Antitumor Activity)

Some of the newly synthesized compounds have been evaluated for their Potential cytotoxicity against two carcinoma cell lines, namely MCF-7 and HCT-116 cells.

The results obtained in table **2** showed that compound **13** possess the significant effect against both breast cancer cell line (MCF7) and Colon cancer cell line((HCT-116), compound **5b** is active against breast cancer cell line but inactive against Colon cancer cell line , compounds **4b,c** possess the significant activity against Colon cancer cell line but less active against breast cancer cell line, compound **5c** is less active against the two cell lines , compound **9** is less active against colon cancer cell line but inactive against breast cancer cell line, in reverse compound **15** is less active against breast cancer cell line but inactive against colon cancer cell line .Finally compounds **3b,c, 17** are inactive against both carcinoma cell lines

Table 2: The IC50 (µg/mL) of some of the selected new compounds against Breast cancer cell line (MCF7) and Colon cancer cell line HCT-116

Tested Compounds	Breast cancer cell line MCF-7(µg)	Colon cancer cell line HCT-116(µg)
3b	> 50	> 50
3c	> 50	> 50
4b	47	24.8
4c	45	32
5b	34.1	> 50
5c	47.4	48
9	> 50	47
13	21.9	22.5
15	47.3	> 50
17	> 50	> 50
Doxorubicin (Standard)	0.426	0.469

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