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Synthesis, Characterization and Biological Applications of Pyrazole-Benzothiazolamine Conjugates

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Present paper describes the synthesis of some new pyrazole-benzothiazolamine conjugates by molecular conjunction by adopting appropriate synthetic routes. Purification of intermediates and final compounds has been done by recrystallization and chromatographic techniques. Characterization of all the newly synthesized pyrazole-benzothiazolamine derivatives was done by physical and spectral data. The experimental procedure for the preparation of seventeen pyrazole-benzthiazolamine conjugates has been incorporated. Data of synthesized compounds like IR, ^{1}H NMR and Mass spectra were neatly presented. All these compounds were evaluated for their activity against Gram-positive and Gram-negative bacteria and various fungal strains. Anticancer activity has been carried out for the synthesized compounds using HeLa, DU145 and A549 cell lines using MTT assay and we found that the pyrazole-benzothiazolamine conjugates were possess antimicrobial, antifungal and anticancer activities.

Keywords: Pyrazole-benzothiazolamine conjugates, Characterization, Evaluation.

INTRODUCTION

Several benzothiazole derivatives synthesis and their SAR studies was carried by Wang *et al.* [1]. X-ray crystallographic studies [2] of benzthiazoles were reported earlier. Literature survey reveals the information that the benzothiazole moiety is responsible for the anticancer activity [3,4], antimicrobial activity [5-9], antiglutamate activity [10], antileishmanial activity [11], DNA binding ability and anticancer activity [12], antidiabetic activity [13] and such compounds were found to be potent inhibitors of HIV-1 protease [14]. In view of importance of the biological activity of bezothiazolamines, an attempt is made to synthesize, characterize and to evaluate the biological activity of derivatives of pyrazole-benzothiazolamine conjugates.

EXPERIMENTAL

Sodium ethoxide, hydrazine hydrochloride, lithium aluminium hydride, tetrahydrofuran, dimethyl sulphoxide, 2-amino-6-methoxy benzothiazole, 2-amino-6-fluoro benzothiazole, 2-amino-6-chloro benzothiazole, 2-amino-6-carbontrifluoro benzothiazole, 2-amino-benzothiazole, ethyl acetate, 2-iodo benzoic acid, acetone, conc. sulphuric acid, hexane, monomethoxy acetophenone, dimethoxy acetophenone, acetophenone, ethanol, acetophenone, anhydrous sodium sulphate, trimethoxy acetophenone, ammonium chloride, potassium bromate from Sigma Aldrich (all were LR grade) were used as received.

The instruments such as infrared spectra are recorded on Perkin Elmer model 283B and Nicolet 740 FT-IR instruments and values are given in cm^{-1}. Proton nuclear magnetic resonance spectra are recorded on varian Gemini-200, varian Unity-200 and advance-300 MHz Bruker UX-NMR instrument. The samples are made in chloroform-d (1:1) or/and DMSO-d_6 using tetramethyl silane (Me_4Si) as the internal standard. ESI mass spectra were recorded on Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS-MS mass spectrometer. Elemental analysis is carried out on VARFIO EL, se elementor. Analytical thin-layer chromatography (TLC) is performed on pre coated silica-gel-60 F254 (0.5 mm) glass plates. Visualization of the sports on TLC plates is achieved either to iodine vapour or UV light. Employing TLC techniques using appropriate solvent system for development monitored all the reasons. Moisture sensitive reactions are carried out by standard syringe-septum techniques. Dry ether, dry toluene are made by distilling them from sodium benzophenone ketyl and dry methanol is prepared by using potassium hydroxide. All extracts are extracted with ethyl

acetoacetate and water and concentrated at reduced Pressure on Buchi-R-3000 rotary evaporated below 50 °C. Yields of materials judged homogenous by TLC and NMR spectroscopy. Melting Points were recorded on Melter Fp-51 instrument and were uncorrected were used for the characterization of synthesized compounds.

Preparation of ethyl 2,4-dioxo-4-(substituted phenyl) butanoates 8(a-d): Diethyl oxalate (1 mol) was added to freshly prepared sodium ethanolate at 0 °C. After 10 min substituted acetophenones **7(a-d)** (1 mol) were added slowly in small portions maintaining the temperature 0 °C. After completion of addition the stirring was continued at room temperature for 4 h, the reaction mixture was neutralized by diluted H_2SO_4 and extracted with ethyl acetate to offer solid products **8(a-d)** (yield 85-90 %) which were taken as such for the next step without purification.

Preparation of ethyl 3-substituted phenyl-1*H*-pyrazole-5-carboxylates 9(a-d): To each ethyl 2, 4-dioxo-4-(substituted phenyl)butanoates **8(a-d)** (1 mol) was added hydrazine dihydrochloride (NH_2-NH_2·2HCl) (1.5 mol) in ethanol and heated to reflux for 3 h. The solvent was removed under vacuum then added water to the residue and the compound was extracted with ethyl acetate (50 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and evaporated the solvent to obtain crude product that was further purified by column chromatography using ethyl acetate and hexane. The pure compounds **9(a-d)** were eluted at 30-40 % of ethyl acetate with good yields.

Preparation of (3-substituted phenyl-1*H*-pyrazol-5-yl)methanols 10(a-d): To the ethyl 3-substituted phenyl-1*H*-pyrazole-5-carboxylates **9(a-d)**, obtained in the above step was added $LiAlH_4$ (0.5 mol) in dry THF at 0 °C and stirred for 1h at room temperature. Added saturated NH_4Cl solution drop wise to quench the unreacted $LiAlH_4$ and removed the THF under vacuum then extracted with ethyl acetate (100 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and evaporated ethyl acetate to obtain colour less solid products of (3-substitutedphenyl-1*H*-pyrazol-5-yl)methanols **10(a-d)** (yield 70-80 %). The alcohols produced in this step were pure and no further purification was required. These compounds were taken as such for the next step.

Preparation of 3-subtitutedphenyl-1*H*-pyrazole-5-carbaldehydes 11(a-d): To the (3-substituted phenyl-1*H*-pyrazol-5-yl)methanols **10(a-d)** produced in the above step was added IBX (1.2 mol) in DMSO and stirred for 1 h at room temperature. Ice cold water was added to the reaction mixture and extracted with ethyl acetate (50 mL × 4). The organic layer was dried on anhydrous Na_2SO_4 and evaporated the ethyl acetate to obtain corresponding 3-subtituted phenyl-1*H*-pyrazole-5-carbaldehydes **11(a-d)** in good yields (80-85 %).

3-(4-Methoxyphenyl)-1*H*-pyrazole-5-carbaldehyde **11a** was prepared using above method by the addition of [3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl]methanol **10a** (2.04 g, 10 mmol) IBX (3.36 g, 1.2 mmol. 3-(3,4-dimethoxyphenyl)-1*H*-pyrazole-5-carbaldehyde **11b** was prepared using above method by the addition of [3-(3,4-dimethoxyphenyl)-1*H*-pyrazol-5-yl]methanol **10b** (2.34 g, 10 mmol) IBX (3.36 g, 1.2 mmol). (3,4,5-Trimethoxyphenyl)-1*H*-pyrazole-5-carbaldehyde **11c** was prepared using above method by the addition of [3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-5-yl]methanol **10c** (2.64 g, 10 mmol) IBX (3.36 g 1.2 mmol). 3-(Benzo[d][1,3]-dioxol-5-yl)-1*H*-pyrazole-5-carbaldehyde **11d** was prepared using above method by the addition of (3-(benzo[d][1,3]dioxol-5-yl)-1*H*-pyrazol-5-yl)methanol **10d** (2.18 g, 10 mmol) IBX (3.36 g, 1.2 mmol). The obtained carbaldehydes were as such taken in the next step for the synthesis of pyrazole-benzothiazole conjugates **12a-12q.**

Preparation of pyrazole and benzothiazole amine conjugates (12a-12q): To 3-subtituted phenyl-1*H*-pyrazole-5-carbaldehydes **11(a-d)** (1 mmol) obtained in the above step was added substituted 2-amino benzothiazoles (1.0 mmol) in ethanol and heated to reflux for 6 h. The completion of reaction was confirmed by TLC in ethyl acetate and hexane solvent system. The product so obtained was filtered and washed twice with ethanol. Futher these solid products were recrystallized in ethanol to get pure conjugates of pyrazole and benzothiazole amine **(12a-12q)** in good yields (**Scheme-I**).

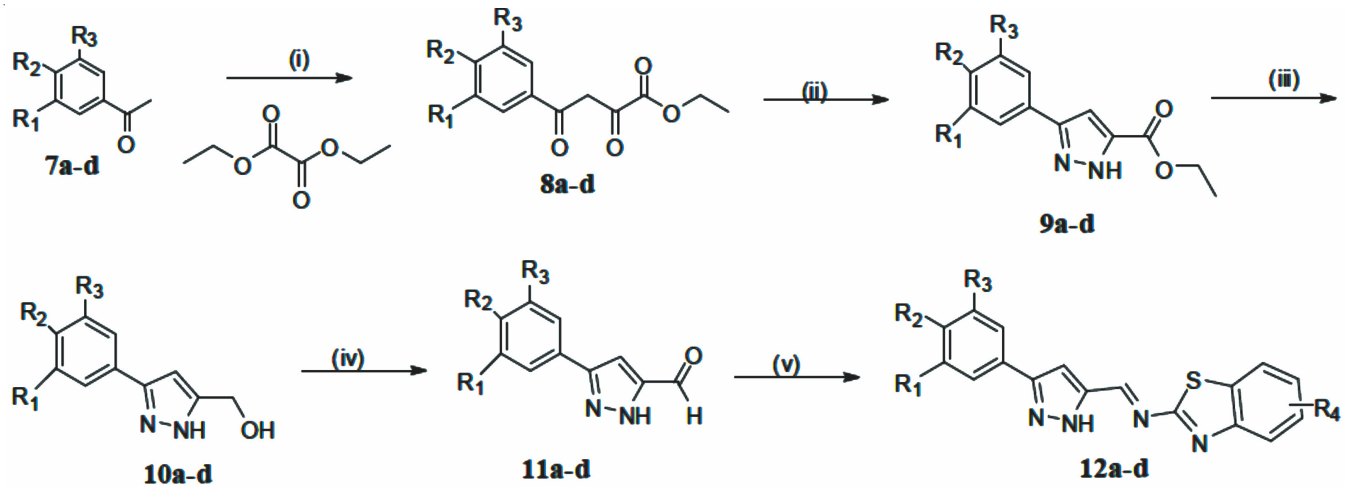
(E)-6-Fluoro-N-[(3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]benzo[d]thiazol-2-amine (12a): Yield: 71 %, brown coloured solid. m.p.: 143-145 °C; IR (Neat, cm^{-1}): 3234, 3000, 2931, 2831, 1526, 1476, 1259, 1197, 1065, 1025, 980, 897, 797, 753, 669; 3243.56 ν(N-H) , 1259.57-ν(C-N) aryl 1065.47 ν(C-F): ^{1}H NMR (DMSO, 300 Hz): δ values: 3.70-3.87 (M, 9H)) –OCH_3; 6.85-7.02 (M, 2H) ArH; 7.06-7.23 (M, 1H) ArH; 7.53-7.79 (M, 3H) ArH; 8.19 (S, 1H) ArH ppm; Mass (ESI) *(m/z):* 365 [M+], 367 [M+2] peak.

(E)-N-[(3-(3,4-Dimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]-6-methoxybenzo[d]thiazol-2-amine (12b): Yield: 79 %, brown coloured solid. m.p.: 148-152 °C; ^{1}H NMR (DMSO, 300 Hz): δ values: 3.79 (s, 3H) –OCH_3, δ = 3.82 (s, 6H) –OCH_3 δ = 6.787 (s, 3H) ArH, δ = 7.07-7-34 (m, 2H) Ar-H δ = 7.28 (s, 2H) Ar-H δ = 7.48-7-63 (m, 1H) Ar-H ppm; Mass (ESI) *(m/z):* 395 [M+] peak.

(E)-N-[(3-(3,4-Dimethoxyphenyl)-1*H*-pyrazol-5-yl)-methylene]-6-fluorobenzo[d]thiazol-2-amine (12c): Yield: 75 %, brown coloured solid; m.p.: 163-165 °C; IR (Neat, cm^{-1}): 3261, 3016, 2937, 2837, 1608, 1545, 1460, 1414, 1331, 1258, 1205, 1159, 1028, 979, 797, 635, 549, 433; 3261 ν(N-H), 1258 ν(C-N) aryl, 1028 ν(C-F), 3016 aromatic C-H stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.65-3.95 (m, 6H) –OCH_3, δ = 6.62-7.03 (m, 2H) ArH, δ = 7.18-7.30 (m, 2H) Ar-H δ = 7.48 (s, 5H) Ar-H ppm; Mass (ESI) *(m/z):* 383 [M+H] peak

(E)-4-Chloro-N-((3-(3,4-dimethoxy phenyl)-1*H*-pyrazol-5-yl)methylene)benzo[d]thiazol-2 amine (12d): Yield: 73 %, light yellow coloured solid; m.p.: 158-160 °C; IR (Neat, cm^{-1}): 3208, 2991, 2830, 1591, 1533, 1434, 1262, 1195, 1107, 1027, 981, 858, 763, 710, 670; 3261 ν(N-H), 1258 ν(C-N) aryl, 3016 -aromatic C-H stretching, 797 -C-Cl stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.65-3.87 (m, 6H) –OCH_3, δ = 7.03 (s, 1H) ArH, δ = 7.08 (s, 1H) Ar-H δ = 7.10 (s, 1H) Ar-H δ = 7.30-7.42 (m, 3H) Ar-H δ = 7.69-7.84 (m, 1H) Ar-H ppm; Mass (ESI) *(m/z):* 399 [M+] peaks.

(E)-N-[(3-(3,4-Dimethoxyphenyl)-1*H*-pyrazol-5-yl)-methylene]-6-methylbenzo[d]thiazol-2-amine (12e): Yield: 78 %, light yellow coloured solid, m.p.: 147-151 °C; IR (Neat, cm^{-1}): 3237, 2931, 2833, 1608, 1524, 1459, 14333, 1259, 1238,



R_1, R_2, R_3, R_4 = Alkyl groups
(i) NaOEt/EtOH/Diethyl oxalate, 4 h, room temperature; (ii) NH_2NH_2·2HCl/EtOH, 3 h, Reflux;
(iii) $LiAlH_4$/THF, 1 h, room temperature; (iv) IBX/DMSO, 1 h, room temperature; (v) 2-Aminobenzothiazoles/EtOH, 4 h, Reflux

Scheme-I

1190, 1142, 1024, 979, 868, 802, 761, 660; 1258 ν(N-H), 1258 ν(C-N) aryl, 3016 -aromatic C-H stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.69-3.77 (m, 6H) –OCH_3, δ = 6.90-7.11 (m, 2H) ArH, δ = 7.13-7.25 (m, 1H) Ar-H δ = 7.33 (s, 3H) Ar-H δ = 7.53-7.63 (m, 1H) Ar-H, δ = 9.60-9.71 (s, 1H) N-H ppm; Mass (ESI) *(m/z):* 379 [M+] peaks.

(E)-6-Fluoro-N-[(3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl)methylene]-benzo[d]thiazol-2-amine (12f): Yield: 79 %, cream coloured solid, m.p.: 163-165 °C; IR (Neat, cm^{-1}): 3012, 2835, 2705, 1611, 1544, 1457, 1287, 1250, 1196, 1114, 1071, 1030, 965, 835, 791, 663; 3261 ν(N-H), 1258 ν(C-N) aryl, 3016 -aromatic ν(C-H), 1071-C-F stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.72-3.76 (m, 3H) –OCH_3, δ = 6.84-6.77 (m, 3H) ArH, δ = 7.10-7.44 (m, 2H) Ar-H, δ = 7.57-7.78 (m, 4H) Ar-H, δ = 9.70 (s, 1H) N-H ppm; Mass (ESI) *(m/z):* 352 [M+] peaks.

(E)-N-[(3-(3,4-Dimethoxyphenyl)-1*H*-pyrazol-5-yl)-methylene]-6-(trifluoromethyl)benzo[d]thiazol-2-amine (12g): Yield: 75 %, white coloured solid, m.p.: 155-157 °C; IR (Neat, cm^{-1}): 3257, 2937, 2834, 1529, 1473, 1325, 1259, 1161, 1107, 1079, 979, 830, 714, 646; 3261 ν(N-H), 1258 ν(C-N) aryl, 3016 -aromatic C-H stretching, 1079 -C-F stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.68-3.84 (m, 3H) –OCH_3, δ = 6.88-7.02 (m, 1H) ArH δ = 7.22 (s, 1H) ArH, δ = 7.34 (s, 2H) Ar-H, δ = 7.59-7.79 (m, 2H) Ar-H, δ = 7.63-7.78 (m, 3H) Ar-H, δ = 8.27 (s, 1H) Ar-H, δ = 9.90 (s, 1H) N-H ppm; Mass (ESI) *(m/z):* 433 [M+] peaks.

(E)-6-methoxy-N-[(3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl)methylene]benzo[d]thiazol-2-amine (12h): Yield: 76 %, brown coloured solid; m.p.: 169-171 °C; IR (Neat, cm^{-1}): 3212, 3021, 2958, 2832, 2707, 1609, 1546, 1467, 1250, 1175, 1058, 964, 831, 793, 659, 534; 3261 ν(N-H), 1258 ν(C-N) aryl, 3016 -aromatic C-H stretching, ^{1}H NMR (DMSO, 300 Hz): δ = 3.72-3.86 (m, 6H) –OCH_3, δ = 6.75-7.04 (m, 4H) Ar, δ = 7.10-7.37 (m, 1H) ArH, δ = 7.42-7.64 (m, 1H) Ar-H, δ = 7.72 (s, 2H) Ar-H ppm; Mass (ESI) *(m/z):* 365 [M+] peaks.

(E)-4-Chloro-N-[(3-(4-methoxyphenyl)-1*H*-pyrazol-5-yl)methylene]benzo[d]thiazol-2-amine (12i): Yield: 78 %, cream coloured solid; m.p.: 166-168 °C; IR (Neat, cm^{-1}): 3443, 3187, 2834, 2704, 1614, 1539, 1412, 1373, 1253, 1178, 1111, 1072, 966, 833, 762, 660, 533; 3187 ν(N-H), 1253 ν(C-N) aryl, 3016 -aromatic C-H stretching, 762-C-Cl stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.74-3.85 (m, 3H) –OCH_3, δ = 6.75-7.06 (m, 4H) ArH, δ = 7.22-7.32 (d, 1H) ArH, δ = 7.45-7.56 (m, 1H) Ar-H, δ = 7.65-7.75 (m, 2H) Ar-H, δ = 9.74-9.95 (m, 1H) N-H ppm; Mass (ESI) *(m/z):* 369 [M+] peak.

(E)-N-[(3-(Benzo[d][1,3]dioxol-5-yl)-1*H*-pyrazol-5-yl)methylene]-6-methoxybenzo[d]thiazol-2-amine (12j): Yield: 78 %, light red coloured solid. m.p.: 152-155 °C; IR (Neat, cm^{-1}): 3188, 3002, 1606, 1548, 1465, 1335, 1288, 1246, 1221, 1166, 1115, 1036, 978, 934, 865, 796, 655; 3188 ν(N-H), 1246 ν(C-N) aryl, 3002 aromatic ν(C-H). ^{1}H NMR (DMSO, 300 Hz): δ = 6.03 (s, 6H) –OCH_2 -O, δ = 6.65-7.0 (m, 3H) ArH, δ = 6.99-7.04 (m, 2H) ArH, δ = 7.12-7.36 (m, 2H) Ar-H, δ = 7.82 (s, 1H) Ar-H ppm; Mass (ESI) *(m/z):* 391 [M+23] peak.

(E)-N-[(3-(Benzo[d][1,3]dioxol-5-yl)-1*H*-pyrazol-5-yl)methylene]-6-fluorobenzo[d]thiazol-2-amine (12k): Yield: 72 %, cream coloured solid; m.p.: 153-158 °C; IR (Neat, cm^{-1}): 3234, 3000, 2931, 2831, 1526, 1476, 1259, 1197, 1065, 1025, 980, 897, 797, 753, 669; 3234 ν(N-H), 1259 ν(C-N) aryl, 3000 aromatic ν(C-H), 1025-C-F stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 5.87-5.99 (m, 2H) –O-CH_2-O, δ = 7.16-7.30 (m, 3H) Ar-H, δ = 7.82 (s, 4H) Ar-H, δ = 6.97-7.20 (s, 1H) Ar-H ppm; Mass (ESI) *(m/z)*: 367 [M+] peaks.

(E)-6-Methoxy-N-[(3-(3, 4, 5-trimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]benzo[d]thiazol-2-amine (12l): Yield: 72 %, brown coloured solid; m.p.: 145-148 °C; IR (Neat, cm^{-1}): 3342, 3204, 2932, 2829, 1600, 1548, 1468, 1422, 1365, 1282, 1226, 1125, 1055, 1003, 903, 832, 764, 661; 3204 ν(N-H), 1282 ν(C-N) aryl, 3204 aromatic C-H stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.73-3.88 (m, 12H) –OCH_3, δ = 6.79-6.84 (m, 1H) ArH, δ = 6.99-7.04 (m, 2H) ArH, δ = 7.22-7.39 (m, 1H) Ar-H, δ = 7.46-7.60 (m, 1H) Ar-H, δ = 8.02 (s, 1H) Ar-H, δ = 9.31-9.36 (m, 1H) N-H ppm; Mass (ESI) *(m/z):* 425 [M+] peaks.

(E)-6-Fluoro-N-[(3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]-benzo[d]thiazol-2-amine (12m): Yield: 73 %, brown coloured solid; m.p.: 167-168 °C; IR (Neat, cm^{-1}): 3171, 2970, 1608, 1554, 1461, 1423, 1340, 1290, 1120, 1010, 905, 842, 764, 659, 566; 3171 ν(N-H), 1290 ν(C-N) aryl, 3000 aromatic ν(C-H), 1010-C-F stretching; ^{1}H NMR (DMSO, 300 Hz): δ = 3.77-3.94 (m, 9H) –OCH_3, δ = 6.79-6.85 (m, 1H) ArH, δ = 6.96-7.08 (m, 3H) ArH, δ = 7.32-7.39 (m, 1H) Ar-H, δ = 7.59 (s, 2H) Ar-H, δ = 9.09-9.17 (m, 1H) N-H ppm; Mass (ESI) *(m/z):* 413 [M+] peaks.

(E)-6-Methoxy-N-[(3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]-benzo[d]thiazol-2-amine (12n): Yield: 74 %, brown coloured solid; m.p.: 158-161 °C; IR (Neat, cm^{-1}): 3208, 3004, 2831, 1594, 1538, 1469, 1423, 1325, 1284, 1236, 1130, 1082, 1046, 1013, 896, 831, 796, 715, 670; 3208 ν(N-H), 1284 ν(C-N) aryl, 3004 aromatic ν(C-H); ^{1}H NMR (DMSO, 300 Hz): δ = 3.71-3.97 (m, 12H) –OCH_3, δ = 6.46 (s, 1H) ArH, δ = 6.82 (s, 1H) ArH, δ = 6.93-6.97 (s, 1H) Ar-H, δ = 7.05-7.16 (s, 2H) Ar-H, δ = 7.25-7.44 (s, 1H) Ar-H, δ = 7.66 (s, 1H) Ar-H, δ = 8.34 (s, 1H) Ar-H ppm; Mass (ESI) *(m/z):* 463 [M+ k] peaks.

(E)-6-Chloro-N-[(3-(3,4,5-trimethoxyphenyl)-1*H*-pyrazol-5-yl)methylene]-benzo[d]thiazol-2-amine (12o): Yield: 72 %, brown coloured solid, m.p.: 144-145 °C; IR (Neat, cm^{-1}): 3180, 2932, 2828, 1595, 1539, 1466, 1421, 1280, 1235, 1203, 1129, 1045, 1000, 890, 811, 781, 652; 3180 ν(N-H), 1235 ν(C-N) aryl, 3016 aromatic ν(C-H), 781 ν(C-Cl); ^{1}H NMR (DMSO, 300 Hz): δ = 3.52-3.81 (m, 9H) –OCH_3, δ = 6.65 (s, 1H) ArH, δ = 6.77-6.88 (m, 2H) ArH, δ = 7.08-7.18 (m, 2H) Ar-H, δ = 7.41 (s, 2H) Ar-H, δ = 8.82 (brs, 1H) N-H ppm; Mass (ESI) *(m/z):* 365 [M+], 367 [M+2] peak.

(E)-N-[(3-(Benzo[d] [1,3]dioxol-5-yl)-1*H*-pyrazol-5-yl)methylene]-6-chlorobenzo[d]thiazol-2-amine (12p): Yield: 71 %, brown coloured solid; m.p.: 155-157 °C; IR (Neat, cm^{-1}): 3180, 3001, 1598, 1541, 1460, 1335, 1288, 1245, 1220, 1167, 1113, 1039, 978, 935, 865, 798, 619, 553; 3180 ν(N-H), 1245 ν(C-N) aryl, 3001 aromatic ν(C-H), 798 ν(C-Cl); ^{1}H NMR (DMSO, 300 Hz): δ = 5.90-6.14 (m, 2H) –O-CH_2 –O-, δ = 6.68-7.04 (m, 3H) ArH, δ = 7.11-7.43 (m, 2H) ArH, δ = 7.88 (s, 3H) Ar-H ppm; Mass (ESI) *(m/z):* 383 [M+] peaks.

(E)-N-[(3-(Benzo[d][1,3]dioxol-5-yl)-1*H*-pyrazol-5-yl)-methylene]-6-(trifluoro methyl)benzo[d] thiazol-2-amine (12q): Yield: 75 %, m.p.: 159-162 °C; IR (Neat, cm^{-1}): 3223, 3021, 2902, 1610, 1572, 1539, 1462, 1324, 1285, 1244, 1163, 1112, 1080, 1040, 35, 863, 830, 801, 717, 645; 3223 ν(N-H), 1244 ν(C-N) aryl, 3021 aromatic ν(C-H), 1080 ν(C-F); ^{1}H NMR (DMSO, 300 Hz): δ = 5.90-6.0 (m, 2H) –O-CH_2-O-, δ = 6.78-6.99 (m, 2H) ArH, δ = 7.24-7.34 (m, 2H) ArH, δ = 7.54-7.84 (m, 3H) Ar-H, δ = 8.08-8.22 (m, 1H) Ar-H ppm; Mass (ESI) *(m/z):* 417 [M+] peaks.

Biological activity study

Antimicrobial activity: The antimicrobial activities of the synthesized compounds were tested using disc diffusion method. Various Gram-positive bacteria like *Bacillus subtilis* MTCC121, *Staphylococcus aureus* MTCC 96, *Staphylococcus* MLS-16 MTCC2940, *Micrococcus luteus* MTCC2470, Gram-negative bacteria like *Escherichia coli* MTCC739, *Pseudomonas aeruginosa* MTCC2453, *Klebsiella planticola* MTCC530, fungal strain like *Candida albicans* MTCC3017 were tested for the synthesized compounds against the standards chloramphinicol and nistatin. 0.1 mL of standard inoculum (1-2 × 10^7 cfu/mL) of each organism was added to freshly prepared sterile Mueller Hilton Agar plates (HiMedia Laboratories) and the compounds dissolved in DMSO was added on the sterile disc, after 18-24 h of incubation the plates were observed for zone of inhibition. The MIC values were determined in each case as the minimum concentration of the compound which inhibited the growth of organism.

Antifungal activity: Selectively **12b** was chosen to check the antifungal activity against selected *Candida* strains (*Issattchenkia orientalis* MTCC 3020, *Candida albicans* MTCC 1637, *Candida albicans* MTCC 3019, *Candida albicans* MTCC 3958, *Candida albicans* MTCC 227, *Candida albicans* MTCC 7315, *Candida albicans* MTCC 854, *Candida albicans* MTCC 183, *Candida albicans* MTCC 3018 and *Candida parapsilosis* MTCC 1744) and showed good activity against *Candida albicans* MTCC3017.

Anticancer activity: Anticancer activity has been carried out for the synthesized compounds using HeLa, DU145 and A549 cell lines by MTT Assay.

RESULTS AND DISCUSSION

Sodium ethoxide acts as a strong base which abstracts proton from methylene carbon of acetophenone to result carbanion. The active carbanion abstracts carbonyl carbon of diethyl oxalate by Claisen-Schmidt condensation. Further removal of ethanol produces **8(a-d)** compounds. Hydrazine dihydro chloride reacts with 2,4-dioxo-4-substituted pentanoic acid ester *via* Knorr condensation to form compound **9(a-d)**. Pyrazole moiety is formed in this step. Ester undergoes reduction with lithium aluminium hydride to form alcohol **10(a-d)**. Then alcohol undergoes selective oxidation with IBX to form aldehyde **11(a-d)**. In the final step, **11(a-d)** reacts with 2-amino benzothiazoles to form pyrazole-benzothiazole conjugates **12(a-d)**.

Biological activity study reveals that few of the synthesized compounds have shown broad spectrum of antimicrobial activity against Gram-positive and Gram-negative organisms. The compound **12h** was found to be most active against Gram-positive, Gram-negative bacteria and fungus (*Candida albicans*), it showed excellent activity against *Bacillus subtilis* MTCC121 (MIC 4 µg/disc), *Staphylococcus* MLS-16 MTCC2940 (MIC 8 µg/disc), *Micrococcus luteus* MTCC2470 (MIC 4 µg/disc), *Staphylococcus aureus* MTCC 96 (MIC 6 µg/disc) and *Escherichia coli* MTCC739 (MIC 16 µg/disc). The compounds **12a**, **12i**, **12j**, **12k** and **12l** also showed moderate to good inhibitory activity but less as compared to **12h**. Overall rest of the synthesized compounds showed average antimicrobial activity when compared to standard compounds and MIC values ranged from (6-80 µg/disc). As per the results observed and thus obtained from the antimicrobial activity, Gram-positive organisms are more affected than Gram-negative organisms by the lead compounds. The antimicrobial activity and inhibitory zone of synthesized compounds against tested strains were incorporated in the Tables 1 and 2, respectively.

TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (MIC, µg/mL)

Compound	*Bacillus subtilis* MTCC121	*Staphylococcus* MLS-16 MTCC2940	*Micrococcus luteus* MTCC2470	*Staphylococcus aureus* MTCC 96	*Escherichia coli* MTCC739	*Pseudomonas aeruginosa* MTCC2453	*Klebsiella planticola* MTCC530	*Candida albicans* MTCC3017
12a	15	12	16	10	25	40	24	24
12b	80	80	80	80	80	80	80	20
12c	41	10	80	80	80	80	80	34
12d	80	80	80	80	80	12	80	80
12e	80	80	80	80	80	80	80	80
12f	80	75	80	75	80	80	80	80
12g	78	80	80	80	75	40	80	80
12h	4	9	5	8	15	26	40	40
12i	10	15	25	10	22	40	25	25
12j	40	80	80	80	78	80	80	80
12k	8	15	12	5	40	25	40	25
12l	20	10	15	40	40	40	24	24
12m	76	80	80	80	80	40	80	80
12n	62	80	80	80	80	60	80	80
12o	40	35	35	35	80	40	80	80
12p	80	60	80	75	80	80	80	80
12q	80	80	80	80	80	80	80	80

a = Gram-positive bacteria; b = Gram-negative bacteria; c = Fungus.

TABLE-2
INHIBITORY ZONE (DIAMETER, mm) OF SYNTHESIZED COMPOUNDS AGAINST TESTED STRAINS

Compound	*Bacillus subtilis* MTCC121	*Staphylococcus* MLS-16 MTCC2940	*Micrococcus luteus* MTCC2470	*Staphylococcus aureus* MTCC 96	*Escherichia coli* MTCC739	*Pseudomonas aeruginosa* MTCC2453	*Klebsiella planticola* MTCC530	*Candida albicans* MTCC3017
12a	20	15	20	16	10	5	10	5
12h	30	25	30	25	20	10	5	5
12i	15	20	12	15	10	6	12	10
12j	5	3	4	3	3	3	3	3
12k	15	15	20	30	6	10	5	10
12l	10	15	15	5	5	5	10	10

a = Gram-positive bacteria; b = Gram-negative bacteria; c = Fungus; Standard (20 µg/disc) was used as positive reference. Synthesized compounds (20 µg/disc).

Antifungal activity: Among the tested *Candida* strains, *Candida albicans* MTCC 1637 and *Candida albicans* MTCC 3020 showed the best activity as compared to others. Overall a very good antifungal activity was observed against most organisms used when compared to standard compounds and MIC values ranged from (4.0-80 µg/disc). Antifungal activity of synthesized compounds were incorporated in Table-3.

Anticancer activity: Few of the compounds have shown moderate to best activity against cancer cell lines. Compounds **12b**, **12n** and **12p** displayed moderate activity against A549 cell lines; **12a**, **12i** and **12o** against HeLa cell lines; **12h** and **12i** against DU145. Best activity has been shown by **12b**, **12c** and **12f** against HeLa cell lines; **12m**, **12n**, **12o** and **12q** against A549 cell lines (Table-4).

Conclusion

In conclusion, the synthesis and characterization of some new pyrazole-benzothiazolamine conjugates are reported and the majority of the synthesized compounds were biological active against Gram-positive and Gram-negative bacteria and various fungal strains. The compound **12h** was found to be most active against Gram-positive, Gram-negative bacteria and

TABLE-3
ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS (MIC, µg/mL)

Organisms	**12a**	**12h**	**12i**	**12j**	**12k**	**12l**
Issattchenkia orientalis MTCC 3020	30	30	35	75	35	35
Candida albicans MTCC 1637	25	40	25	80	25	25
Candida albicans MTCC 3019	40	45	40	80	25	40
Candida albicans MTCC 3958	25	50	40	65	30	35
Candida albicans MTCC 227	40	40	50	60	45	35
Candida albicans MTCC 7315	40	60	40	80	40	45
Candida albicans MTCC 854	30	40	35	80	40	40
Candida albicans MTCC 183	30	40	50	80	50	40
Candida albicans MTCC 3018	40	50	35	70	35	35
Candida parapsilosis MTCC 1744	25	30	30	65	35	30

TABLE-4
ANTICANCER ACTIVITY OF SYNTHESIZED COMPOUNDS (MIC, µg/mL)

Compound	HeLa	DU145	A549
12a	12.2 ± 2.4	16.2 ± 2.0	52.1 ± 1.1
12b	3.6 ± 1.9	26.8 ± 2.4	11.5 ± 1.8
12c	3.6 ± 2.6	20.5 ± 1.4	14.1 ± 2.8
12d	15.1 ± 0.4	32.6 ± 2.4	19.6 ± 1.1
12e	16.2 ± 1.7	9.1 ± 2.0	8.5 ± 2.6
12f	9.3 ± 1.5	16.8 ± 1.3	12.7 ± 2.2
12g	21.6 ± 1.4	15.1 ± 3.4	33.5 ± 3.1
12h	23.9 ± 2.6	12.0 ± 1.9	45.1 ± 1.4
12i	12.0 ± 4.8	28.7 ± 2.0	16.1 ± 3.2
12j	14.8 ± 4.9	28.1 ± 3.2	53.2 ± 2.4
12k	18.3 ± 1.0	15.2 ± 1.4	26.1 ± 2.9
12l	15.1 ± 4.6	17.1 ± 3.8	24.4 ± 3.5
12m	31.1 ± 1.6	26.9 ± 2.6	20.1 ± 1.4
12n	16.9 ± 3.2	32.0 ± 2.6	10.7 ± 1.2
12o	13.5 ± 2.3	15.1 ± 2.9	4.1 ± 1.0
12p	26.5 ± 1.7	19.1 ± 1.2	12.1 ± 1.4
12q	28.9 ± 3.4	22.8 ± 2.7	4.1 ± 1.8

Each data represents mean ± S.D. from three different experiments conducted in triplicates; HeLa: Human cervix cancer cell line; DU145: Human prostate cancer cell line; A549: Human lung adenocarcinoma epithelial cell line.

fungus (*Candida albicans*). The compounds **12a**, **12i**, **12j**, **12k** and **12l** also showed moderate to good inhibitory activity but less as compared to **12h**. Then, **12b**, **12n** and **12p** displayed moderate activity against A549 cell lines; **12a**, **12i** and **12o** against HeLa cell lines; **12h** and **12i** against DU145. Best activity has been shown by **12b**, **12c** and **12f** against HeLa cell lines; **12m**, **12n**, **12o** and **12q** against A549 cell lines. Anticancer effects of these compounds in tumor cells indicated that they are good candidates for further pharmacological studies to discover effective chemotherapeutic for the treatment of cancer diseases.

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