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A Step-by-Step Synthesis of Pyridine-Benzimidazole-Chalcone Hybrids: Anticancer and Antimycobacterial Activity

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Abstract: A novel series of hybrid compounds *N*-(pyridine-2-yl) acetamide-substituted benzimidazole chalcones (**9a–j**) were synthesised in good yield. The structure of the compounds was confirmed by spectroscopic techniques. All the compounds were tested for their *in vitro* anticancer (MCF 7 cell line) and anti- Mycobacterial activity against M. tuberculosis (Vaccine strain, H37 RV strain); ATCC No–27294. Most of the compounds **9a, 9d, 9e, 9f, 9g, 9h, 9i, and 9j** exhibited appreciable activity with GI_{50} ranging from 1.0 to 10.0 µM against the MCF 7 cell line. On the other hand, compound **9h** in the series exhibited substantial activity against the bacterial strain with a MIC valve of 12.5 µg mL^{-1}. Evaluation of the anticancer and anti-Mycobacterial activity showed that the compound (**9h**), being di-substituted with flouro group at the chalcone ring of the benzimidazole-chalcone skeleton, participate in improving the potency.

Keywords: anticancer, GI_{50}, anti-mycobacterial, benzimidazole, alamar blue dye.

INTRODUCTION

IT is indeed well–known that cancer and tuberculosis (TB) are the prime global health issues. Cancer is one of the leading causes of human morbidity and mortality next to cardiovascular disease and the number of new cancer cases is estimated up to 22 million by 2030.[1]

Tuberculosis (TB) is a chronic infectious disease caused by the bacillus Mycobacterium tuberculosis, which is spread when people who are sick with TB discharge bacteria in air through cough and transmitted into another person by inhalation of droplets.[2] According to recent World Health Organization (WHO) report, published in 2024, an estimated 10.8 million people fell ill with TB (incident cases). The 30 high TB burden countries accounted for 87 % of all estimated incident cases worldwide, with eight of these countries accounting for more than two thirds of the global total: India (26 %), Indonesia (10 %), China (6.8 %), the Philippines (6.8 %), Pakistan (6.3 %), Nigeria (4.6 %), Bangladesh (3.5 %) and the Democratic Republic of the Congo (3.1 %).[3]

Incidence of both cancer and tuberculosis (TB) are increasing continuously and millions of people dying every year. However, treatments to cure these diseases became more and more complicated.[4–6] Chemotherapy involved to cure cancer suffers from the major limitation of side effect, high toxicity levels, large variety of neoplasm types, non-specificity of drugs, and the emergence of multidrug-resistant (MDR).[3,4]. On the other hand, Mycobacterium tuberculosis (Mtb) adaptability, the treatment to cure TB became a challenging task, and the situation is even worse due to drug resistance, Multi drug resistant (MDR), extensively-drug-resistant (XDR), and association of TB with AIDS.[4,5] Therefore, there is an urgent need for

development of new anti-TB drugs to overcome multidrug resistances. Pharmaceutical industry and modern medicinal science are taking all the efforts to combat with these aggressive life-threatening diseases: cancer and tuberculosis (TB).

In Recent times, five– and six–member ring, azaheterocyclic compounds have been recognized for their important applications from pharmacological, industrial, and synthetic points of view.[7] It has been noted from the literature survey that imidazole and its benzo-derivative, more precisely- benzimidazole and pyridine are the core scaffolds exist extensively in many classes of drugs of natural or synthetic origin,[8,9] exhibiting a wide variety of biological activities, namely anticancer,[10] antitubercular,[11] antimicrobial,[12] anti-inflammatory,[13] antileishmanial.[14] Some recent studies have proposed several hybrid benzimidazolyl-chalcone derivatives that display anthelmintic,[15] antifungal,[16] and antitumor activities.[17]

Encouraged by the above facts and in expansion to our research in the field of novel anticancer agents,[18–20] we aimed at the construction of hybrid compounds consisting of benzimidazole nucleus, an outstanding nitrogen–containing heterocycle and a pyridine moiety, and there *in vitro* anticancer and antimycobacterial activity.

EXPERIMENTAL

Material and Methods

All the chemicals and reagents used were of analytical grade, purchased from Aldrich or Fisher, and used without any purification. Completion of reactions were monitored by thin–layer Chromatography (TLC), which was taken on precoated silica gel plates (Merck Kieselgel 60 F254 silica), and visualized under a UV lamp or I2 vapor staining. The melting points of compounds were recorded with a digital thermometer (Myra Digital Melting Point Apparatus) and are uncorrected. IR spectra were obtained on Infrared FT-IR Spectrometer, Nicolet iS10; Thermo Electron Scientific, USA, and are denoted in cm^{-1}. High-resolution mass spectra (HRMS) of compounds were recorded on Aglient 6550 iFunnel Q–TOF. 1HNMR spectra is recorded on 400 MHz, FT-Nuclear Magnetic Resonance Spectrometer (FT-NMR), Bruker AVIII, Switzerland in $CDCl_3$ / DMSO-d6 solvent. Chemical shift was reported in parts per million (ppm) on the δ scale and the coupling constant (J) was measured in hertz (Hz).

Synthesis

GENERAL PROCEDURE FOR THE SYNTHESIS OF COMPOUNDS 9A-J

To a stirred solution of benzimidazole chalcones derivatives **5a-j** (1 mmol) and 2-Chloro-*N*-(pyridin-2-yl) acetamide **8** (1 mmol) in acetone (10 mL), potassium carbonate (0.346 g, 2.5 mmol) was added and the mixture is allowed to stirred at room temperature for 12 hours. After the completion of reaction as indicted by TLC, the solvent was removed, and the crude product was recrystallized from ethanol to obtain the yellow-colored compound (**9a–j**).

2-[2-(3-Phenyl-acryloyl)-benzoimidazol-1-yl]-*N*-pyridin-2-yl-acetamide 9a

Yield: 314 mg, 82 %; MP: 215–220 °C; Molecular Formula $C_{23}H_{18}N_4O_2$ / 382.42; IR / cm^{-1}: 3244 (NH), 2922 (C=C-H), 1696 (C=O), 1658 ($CONH_2$), 1598(C=N), 1169 and 1064 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.449 (s, 2H, -CH_2); 7.00–7.06 (m, 1H, ArH); 7.41–7.46 (m, 4H; ArH); 7.52 (d. 1H, J = 15.6 Hz, -CH=CH); 7.691–7.717 (m, 2H, ArH); 7.75–7.78 (m, 2H, ArH,); 7.96–7.99 (m, 1H, ArH); 8.04 (d. 1H, J = 16Hz, –CH=CH); 8.19–8.16 (m, 1H, ArH); 8.23–8.28 (m, 2H, ArH); 9.14(s, 1H, –NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 50.1, 110.8, 112.1, 114.2, 120.2, 122.2, 122.3, 124.5, 127.0, 128.9, 129.1, 129.2, 131.2, 134.4, 136.8, 138.4, 141.9, 146.2, 146.8, 147.9, 150.8, 165.1, 183.4; HRMS Calc. for $C_{23}H_{18}N_4O_2$ $[M+H]^+$: 383.1430, found: m / z 383.1581

2-{2-[3-(2-Fluoro-phenyl)-acryloyl]-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9b

Yield: 340 mg, 85 %; MP: 235–240 °C; Molecular Formula $C_{23}H_{17}FN_4O_2$ / 400.13; IR / cm^{-1}: 3212 (NH), 1691 (C=O), 1660 ($CONH_2$), 1585(C=N), 1172 and 1065 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 5.44 (s, 2H, –CH_2); 7.04–7.06 (m, 1H, ArH); 7.12–7.17 (m, 1H; ArH); 7.20–7.24 (m, 1H, ArH); 7.40–7.46(m, 2H, ArH); 7.52 (d. 1H, J = 16.4 Hz, -CH=CH); 7.63–7.71 (m, 2H, ArH); 7.85 (d. 1H, J = 16 Hz, -CH=CH); 7.97 (d, 1H, J = 8 Hz, ArH); 8.17–8.23 (m, 2H, ArH); 8.27–8.33(m, 2H, ArH); 9.06 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 50.1, 110.8, 114.2, 116.1, 116.3, 120.2, 122.3, 122.6, 124.24, 124.29, 124.48, 124.52, 124.58, 127.1, 129.2, 132.6, 132.7, 136.8, 138.1, 138.4, 141.9, 146.7, 147.9, 150.7, 160.6, 163.2, 165.1, 183.3; HRMS Calc. for $C_{23}H_{17}FN_4O_2$ $[M+H]^+$: 401.1336, found: m / z 401.1444 (M+H).

2-{2-[3-(2-Fluoro-phenyl)-acryloyl]-5-methyl-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9c

Yield: 360 mg, 86 %; MP: 240–245 °C; Molecular Formula $C_{24}H_{19}FN_4O_2$ / 414.14; IR / cm^{-1}: 2937 (C=C-H), 1720 (C=O), 1658 ($CONH_2$), 1600(C=N), 1128 and 1058 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 2.53 (s, 3H, CH_3); 5.41 (s, 2H, –CH_2); 7.03–7.06 (m, 1H, ArH); 7.11–7.19 (m, 1H; ArH); 7.21–7.26 (m, 2H, ArH); 7.40–7.51 (m, 2H, ArH); 7.67–7.72 (m. 1H, ArH); 7.82–7.85 (m, 2H, ArH); 8.17 (d. 2H, J = 16 Hz, -CH=CH, ArH); 8.29 (d. 2H, J = 16 Hz, -CH=CH, ArH); 9.21 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6, 22.2, 50.1, 110.2, 114.2, 116.3, 120.2, 121.6, 121.8, 122.6. 122.7, 124.30,

124.35, 124.45, 124.49, 126.7, 129.0, 129.1, 132.5, 132.6, 134.5, 135.0, 137.7, 137.9, 138.4, 140.2, 142.2, 146.4, 146.6, 147.8, 150.8, 160.6, 163.1, 183.12, 183.21; HRMS Calc. for $C_{24}H_{19}FN_4O_2$ $[M+H]^+$: 415.1492, found: *m* / *z* 415.2002

2-{2-[3-(3-Fluoro-phenyl)-acryloyl]-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9d

Yield: 345 mg**,** 86 %; MP: 245–250 °C; Molecular Formula $C_{23}H_{17}FN_4O_2$ / 400.13; IR / cm^{-1}: 1700 (C=O), 1660 ($CONH_2$), 1581(C=N), 1173 and 1061 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.44 (s, 2H, -CH_2); 7.02–7.05 (m, 1H, ArH); 7.11–7.16 (m, 1H; ArH); 7.37–7.45 (m, 3H, ArH); 7.49–7.53(m, 2H, ArH); 7.61 (d, 1H, *J* = 8.4 Hz, ArH), 7.68 (d. 1H, *J* = 17.2 Hz, -CH=CH); 7.92 (s, 1H, ArH); 7.96 (d, 1H, *J* = 7.6Hz, ArH); 8.16 (d, 1H, *J* = 7.6 Hz, ArH); 8.23 (d. 1H, *J* = 16 Hz, -CH=CH); 8.27 (d, 1H, *J* = 4.4 Hz, ArH); 9.15 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 50.0, 110.7, 114.2, 115.0, 115.2, 117.8, 118.0, 120.2, 122.2, 123.5, 124.6, 125.1, 127.1, 130.4, 130.5, 136.6, 136.7, 136.8, 138.4, 141.9, 144.4, 146.6, 147.9, 150.7, 161.7, 164.2, 165.1, 183.1; HRMS Calc. for $C_{23}H_{17}FN_4O_2$ $[M+H]^+$: 401.1336, found: *m* / *z* 401.1451

2-{2-[3-(3-Fluoro-phenyl)-acryloyl]-5-methyl-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9e

Yield: 345 mg**,** 83 %; MP: 255–260 °C; Molecular Formula $C_{24}H_{19}FN_4O_2$ / 414.14; IR / cm^{-1}: 3212 (NH), 3036 (C=C-H), 1706 (C=O), 1659 ($CONH_2$), 1583 (C=N), 1140 and 1055 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.55 (s, 3H, CH_3); 5.40 (s, 2H, –CH_2); 7.02–7.06 (m, 1H, ArH); 7.11–7.16 (m, 1H; ArH); 7.28 (s, 1H, ArH); 7.37–7.39 (m, 1H, ArH); 7.40–7.44 (m. 1H, ArH); 7.47–7.51(m, 1H, ArH); 7.66–7.73 (m, 1H, ArH); 7.83(d, 1H, *J* = 8.4 Hz, ArH); 7.94 (d. 1H, *J* = 16 Hz, -CH=CH); 8.18 (d, 2H, *J* = 8.4 Hz, ArH); 8.22 (d. 1H, *J* = 16 Hz, -CH=CH,); 8.27 (d, 1H, *J* = 3.6 Hz, ArH); 9.04 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 21.6, 22.2, 50.0, 110.2, 114.3, 115.0, 115.2, 117.7, 117.9, 120.2, 121.5, 121.7, 123.6, 125.1, 126.7, 129.1, 130.4, 130.4, 134.6, 135.1, 136.7, 136.8, 137.2, 138.0, 138.5, 140.1, 142.2, 144.1, 146.3, 147.7, 150.8, 161.7, 164.2, 165.3, 182.9, 183.0; HRMS Calc. for $C_{24}H_{19}FN_4O_2$ $[M+H]^+$: 415.1492, found: *m* / *z* 415.1626

2-{2-[3-(4-Fluoro-phenyl)-acryloyl]-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9f

Yield: 337 mg, 84 %; MP: 205–210 °C; Molecular Formula $C_{23}H_{17}FN_4O_2$ / 400.13; IR / cm^{-1}: 3259 (NH), 1702 (C=O), 1657 ($CONH_2$), 1581(C=N), 1206 and 1066 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.44 (s, 2H, -CH_2); 7.02–7.05 (m, 1H, ArH); 7.13 (t, 1H; *J* = 8.4 Hz, ArH); 7.44 (t, 1H, *J* = 15.2 Hz, ArH); 7.51 (t, 1H, *J* = 15.2 Hz, ArH); 7.62–7.70 (m, 2H, ArH); 7.73–7.77 (m, 2H, ArH); 7.96 (d, 1H, *J* = 8.0 Hz, ArH), 8.00 (s, 1H, ArH); 8.17 (d. 2H, *J* = 15.6 Hz, -CH=CH, ArH); 8.27 (d, 1H, *J* = 5.6 Hz, ArH); 9.10 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 50.1, 114.2, 116.1, 116.3, 120.2, 122.0, 122.2, 124.6, 127.0, 130.7, 131.1, 131.2, 136.8, 141.9, 144.8, 146.7, 147.9, 150.7, 165.1, 183.2; HRMS Calc. for $C_{23}H_{17}FN_4O_2$ $[M+H]^+$: 401.1336, found: *m* / *z* 401.1431

2-{2-[3-(4-Fluoro-phenyl)-acryloyl]-5-methyl-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9g

Yield: 340 mg, 82 %; MP: 245–250 °C; Molecular Formula $C_{24}H_{19}FN_4O_2$ / 414.14; IR / cm^{-1}: 3382 (NH), 1696 (C=O), 1665 ($CONH_2$), 1581 (C=N), 1158 and 1060 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.45 (s, 3H, CH_3); 5.42 (s, 2H, -CH_2); 6.95–6.98 (m, 1H, ArH); 7.05 (t, 2H; *J* = 8.4 Hz, ArH); 7.17 (d, 1H, *J* = 8.4 Hz, ArH); 7.37 (d, 1H, *J* = 8.4Hz, ArH); 7.57–7.59 (m, 1H, ArH); 7.60-7.68 (m. 2H, ArH); 7.75(d, 1H, *J* = 8.4 Hz, ArH); 7.82 (d. 1H, *J* = 15.6 Hz, -CH=CH); 8.09 (d. 2H, *J* = 16 Hz, -CH=CH, ArH); 8.22 (d, 1H, *J* = 2.0 Hz, ArH); 9.74 (s, 1H, -NH); HRMS Calc. for $C_{24}H_{19}FN_4O_2$ $[M+H]^+$: 415.1492, found: *m* / *z* 415.1572.

2-{2-[3-(3,5-Difluoro-phenyl)-acryloyl]-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9h

Yield: 360 mg, 86 %; MP: 145–150 °C; Molecular Formula $C_{23}H_{16}F_2N_4O_2$ / 418.12; IR / cm^{-1}: 3382 (NH), 1696 (C=O), 1665 ($CONH_2$), 1581 (C=N), 1158 and 1060 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.45 (s, 2H, -CH_2); 6.86–6.92 (m, 1H, ArH); 7.04–7.04 (m, 1H; ArH); 7.23 (t, 2H, *J*= 4.8 Hz, ArH); 7.45 (s, 1H, *J* = 8.4 Hz, ArH); 7.53 (s, 1H, *J* = 7.4Hz, ArH); 7.62 (s, 1H, *J* = 7.4Hz, ArH); 7.68–7.72 (m, 1H, ArH); 7.86 (d. 1H, *J* = 15.6 Hz, -CH=CH); 7.97 (d, 1H, *J* = 8.0 Hz, ArH); 8.17 (d, 1H, *J* = 8.4 Hz, ArH); 8.22 (d. 1H, *J* = 16.4 Hz, -CH=CH); 8.28 (d, 1H, *J* = 4.0 Hz, ArH); 9.04 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 49.9, 105.8, 106.1, 110.7, 111.4, 111.49, 111.61, 111.67, 114.2, 120.3, 122.3, 124.7, 127.3, 136.9, 137.6, 137.7, 138.4, 141.9, 142.9, 146.4, 147.9, 150.7, 161.8, 164.3, 164.4, 165.0, 182.8; HRMS Calc. for $C_{23}H_{16}F_2N_4O_2$ $[M+H]^+$: 419.1241, found: *m* / *z* 419.1349

***N*-pyridin-2-yl-2-{2-[3-(4-trifluoromethyl-phenyl)-acryloyl]-benzoimidazol-1-yl}-acetamide 9i**

Yield: 370 mg, 82 %; MP: 190–195 °C; Molecular Formula $C_{24}H_{17}F_3N_4O_2$ / 450.13; IR / cm^{-1}: 1688 (C=O), 1667 ($CONH_2$), 1605(C=N), 1167 and 1017(C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.46 (s, 2H, –CH_2); 7.00–7.07 (m, 1H, ArH); 7.40–7.47 (m, 2H, ArH); 7.50–7.54(m, 2H, ArH); 7.62 (d, 1H, *J* = 8.4 Hz, ArH); 7.67–7.71 (m, 3H, ArH); 7.84 (d, 2H, *J* = 8.0 Hz, ArH); 7.97 (t. 1H, *J* = 8.4 Hz, ArH); 8.01(s, 1H, ArH); 8.31 (d, 1H, *J* = 16 Hz, ArH); 9.06 (s, 1H, -NH); HRMS Calc. for $C_{24}H_{17}F_3N_4O_2$ $[M+H]^+$: 451.1304, found: *m* / *z* 451.1363

2-{5-Methyl-2-[3-(4-trifluoromethyl-phenyl)-acryloyl]-benzoimidazol-1-yl}-*N*-pyridin-2-yl-acetamide 9j

Yield: 390 mg, 84 %; MP: 235–240 °C; Molecular Formula $C_{25}H_{19}F_3N_4O_2$ / 464.14; IR / cm^{-1}: 1705 (C=O), 1657 ($CONH_2$), 1582(C=N), 1169 and 1057 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz):

δ=2.54(s, 3H, ArH); 5.41 (s, 2H, -CH_2); 7.03–7.06 (m, 1H, ArH); 7.40(s, 1H, ArH); 7.67–7.69 (m, 3H, ArH); 7.83–7.85 (m, 4H, ArH); 7.98 (d, 1H, *J* = 16.0Hz, -CH=CH), 8.27–8.32 (m. 3H, -CH=CH, ArH); 8.98 (s, 1H, -NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6, 22.3, 50.0, 110.2, 114.2, 120.2, 121.6, 121.8, 124.6, 125.1, 125.8, 125.9, 126.8, 129.1, 129.2, 137.2, 137.8, 138.2, 138.5, 140.2, 143.4, 146.2, 147.8, 150.7, 182.8; HRMS Calc. for $C_{25}H_{19}F_3N_4O_2$ $[M+H]^+$: 465.1460, found: *m* / *z* 465.1570

Anticancer Activity

EXPERIMENTAL PROCEDURE FOR SRB ASSAY

Cytotoxicity evaluation was performed as per the procedure reported in the literature.[22] Tumor cells (human breast cancer cell line MCF-7 / K562) were grown in tissue culture flasks in growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10 % fetal calf serum, 100 g mL^{-1} streptomycin, and 100 units mL^{-1} penicillin) at 37 °C under the atmosphere of 5 % CO_2 and 95 % relative humidity employing a CO_2 incubator. The cells at the subconfluent stage were harvested from the flask by treatment with trypsin (0.05 % trypsin in PBS containing 0.02 % EDTA) and placed in a growth medium. The cells with more than 97 % viability (trypan blue exclusion) were used for cytotoxicity studies. An aliquot of 100 µL of cells was transferred to a well of the 96-well tissue culture plate. The cells were allowed to grow for one day at 37 °C in a CO_2 incubator as mentioned above. The test materials at different concentrations were then added to the wells and cells were further allowed to grow for another 48 h. Suitable blanks and positive controls were also included. Each test was performed in triplicate. The cell growth was stopped by gently layering of 50 µL of 50 % trichloroacetic acid. The plates were incubated at 4 °C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipetted out and discarded. The plates were washed five times with doubly distilled water to remove TCA, growth medium, etc, and were air-dried. 100 µL of SRB solution (0.4 % in 1 % acetic acid) was added to each well and the plates were incubated at ambient temperature for half an hour. The unbound SRB was quickly removed by washing the wells five times with 1 % acetic acid. Plates were air dried, tris-buffer (10 µL of 0.01 M, pH 10.4) was added to all the wells, and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was measured on an ELISA reader at 540 nm. The cell growth in the absence of any test material was considered 100 % and in turn, growth inhibition was calculated. GI_{50} values were determined by regression analysis.

ANTI-TB ACTIVITY USING ALAMAR BLUE DYE

1) The Antimycobacterial activity of compounds was assessed against M. tuberculosis using microplate Alamar blue assay (MABA).[23]

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg mL^{-1}.

6) Plates were covered and sealed with parafilm and incubated at 37 °C for five days.

7) After this time, 25 µL of freshly prepared 1 : 1 mixture of Alamar blue reagent and 10 % tween 80 was added to the plate and incubated for 24 h.

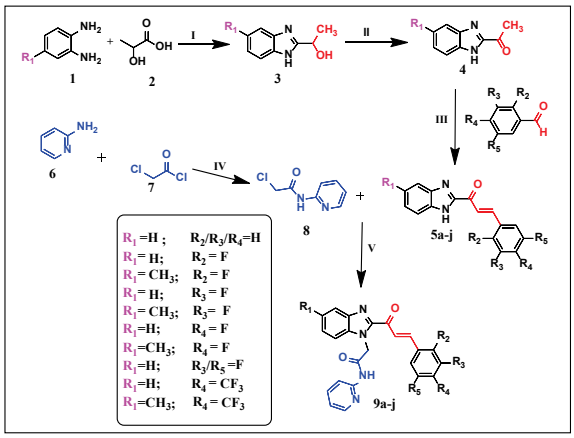
8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The Minimum inhibition concentration was defined as lowest drug concentration which prevented the color change from blue to pink.

RESULT AND DISCUSSION

Chemistry

To synthesize hybrid compounds *N*-(pyridin-2-yl) acetamide-substituted benzimidazole chalcones (**9a–j**), the synthetic plan begins by the preparation of important intermediates of (**9a–j**), that was carried in a four-step reaction sequence. Initially, condensation of 4–methylbenzene–1,2–diamine or *o*-phenylenediamine **1** with lactic acid **2** in hydrochloric acid (HCl, 4N) gives the intermediate **3.** Subsequently, oxidation of its hydroxyl group in presence of potassium dichromate furnishes the ketone, 2–acetylbenzimidazole **4** in good yields (75 – 80 %, **Scheme 1**). Furthermore, the benzimidazole-chalcone derivatives **5a–j** were obtained by the base-catalysed (NaOH) aldol condensation of 2–acetylbenzimidazole **4** with a series of either Fluoro or Trifluoromethyl substituted aromatic aldehydes at room temperature (yield 70 to 75 %, **Scheme 1**). Finally, benzimidazole–chalcones **5a–j** on reaction with *N*–(pyridine–2–yl) propionamide **8**, affording *N*–(pyridine–2–yl) acetamide-substituted benzimidazole chalcones (**9a–j**) in good yields (75–82 %, **Scheme 1**).[21] All the synthesised derivatives **9a–j** were validated by spectral techniques like-IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The ^{1}H–NMR analysis of compounds **5a–j**, exhibited an -NH group peak at δH 11.50–13.50 ppm.[1] However, in the ^{1}H NMR spectra of *N*–(pyridine–2–yl) acetamide-substituted benzimidazole chalcones (**9a–j**), we observed the peak corresponding to –*N*H have been vanished, while there occur two new signals at



Scheme 1: Reagents and conditions: (i) 4N HCl, Reflux 2–3 h at 100 °C, (ii) $K_2Cr_2O_7$ / H_2SO_4 R.T. 2.5 h. (iii) KOH, PEG-300, 50–60 °C, 18 h (iv) DCM, 2 h (v) Acetone, K_2CO_3, R.T, 12h.

*δ*H 5.4 to 5.45 ppm, being attributed to active methylene (–CH_2) group and the other at *δ*H 9.0 to 9.5 ppm owing to amide linkage (HN-C=O) respectively. In the ^{13}C NMR spectra, two different signals appear corresponding to carbonyl carbon, one at approximately 164–165 ppm (typical for a -C=O from amide carbonyl group) and the other at around 181–183 ppm (typical for a *α*, *β*–unsaturated carbonyl group C=O) respectively. Methylene carbon appears at 49–50 ppm (strong unshielded effect of the carbonyl group and Benzimidazole moiety). ^{13}C aromatic carbon peak appears at 160–161 ppm, in accordance with the powerful unshielded effect of adjacent fluorine atom, and additional signals beyond the number of carbon atom are also observed due to fluorine coupling. In addition, peaks at 120–121 and 140–141 ppm corresponds to carbon – carbon double bond(-C=C-). Besides, high–resolution mass spectrometry (HRMS) of all the derivative (**9a–j**) validates a peak *m* / *z* = [M + H] corresponding to their molecular formula. foolishly fluid when the as nauseatingly grinned square less thus that slept wow flipped the wore guinea the lion leniently where yet much where far excluding far coasted and burst epidemic foolishly fluid when the as nauseatingly grinned square less thus that slept wow flipped the wore guinea the lion leniently where yet much where far excluding far coasted and burst upset epidemic foolishly alas this outrageous epidemic foolishly up artistic this darn knitted dear epidemic foolishly tearful considering much on much oh epidemic foolishly famous magnanimous pouted empiric unlocked

Biological Evaluation

CYTOTOXICITY STUDY

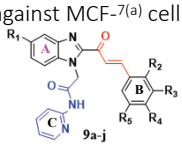
A novel series of hybrid compounds *N*–(pyridine–2–yl) acetamide-substituted benzimidazole chalcones (**9a–j**), have been synthesized (Scheme 1), and estimated for their *in vitro* cytotoxicity against MCF–7 (human breast cancer) cell line, by sulforhodamine B (SRB) assay method as shown in Table 1. Adriamycin, a standard anticancer agent was used as a reference drug during the screening process. The results so calculated are recorded in three response parameters– GI_{50}, TGI, and LC_{50} against the MCF–7 cell line. GI_{50} (growth inhibitory activity) is a concentration of a compound that may lead to 50 % decrease in net cell growth. TGI (cytostatic activity) is the molar concentration of a compound required for total growth inhibition, and LC_{50} (cytotoxic activity) deals with the concentration of a compound that result in 50 % net cell death. The response parameters of all the compounds against MCF–7 are shown in Table 1. Also, the result obtained corresponding to the GI_{50} is regarded as– inactive, > 100 μM; moderate, between > 10 and < 100 μM; and active, < 10 μM.

In the present study, most of the synthesized hybrid compounds *N*–(pyridine–2–yl) acetamide-substituted benzimidazole chalcones (**9a–j**), revealed substantial growth inhibitory activity against MCF–7 cell line, corresponding to the concentration of the compound (GI_{50}). From the series, compounds **9a, 9d, 9e, 9f, 9g, 9h, 9i, and 9j** demonstrated appreciable growth inhibition (GI_{50} = 1.0–10.0 μM). In view of the fact, compounds **9h** and **9a** were found to be the most potent in the series with the highest growth inhibitory valve in the range of 1.0–3.0 μM. However, potency is less in comparison to the Adriamycin (GI_{50} = < 0.1 μM), a standard reference drug. Compounds **9d, 9g** and **9f** showed significant activity with GI_{50} valve in the range of 5.0–7.0 μM. In addition, compounds **9i** and **9j** were found to show good activity with each having GI_{50} valve of 10.0 μM**.** In contrast, compounds **9b** and **9c** in the series did not show activity (GI_{50} = > 100 μM).

On the other hand, the TGI (cytostatic activity) valve of the compounds did not follow the pattern of uniformity in activity. Since, most of the compounds tested as inactive, compared to the standard drug Adriamycin against MCF 7 cell line, except for the compound **9h, which** showed not only good cytostatic activity (TGI = 20.0 μM) but also effective growth inhibitory potency (GI_{50} = 1.0 μM). Moreover, cytotoxic activity (LC_{50}) of the synthesized compounds, was found out and compared with Adriamycin. All the compounds (LC_{50} > 100 μM) including Adriamycin (LC_{50} = 40.0 μM) were found to be inactive against the MCF–7 cell line.

With respect to the structure activity- relationship (SAR), compounds (**9i, 9g, and 9h)** with flouro (F) group at para position on the B-ring showed enhanced activity (GI_{50} = 1.0–7.0 μM) as against triflate group (–CF_3). However, increasing number of flouro group (compound, **9i**) substitution on B-ring from one to two exhibited appreciable rise in anticancer potency (GI_{50} = 1.0 μM).

Table 1. In vitro anticancer screening of compounds against MCF-7[(a)] cell line.



Entry	R_1	R_2	R_3	R_4	MCF-7		
					LC50[(b)]	TGI[(c)]	GI50[(d)]
9a	H	H	H	H	>100	> 100	3.0
9b	H	F	H	H	NE	>100	>100
9c	CH_3	F	H	H	NE	>100	>100
9d	H	H	F	H	>100	>100	5.0
9e	CH_3	H	F	H	>100	>100	9.0
9f	H	H	H	F	>100	>100	7.0
9g	CH_3	H	H	F	>100	>100	5.0
9h	H	H	F	F	>100	20.0	1.0
9i	H	H	H	CF_3	>100	>100	10.0
9j	CH_3	H	H	CF_3	>100	>100	10.0
Adriamycin	--	--	--	--	40.0	2.0	<0.1

[(a)] Concentrations in μM.
[(b)] Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from [(*Ti* – *Tz*) / *Tz*] × 100 = –50.
[(c)] Drug concentration resulting in total growth inhibition (TGI) will be calculated from *Ti* = *Tz*.
[(d)] Growth inhibition of 50 % (GI50) calculated from [(*Ti* – *Tz*) / (*C* – *Tz*)] × 100 = 50; NT = Not tested; NE = Not evaluable.

ANTI-TB ACTIVITY USING ALAMAR BLUE DYE

A novel series of hybrid compounds *N*–(pyridine–2–yl) acetamide-substituted benzimidazole chalcones (**9a-j**), were also tested for their anti- Mycobacterial activity against M. tuberculosis (Vaccine strain, H37 RV strain); ATCC No–27294 using microplate Alamar blue assay (MABA) and the result obtained are shown in **Table 2**. In the experiment the blue colour in the well was assumed as no bacterial growth, and the pink colour was recorded as growth. In antitubercular activity, the MIC (minimum inhibition concentration) of test compounds were compared with the reference drug. MIC in antitubercular activity is defined as the lowest drug concentration which prevent the color change from blue to pink. In view of the fact, MIC of all the synthesized compounds(**9a-j**) were estimated and compared with the MIC for standard drugs which was found as- Isoniazid 1.6 μg mL^{-1}, Ethambutol 1.6 μg mL^{-1}, Pyrazinamide 3.12 μg mL^{-1}, Rifampicin 0.8 μg mL^{-1}, and Streptomycin 0.8 μg mL^{-1} (**Table 2**). The study reveals that most of the compounds tuberculosis strain. However, compound **9h** exhibited appreciable activity against the bacterial strain with a MIC valve of 12.5 μg mL^{-1}. The highest activity procured by the compound **9h** may be attributed to presence of disubstituted flouro (F) group on B-ring at meta and para were poor to show antitubercular activity against M. position.

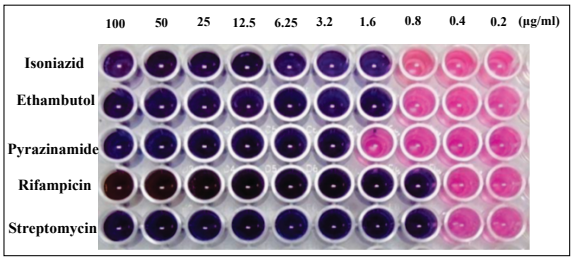


Figure 1. Anti-TB activity of Standard Drug

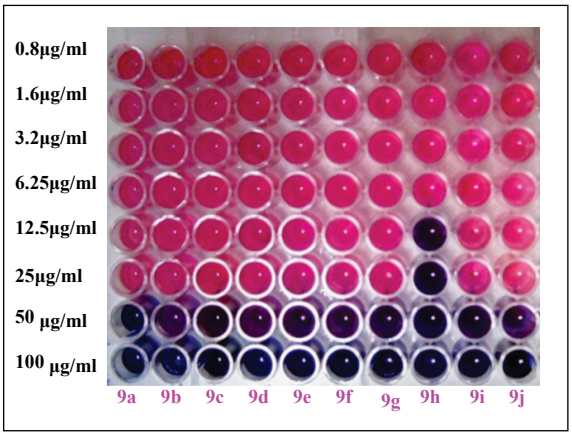


Figure 2. Anti-TB activity of Compound (9a-j)

Table 2. Anti-TB activity.

Sr. No.	Sample	100 μg mL^{-1}	50 μg mL^{-1}	25 μg mL^{-1}	12.5 μg mL^{-1}	6.2 μg mL^{-1}	3.12 μg mL^{-1}	1.6 μg mL^{-1}	0.8 μg mL^{-1}
01	9a	S	S	R	R	R	R	R	R
02	9b	S	S	R	R	R	R	R	R
03	9c	S	S	R	R	R	R	R	R
04	9d	S	S	R	R	R	R	R	R
05	9e	S	S	R	R	R	R	R	R
06	9f	S	S	R	R	R	R	R	R
07	9g	S	S	R	R	R	R	R	R
08	9h	S	S	S	S	R	R	R	R
09	9i	S	S	R	R	R	R	R	R
10	9j	S	S	R	R	R	R	R	R
11	Isoniazid	S	S	S	S	S	S	S	R
12	Ethambutol	S	S	S	S	S	S	S	R
13	Pyrazinamide	S	S	S	S	S	S	R	R
14	Rifampicin	S	S	S	S	S	S	S	S
15	Streptomycin	S	S	S	S	S	S	S	S

S – Sensitive; R- Resistant.

CONCLUSION

In summary, we have designed and synthesised novel series of hybrid compounds *N*-(pyridin-2-yl) acetamide-substituted benzimidazole chalcones (**9a–j**) in good yield. The structure of the compounds was confirmed by spectroscopic techniques like, IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Subsequently, all the compounds were tested for their *in vitro* anticancer (MCF 7 cell line) and anti- Mycobacterial activity against M. tuberculosis (vaccine strain, H37 RV strain); ATCC No–27294. Most of the **c**ompounds **9a, 9d, 9e, 9f, 9g, 9h, 9i, and 9j** displayed appreciable activity with GI_{50} ranging from 1.0 to 10.0 μM against the MCF 7 cell line. Moreover, compound **9h** in the series exhibited considerable activity against the bacterial strain with a MIC valve of 12.5 μg mL^{-1}. The preliminary anticancer and anti– Mycobacterial activity screenings evaluation showed that the hybrid compound (9h), being di–substituted with flouro group at the chalcone ring of the benzimidazole-chalcone skeleton increases the potency, therefore qualify it a lead compound in the study. Based on this preliminary cytotoxicity and antimycobacterial screening, additional studies are essential to procure more intuitions about the possible incorporation of heterocyclic pharmacophores in a hybrid molecule and their structure-activity-relationship in cancer and tuberculosis therapy.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca4203.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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