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SYNTHESIS AND IN VITRO CYTOTOXIC EVALUATION OF NOVEL TRIAZOLE-BENZIMIDAZOLE EMBODIED PYRAZOLE DERIVATIVES AGAINST BREAST CANCER

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ABSTRACT

A new series of novel triazole – benzimidazole containing pyrazole derivatives (10a-m) have been designed, synthesized, and characterized by their spectral data. These newly synthesized compounds (10a-m) were tested for their *in vitro* cytotoxic activity against MCF-7 human breast cancer cell lines. This shows that compound 10b proved to be the most prominent cytotoxic agent against MCF-7 cell lines.

Keywords: Triazole-benzimidazopyrazoles, VilsmeierHaack Reaction, Cytotoxic Assay, Breast Cancer.

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INTRODUCTION

Design and synthesis of new bioactive molecules by molecular hybridization by combining individual pharmacophore moieties into a unique pharmacophore has gained considerable interest in medicinal chemistry. These hybridized molecules showed improved efficacy, selectivity, affinity, and lowered side effects than parent drugs.[1-4] Heterocyclic compounds are well known for their biological activity and play a significant role in drug design and discovery. Synthesis of novel heterocyclic compounds by molecular hybridization approach in which combining the lead scaffold of two or more biologically active heterocycles into a single moiety is challenging due to the existence of various functional groups with different reactivities.[5,6] Continuing our investigation towards developing new heterocyclic compounds with biological significance, we report the synthesis of pyrazole containing benzimidazole-triazoles and their evaluation as anti-cancer agents. Pyrazole-based heterocycles have attracted much attention in recent years because of their wide range of biological activities, such as anti-inflammatory[7], anti-microbial[8], anti-oxidants[9], anti-depressant[10], anti-influenza[11], and anti-cancer[12] activities. Among these 1,3-diphenyl pyrazoles have been reported as promising medicinally active substances.[13-16] Besides, benzimidazole derivatives also have occupied a prominent position in the field of medicinal chemistry and the pharmaceutical industry because of their high ability to interact with biomolecules of the living system. Specifically, this nucleus is a constituent of vitamin B_{12}[17], and exhibits anti-microbial[18], anti-cancer[19], anti-histaminic[20], anti-proliferative[21], anti-coagulant[22], anti-hepatitis[23], analgesic[24], and anti-inflammatory[25] activities.

EXPERIMENTAL

Synthesis of Phenyl Hydrazone (3)

Condensation of 4-hydroxy acetophenone [14.7 mmol] with phenylhydrazine [17.6 mmol] in the presence of glacial acetic acid [3ml], and ethanol as a solvent medium under reflux conditions for 3 hours results in the disappearance of starting materials as monitored by the TLC. Cooling the reaction mass to room temperature followed by slowly pouring it onto crushed ice with stirring gives precipitate. Filtration of the precipitate followed by recrystallization from ethanol produces hydrazone (3) as a white solid, yielding 93%, m-p: 146-148°C.

Synthesis of 3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (4)

To an oven-dried R.B flask was charged with hydrazone 3 [4.4 mmol] in dry DMF [10ml] to this $POCl_3$ [5ml] was added dropwise over a period of 5 minutes. Stirring was continued at 100ºC for 12 hours, followed by distillation of $POCl_3$ under reduced pressure, the addition of this reaction mass slowly to crushed ice with stirring gives the solid material. Filtration followed by drying the solid material gives the pyrazole aldehyde a Light yellow solid, yield: 85%, m.f: $C_{16}H_{12}N_2O_2$, m.p: 148-150ºC, (IR neat, Vmax, cm^{-1}): 3558(O-H), 1730(C=O). ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 9.53 (s, 1H), 7.92 (s, 1H), 7.36-6.80(m,9H), 5.23(s,1H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 191.5, 157.5, 152.3, 140.0, 130.3, 129.8, 128.6, 128.4, 128.0, 125.7, 121.2, 121.0, 138.2, 137.1, 107.5. Mass: ESI-MS: m/z = 265$[M+H]^+$.

Synthesis of 1-phenyl-3-(4-(prop-2-ynyloxy) phenyl)-1H-pyrazole-4-carbaldehyde (6)

To a solution of compound 4 [3.7mmol] and K_2CO_3 [12mmol] in acetone was added propargyl bromide [5.5mmol], the reaction mass was stirred under reflux condition for a period of 5 hours. Coolng of reaction mass to RT and passing it through a cotton plug to remove excess K_2CO_3. Distil off the acetone under reduced pressure gives compound **6** as brown solid, yield: 87%, m.f: $C_{19}H_{14}N_2O_2$, m.p: 124-126 ºC. (IR neat, v_{max}, cm^{-1}):2256(C≡C), 1742 (C=O).^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 9.50 (s, 1H), 7.84 (s, 1H), 7.37-6.83(m,9H), 4.50(d,2H), 2.55(t, 1H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 190.9, 161.0, 159.9, 140.5, 130.5, 130.3, 129.1, 128.5, 126.5, 125.4, 120.8, 120.5, 108.5, 79.9, 77.5, 59.0,. Mass: ESI-MS: m/z = 303 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 8a-c

To a solution of alkyne compound 6 and phenyl azide (7a-c) in acetonitrile was added copper turnings [10mol], $CuSO_4$ solution [0.5mL, 1M] and sodium ascorbate [10mol]. The reaction mixture was refluxed at 65ºC for 4-6 hours. After completion of the reaction [monitored by TLC], the reaction mixture was filtered through the celite bed to remove the undissolved materials. Later, the solvent was removed on a rotary evaporator under reduced pressure. Extraction of the reaction mixture using ethyl acetate and water followed by purification with column chromatography over 60-120 silica gel gives the traizole derivatives (8a-c) in 60-75% yields. Spectral data of compound (8a) Brown solid, yield: 72%, m.f.:$C_{25}H_{19}N_5O_2$, m.p.: 130-132 ºC. (IR neat, v_{max}, cm^{-1}): 1730 (C=O).^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 9.60 (s, 1H), 7.85 (s,1H),7.66 (s,1H),7.38-6.82(m,14H), 5.54 (d,2H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 191.0, 160.7, 150.4, 146.0, 140.1, 131.5, 129.9, 129.5, 128.9, 128.5, 128.1, 127.8126.8, 125.7, 121.5, 120.8, 115.6, 114.9, 108.7, 68.7 .Mass: ESI-MS: m/z = 422 $[M+H]^+$.

General Procedure for the Synthesis of Compounds (10a-m)

To a solution of aldehyde compounds (8a-c) and orthophenylene diamine derivatives (9a-d) in $CHCl_3$ was added a catalytic amount [0.01mol] of acetic acid, and the reaction was continued at room temperature overnight. After completion of the reaction, the reaction mixture was diluted with 15ml of $CHCl_3$ and 20 ml of water. The separated and collected chloroform layer is washed with sodium bicarbonate solution to remove the traces of acetic acid. Later the combined organic layers are extracted with brine solution. Drying the organic layers over anhydrous Na_2SO_4 followed by evaporation under reduced pressure gave the crude reaction mass. Purification of the crude compounds on silica gel column chromatography (60-120 mesh) using EtOAc/Hexane as eluents gave compounds 10a-m with 74-89% yield.

2-(3-(4-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole 10(a)

White solid, yield: 74%, m.f.: $C_{31}H_{21}Cl_2N_7O$, m.p.:156-158ºC. (IR neat, v_{max}, cm^{-1}): 2980, 2833, 2790, 1710, 1620, 1550, 1470, 1250, 1120, 985. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.56 (s,1H), 8.22 (s,1H),7.91 (s,2H),7.71-7.55(m,8H),7.31 (d, J=16.2 Hz, 6H), 6.73-6.68 (m,1H), 4.96 (d,2H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 152.6, 149.4, 144.0, 142.3, 140.8, 138.2, 137.1, 136.3, 134.4, 134.3, 133.4, 131.8, 127.7, 126.8, 125.7, 124.4, 123.6, 119.5, 66.3.Mass: ESI-MS: m/z = 579 $[M+H]^+$.

5-chloro-2-(3-(4-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole(10b)

White solid, yield: 79%, m.f.: $C_{31}H_{20}Cl_3N_7O$, m.p.:160-162ºC. (IR neat, v_{max}, cm^{-1}): 2890, 2820, 2750, 1720, 1610, 1540, 1500, 1250, 1120, 996. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.53 (s,1H), 8.02

(s,1H),7.86(s,2H), 7.74 (d, J=8.5 Hz, 2H), 7.59 (dd, J=5.9, 2.5 Hz, 5H), 7.47 (s,2H), 7.32 (t, J=7.0 Hz,2H), 7.21 (s,2H), 7.01 (s,2H),5.23 (s,2H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 155.2, 152.6, 144.0, 142.3, 140.8, 139.7, 138.2, 137.1, 136.3, 134.3, 131.8, 127.7, 126.8, 125.7, 124.4, 123.6, 119.5, 66.3. Mass: ESI-MS: m/z = 612 $[M+H]^+$.

2-(3-(4-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-methyl-1H-benzo[d]imidazole (10c)

White solid, yield: 77%, m.f.: $C_{32}H_{23}Cl_2N_7O$, m.p.:154-156 ºC.(IR neat, ν_{max}, cm^{-1}): 2980, 2910, 2860, 1730, 1620, 1580, 1520, 1250, 1140, 985. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.55 (s,1H), 8.08 (s,1H),7.88(d, J=12.5 Hz, 3H), 7.74 (d, J=8.5 Hz, 1H), 7.62-7.54(m,5H), 7.47(s,2H), 7.41-7.28(m,2H), 7.21(s,1H), 7.01 (s,2H), 5.23 (s,2H), 2.08 (s,3H),. ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 155.8, 152.2, 147.5, 145.8, 138.9, 135.8, 134.0, 133.0, 131.5, 129.6, 129.4, 127.1, 122.2, 119.5, 118.9, 114.7, 61.4, 21.5. Mass: ESI-MS: m/z = 592 $[M+H]^+$.

2-(3-(4-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (10d)

White solid, yield: 80%, m.f.: $C_{33}H_{27}N_7O$, m.p.:158-160ºC. (IR neat, ν_{max}, cm^{-1}): 2945, 2913, 2820, 1720, 1640, 1540, 1500, 1250, 1115, 1010. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.48 (s,1H), 7.98(d, J=6.7 Hz, 2H), 7.55(s,1H), 7.47-7.40(m, 9H), 7.34-7.30(m,2H),7.14 (d, J=7.7 Hz, 1H), 6.92 (d, J=6.6 Hz, 2H), 5.58 (s,1H), 5.15 (s, 2H), 2.37 (s,3H), 2.34 (s,3H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 158.6, 158.3, 151.1, 150.3, 144.5, 139.3, 136.9, 129.7, 129.5, 129.2, 128.9, 127.2, 126.5, 125.3, 123.6, 121.0, 120.6, 119.2, 118.7, 115.7, 114.9, 110.9, 63.0, 22.6, 17.6. Mass: ESI-MS: m/z = 538 $[M+H]^+$.

5-chloro-2-(3-(4-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole(10e)

White solid, yield: 78%, m.f.: $C_{33}H_{26}ClN_7O$, m.p.:152-154ºC. (IR neat, ν_{max}, cm^{-1}): 2969, 2924, 2852, 1695, 1595, 1535, 1421, 1219, 1080, 995. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm: 8.54 (s,1H), 8.07(d, J=8.1 Hz, 2H), 7.82 (dd, J=10.6, 8.0 Hz, 2H), 7.54 (d, J=7.6 Hz, 2H), 7.43 (dd, J=8.2, 3.8 Hz, 3H),7.35(d, J=14.1 Hz, 3H), 7.27-7.24(m, 3H), 7.15 (s, 2H), 6.82 (s, 2H), 5.37 (s,2H), 5.09 (s, 1H), 2.33 (s,6H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 158.6, 154.2, 150.6, 144.1, 138.9, 138.4, 137.8, 134.7, 131.4, 130.6, 130.3, 129.6, 129.4, 127.9, 127.1, 123.5, 122.3, 121.7, 121.2, 119.7, 118.9,117.8, 115.0, 61.7, 19.9, 19.4. Mass: ESI-MS: m/z = 572 $[M+H]^+$.

2-(3-(4-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-methyl-1H-benzo[d]imidazole (10f)

White solid, yield: 81%, m.f.: $C_{34}H_{29}N_7O$, m.p.:150-152ºC. (IR neat, ν_{max}, cm^{-1}): 2995, 2910, 2840, 1650, 1575, 1520, 1480, 1220, 1115, 991. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.09 (s,1H), 7.78(d, J=6.7 Hz, 2H), 7.56(s,1H), 7.48-7.40(m,8H), 7.34-7.30(m,2H), 7.14 (d, J=7.9 Hz, 1H), 6.92 (d, J=6.6 Hz, 2H),5.58 (s,1H), 5.16 (s, 2H), 2.42 (s,3H), 2.37 (s,3H) , 2.34 (s,3H) . ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 158.7, 150.6, 144.1, 139.0, 138.4, 137.8, 134.7,133.7, 130.7, 130.1, 129.8, 129.5, 127.2, 125.1, 124.7, 121.7, 121.1, 119.0, 117.8, 115.1, 61.8, 21.6, 19.9, 19.4. Mass: ESI-MS: m/z = 552 $[M+H]^+$.

2-(3-(4-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (10g)

White solid, yield: 80%, m.f.: $C_{34}H_{26}F_3N_7O$, m.p.:164-166ºC. (IR neat, ν_{max}, cm^{-1}): 2965, 2923, 2862, 1651, 1554, 1500, 1475, 1250, 1191, 1012. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.40 (s,1H): 8.10 (s,1H),7.81(dd, J=4.5,2.1 Hz, 2H), 7.66(s,1H), 7.55-7.43(m,7H), 7.41-7.33(m,3H), 7.04-6.95(m,2H),5.31 (s,1H), 5.16 (s, 2H), 2.35 (s,3H), 2.34 (s,3H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 159.0,155.6,147.6,138.5,136.5,135.9,130.7,129.6,127.7,125.9,124.1,121.7,119.2,117.8,115.5,115.0,112.1, 61.7,19.9,19.4. Mass: ESI-MS: m/z = 606 $[M+H]^+$.

2-(1-phenyl-3-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl)-1H-pyrazol-4-yl)-1H-benzo[d]imidazole(10h)

White solid, yield: 81%, m.f.: $C_{31}H_{23}N_7O$, m.p.:148-150ºC. (IR neat, ν_{max}, cm^{-1}): 2953, 2930, 2852, 1698, 1597, 1531, 1481, 1225, 1099, 1001. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.10(s,1H), 7.89(s,1H),

7.63(d, J=7.8 Hz, 1H), 7.45 (d, J=7.9 Hz, 1H), 7.30-7.10(m,5H), 7.00-6.85(m,5H), 6.93(d, J=7.4 Hz, 2H), 6.73(d,J=7.5 Hz, 2H), 5.22(s,2H). ^{13}C NMR(100 MHZ, $CDCl_3$,δ ppm): 156.3, 144.3, 139.8, 137.1, 132.8,131.2, 130.3, 129.3, 128.3, 127.7, 127.0, 126.9, 123.2, 121.6, 120.7, 114.3, 62.6, Mass: ESI-MS: m/z = 510 $[M+H]^+$.

2-(3-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl)-1H-pyrazol-4-yl)-5-(trifluoromethyl)-1H-benzo[d]imidazole(10i)

White solid, yield: 83%, m.f.: $C_{32}H_{22}F_3N_7O$, m.p.:140-145ºC. (IR neat, ν_{max}, cm^{-1}): 2980, 2873, 2845, 1600, 1555, 1540, 1500, 1319, 1100, 1000. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm) : 8.34(s,1H), 8.08(s,1H), 7.95(s,1H),7.85(d, J=7.9 Hz, 1H), 7.68(d, J=7.5 Hz, 3H), 7.38(dd, J=7.7,3.0 Hz, 7H), 7.28-7.24(m,3H), 7.14-7.11(m,2H), 6.93(d, J=8.7 Hz, 2H), 5.22(s, 2H). ^{13}C NMR(100 MHZ, $CDCl_3$, δ ppm): 160.37, 145.9, 139.8, 139.7, 132.8, 131.5, 131.2, 129.9, 128.8, 127.5, 127.3, 126.9, 125.2, 121.7, 121.7, 115.4, 68.3. Mass: ESI-MS: m/z = 578 $[M+H]^+$.

2-(3-(4-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-(trifluoromethyl)-1H-benzo[d]imidazole (10j)

White solid, yield: 85%, m.f.: $C_{32}H_{20}Cl_2F_3N_7O$, m.p.:168-173ºC. (IR neat, ν_{max}, cm^{-1}): 2987, 2900, 2878, 1700, 1630, 1554, 1510, 1250, 1100. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 7.89 (s,1H), 7.70-7.54(m,3H), 7.40(d, J=6.7 Hz, 2H), 7.30-6.89(m,10H), 6.55 (d, J=6.5 Hz, H), 5.23(s,2H).13C NMR (100 MHZ,$CDCl_3$,δ,ppm):
158.5,153.6,150.5,148.5,146.1,142.3,140.5,139.5,138.1,137.8,135.5,133.2,130.7,129.7,127.8,125.5,125.0, 122.8,120.3,68.5 Mass: ESI-MS: m/z = 646 $[M+H]^+$.

2-(3-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-methoy-1H-benzo[d]imidazole(10k)

White solid, yield: 81%, m.f:$C_{32}H_{25}N_7O$, m.p.:145-148ºC. (IR neat, ν_{max}, cm^{-1}): 2940, 2910, 2654, 1700, 1620, 1540, 1475, 1250, 1111. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.15 (s,1H), 7.98(d, J=6.2 Hz, 2H), 7.56(s,1H), 7.50-7.30(m,11H), 7.14 (d, J=7.9 Hz, 2H), 6.92 (d, J=6.6 Hz, 2H), 5.58 (s,1H), 5.16(s,2H),
3.80(s,3H).^{13}CNMR(100MHZ,$CDCl_3$,δppm):160.5,158.6,148.3,138.5,138.3,135.7,135.2,133.7,132.8,130.5,129.3,124.5,120.2,118.5,116.7,115.5,63.0,54.2 Mass: ESI-MS: m/z = 540 $[M+H]^+$.

2-(3-(4-((1-(2,4-dichlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-methoxy-1H-benzo[d]imidazole (10l)

White solid, yield: 89%, m.f.: $C_{32}H_{23}Cl_2N_7O_2$, m.p.:158-160ºC. (IR neat, ν_{max}, cm^{-1}): 2900, 2870, 1650, 1600, 1550, 1460, 1250, 1120, 980. ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm): 8.55 (s,1H), 8.12 (s,1H), 7.96(d, J=11.8 Hz, 3H), 7.68(d, J=9.2 Hz, 1H),7.65-7.34(m,5H),7.20(S,2H), 7.11-7.00(m,2H),6.95 (s, 1H), 6.80 (s,2H), 5.55 (s,2H) , 3.70 (s,3H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 158.6, 156.4, 149.1, 147.1, 140.8, 138.5, 135.0, 134.2, 133.5, 130.5, 129.3, 125.5, 120.4, 119.9, 115.6, 69.8, 57.5 Mass: ESI-MS: m/z = 608 $[M+H]^+$.

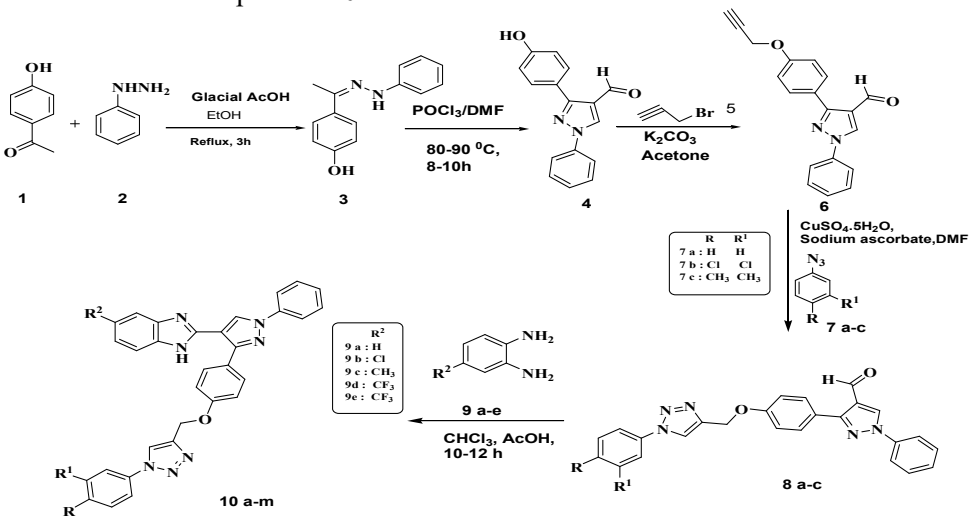
2-(3-(4-((1-(3,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-5-methoxy-1H-benzo[d]imidazole (10m)

White solid, yield: 78%, m.f.: $C_{34}H_{29}N_7O_2$, m.p.:145-147ºC. (IR neat, ν_{max}, cm^{-1}): 2940,2872,1670,1598,1523,1490,1200,1100,1010 ^{1}H NMR data (400 MHz, $CDCl_3$, δ ppm) : 8.19 (s,1H), 7.90(d, J=6.9 Hz, 2H), 7.80(s,1H), 7.70-7.50(m,9H), 7.44-7.10(m,2H), 7.00 (d, J=7.9 Hz, 1H), 6.85 (d, J=5.8 Hz, 2H), 5.45 (s,2H), 3.73(s, 3H), 2.35 (s,3H), 2.20 (s,3H). ^{13}C NMR (100 MHZ, $CDCl_3$, δ ppm): 159.5,153.6,147.3,140.3,139.5,138.9,133.7,131.5,130.5,130.1,128.9,128.5,127.5,124.0,121.5,120.5,118.9, 114.2,58.9,55.7,20.9,19.3 Mass: ESI-MS: m/z = 568 $[M+H]^+$.

RESULTS AND DISCUSSION

Synthesis of the title compounds (10a-m) was accomplished by the synthetic procedure outlined in scheme-1. Initially, condensation of 4-hydroxy acetophenone with phenylhydrazine gives the hydrazone derivative 3. In order to obtain the 1,4-diphenyl substituted pyrazole-4-aldehyde, the hydrazone compound 3 was subjected to a vilsmeier Haack reaction with $POCl_3$ and DMF. The reaction underwent

smoothly to give aldehyde compound 4. The hydroxyl functional group of compound 4 on O-alkylation with propargyl bromide in the presence of K_2CO_3 and acetone under reflux conditions yields the pyrazole derivative 6. 1,3 dipolar cycloaddition of compound 6 with azide (7a-c) under click reaction conditions gives the 1,2,3-triazole compounds (8a-c). The aldehyde functional group present on the pyrazole ring of compounds 8a-c undergoes a cyclization reaction with aromatic orthodiamine compounds 9 a-e to give the targeted benzimidazole compounds 10a-m.



Scheme-1: Synthetic Scheme for the Preparation of Compounds (10a-m)

Anticancer Activity-Cytotoxicity Assay

All the newly synthesized triazole–benzimidazole pyrazole derivatives (10a-m) are screened for their *in vitro* cytotoxic activity against human breast MCF -7 cancer cells using MTT colorimetric assay[26] using Cisplatin as standard. Cells treated with increasing concentrations of compounds ranging from 2, 5, 10, and 20 µg/ml resulted in dose-responsive cellular cytotoxicity data in the form of IC_{50} (half-maximal concentration of compound required to inhibit the cellular growth) on MCF-7 cell lines. The results obtained infer an inverse relationship between the drug concentration and cell viability. The experiment was performed in triplicate, and the absorbance determined spectrophotometrically at 620nm was recorded using a microplate reader (Bio-Rad, San Diego, USA). The Cytotoxic studies of triazole-benzimidazole containing pyrazole derivatives (10a-m) against MCF-7 breast cancer cell line are outlined in table-1. In this, the compound (10b) shows better activity than cisplatin at 20 µg/ml concentration, whereas the remaining compounds did not show any promising activity compared to cisplatin.

Table-1:- Cell Viability Data after 48 Hours of Drug Treatment

Compound	% Viability of MCF-7 cells			
	2 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml
10a	94.12	89.56	85.47	80.36
10b	75.28	61.38	50.58	28.75
10c	93.25	86.17	72.18	59.18
10d	93.26	88.54	81.26	75.48
10e	92.88	87.12	81.22	72.19
10f	93.65	86.22	81.69	74.56
10g	81.45	65.38	52.44	29.68
10h	96.81	90.09	84.23	79.85
10i	91.50	88.53	85.50	69.80
10j	95.88	87.92	80.23	78.50
10k	92.33	86.54	77.80	66.87
10l	88.60	78.82	70.13	65.87
10m	90.15	85.32	79.90	70.12
Cisplatin	44.03	41.36	38.92	29.68

CONCLUSION

In the present work, a series of triazole benzimidazole containing pyrazole compounds (10a-m) were successfully synthesized in good yields using the Vilsmeier Haack reaction and click chemistry approach. The Cytotoxicity assay of the compounds was evaluated; out of this, compound 10b is found to be more active among the tested.

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