INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

ACETAMIDE LINKED AZETIDINONE-BENZIMIDAZOLE DERIVATIVES: SYNTHESIS AND ANTIBACTERIAL ACTIVITY

Gurvinder Singh [1,2], Charanjit Kaur [1,2], Pardeep Kumar Sharma [1], Rajesh Kumar [1,2], Chander Mohan *[3]
[1]Department of Pharmaceutical Sciences, Lovely Faculty of Applied Medical Sciences, Lovely Professional University, Phagwara (Punjab)-144401, India
[2]IKG Punjab Technical University, Kapurthala (Punjab)-144603, India
[3]Rayat Bahra Institute of Pharmacy, Hoshiarpur, (Punjab)-146001, India
*Corresponding Author Email: guri_ph@yahoo.co.in

Article Received on: 09/12/18 Approved for publication: 22/01/19

DOI: 10.7897/2230-8407.100394

ABSTRACT

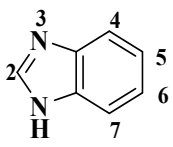
Benzimidazoles have been considered as one of the most potent and biologically active class in medicinal chemistry. A wide variety of benzimidazole analogs have been synthesized so far and screened for their chemotherapeutic importance. In this study, a novel series of acetamide linked azetidinone-benzimidazole analogs have been synthesized to explore the improvement in antibacterial potential with variations in its structure. The derivatives were synthesized by reaction of the Schiff base of 2-phenylbenzimidazole with triethylamine, 1,4-dioxane and chloroacetyl chloride. Thin layer chromatography was used to check the purity of synthesized derivatives and structure of compounds was elucidated using IR, ^{1}HNMR and mass spectrometry. Both Gram-negative strains (*E. coli* and *P. aeruginosa*) and Gram positive strains (S. *aureus* and *B. subtilis*) were used to check the antibacterial activity of synthesized derivatives with cup and plate method. Against the above-mentioned microbes, all the synthesized azetidinone-benzimidazole derivatives exhibited good activity.

Keywords: Benzimidazole, Azetidinones, Hydrazone, Antimicrobial, Antibacterial

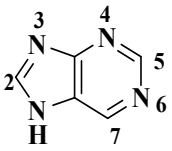
INTRODUCTION

Infectious diseases caused by clinically significant species of microbes remain persistent problem world-wide because of emerging resistance to large number of existing antimicrobial agents such as β-lactam antibiotics, quinolones, vancomycin and macrolides. One way to battle with this challenge is to develop new molecules with antimicrobial activity and to combat with emergence of resistance along with the duration of therapy[1]. Due to vast chemotherapeutic potential, benzimidazoles have received greater attention in the last few decades[2]. Benzimidazoles **(1)** are medicinally important moieties amongst heterocyclics which possess biologically activity. Extensive biochemical and pharmacological studies have confirmed their potential against various strains of microorganisms[3-7] like astemizole (antihistaminic), flubendazole and thiabendazole (anthelmintic) and lansoprazole and omeprazole (antiulcerative). The pharmacology and chemistry of benzimidazoles have been of great interest to medicinal chemist because its derivatives possessed various biological activities such as antimicrobial[8], antioxidant[9], anticancer[10], anthelmintic[11], anti-inflammatory[12], antihypertensive[13], antiprotozoal[14], analgesic[15], antiulcer[16], anticonvulsant[17], anti-hepatitis B virus[18], antifungal[19] and antiviral[20] activity. Amongst these derivatives, the substitution at 2nd position leads to pharmacologically more potent molecules, thus the design and synthesis of 2-substituted benzimidazoles has become a key focus for synthesis of molecules with improved potential in terms of anti-microbial activity[21].

Benzimidazoles, having structural similarity with purines **(2)**, have proven to be a potential competitor to purines in the bacterial protein and nucleic acid synthesis[22].



1*H*-benzo[*d*]imidazole
(1)



7*H*-purine
(2)

Therefore, preparation of benzimidazole derivatives has attracted considerable attention in recent years.

Another important heterocyclic moiety containing valuable antimicrobial activity is azetidinone. Azetidinone, a four membered cyclic amide, is a very well-known compound for the medicinal chemist as it is a part of numerous antibiotic molecules. The azetidinone moiety in form of β-lactam drugs (Penicillins, Nocardicin and Cephalosporin) has been reported to possess antibacterial activity[23]. In addition, Azetidinones have been reported to exhibit anti-tubercular[24], antifungal[25], anti-inflammatory[26], anticonvulsant[27], analgesic[28] and CNS depressant[29] activities.

In the present study, the synthesis of novel acetamide linked azetidinone-benzimidazole hybrid molecule derivatives was carried out along with *in vitro* screening of their antimicrobial activity. Cup and plate method was used for *in vitro* antibacterial

evaluation against gram-negative (*E. coli* and *P. aeruginosa*) and gram positive (S. *aureus* and *B. subtilis*) bacteria.

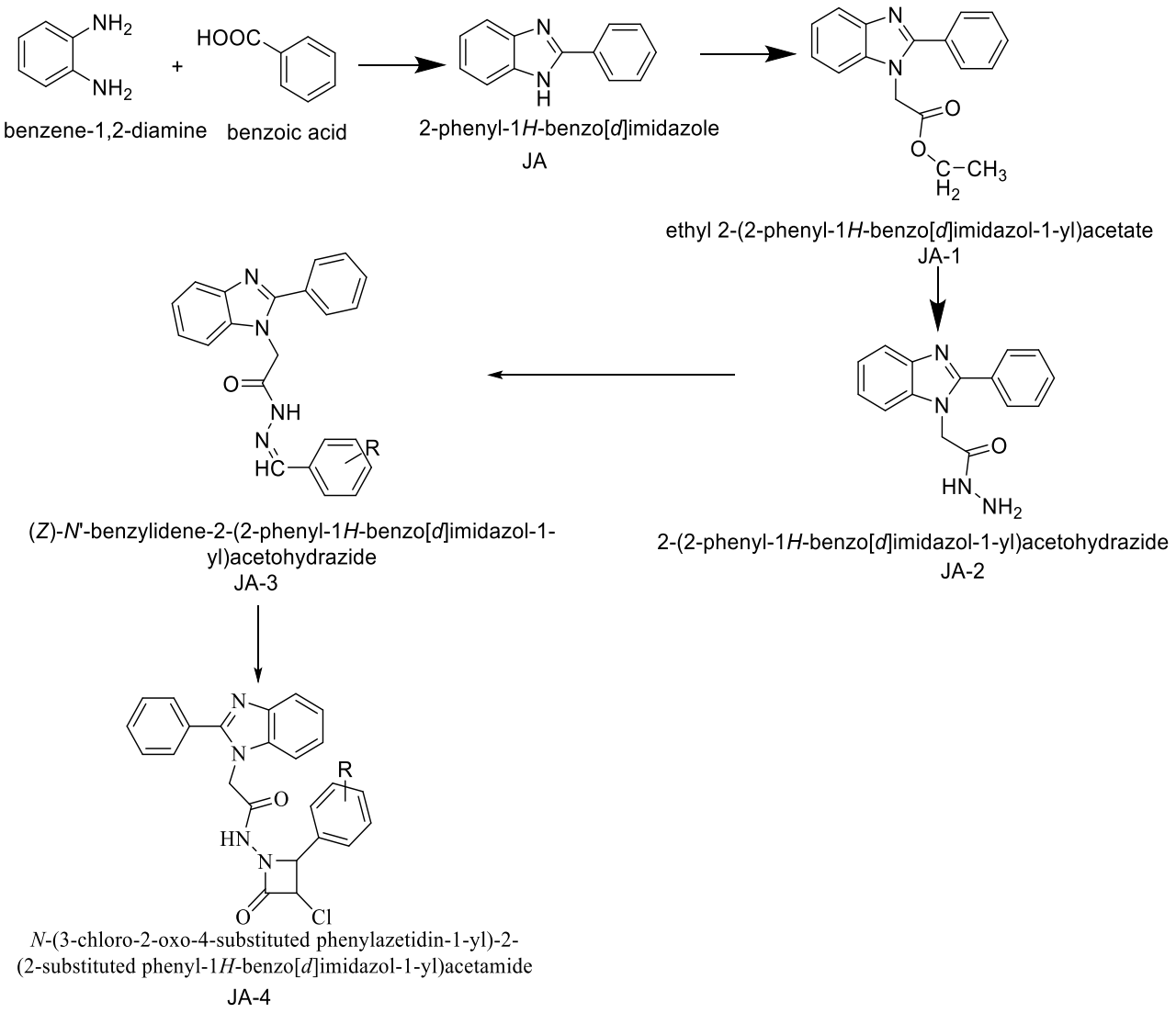
MATERIALS AND METHODS

Chemistry

The chemicals used for the synthesis of acetamide linked azetidinone-benzimidazole derivatives were purchased from Mumbai, Loba Chemie Pvt. Ltd., Mumbai, India; Merck, Electron LLS India Pvt. Ltd.; Qualikems Fine Chem Pvt. Ltd. Vadodara, India and Thermo Electron LLS India Pvt. Ltd. Mumbai.

Digital melting point apparatus (Popular, India) was used to determine the melting point by open capillary method. Shimadzu 8400 FTIR was used to scan IR spectra of the samples using pellets of Potassium Bromide (KBr). Bruker Avance II 400 NMR spectrometer was used to record ^{1}H-NMR spectra. Waters Q-Tof micromass spectrometer was used to record Mass spectra. The elements like C, H and N were analyzed using Thermo Flash 2000 analyzer. The advancement of reaction was checked through thin layer chromatography (TLC) analysis using pre-coated aluminium plates (silica gel 60 F254, Merck-Germany).

Scheme



Scheme: Synthesis of acetamide linked azetidinone-benzimidazole derivatives.

General Procedure

An equimolar quantity of o-phenylenediamine and benzoic acid was allowed to react using polyphosphoric acid to form 2-phenylbenzimidazole, which on further reaction with ethylchloroacetate and potassium carbonate solution in dry acetone, produced ethyl-2-(2-phenyl-1H-benzimidazole-1-yl) acetate. The later was mixed with hydrazine hydrate using ethanol as solvent to form 2-(2-phenyl-IH-benzimidazol-1-yl) acetohydrazide. Schiff base was produced by condensing hydrazide produced in above reaction with substituted aromatic aldehydes. The final hybrid compound was synthesized by reaction of Schiff base with triethylamine,1,4-dioxane and chloroacetyl chloride.

Experimental

2-Phenyl Benzimidazole (JA): FT-IR (KBr, cm^{-1}): 3404 (N-H str), 3047 (=C-H str), 1664 (C=N), 1589 and 1464 (C=C str), 1276 (C-N str) ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.5 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.45 (t, 2H, benzene), 12.8 ((s, 1H, NH), ES-MS (m/z): 194 [M+1]; Anal. for $C_{13}H_{10}N_2$: C;80.39, H;5.19, N; 14.49.

Ethyl-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetate (JA-1): FT-IR (KBr, cm^{-1}): 3047 (=C-H str), 2966 (C-H str), 1772 (C=O str), 1683 (C=N str), 1622 and 1462 (C=C str), 1276 (C-N str), 1226 (C-O-C str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.49 (t, 2H, benzene), 7.39 (t, 1H, benzene), 8.23 (d, 2H, benzene), 7.47 (t, 2H, benzene), 4.7 (s, 2H, -CH_2), 4.3 (q, 2H, -CH_2), 2.0 (t, 3H, -CH_3), ES-MS (m/z): 280 [M+1]; Anal. for $C_{17}H_{16}N_2O_2$: C; 72.84, H; 5.75, N; 9.99, O; 11.41.

2-(2-phenyl-1H-benzo[d]imidazole-1-yl) acetohydrazide (JA-2): FT-IR (KBr, cm^{-1}): 3479 and 3416 (N-H Str), 3047 (=C-H str), 2986 (C-H str), 1637 (C=N str), 1618 (C=O str). 1591 and 1475 (C=C str), 1541 (N-H bend), 1276 (C-N str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.50 (t, 2H, benzene), 7.40 (t, 1H, benzene), 8.22 (d, 2H, benzene), 7.48 (t, 2H, benzene), 4.7 (s, 2H, -CH_2), 9.15 (t, 1H, -Sec NH), 2.15 (d, 2H, -NH_2), ES-MS (m/z): 266 [M+1]; Anal. for $C_{15}H_{14}N_4O$: C; 67.65, H; 5.30, N; 21.04, O; 6.01.

N'-benzylidine-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3): FT-IR (KBr, cm^{-1}): 3117 (N-H Str), 3047 (=C-H str), 2918 (C-H str), 1668 (C=O str), 1622 (C=N str), 1591 and 1460 (C=C str), 1274 (C-N str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.49 (t, 2H, benzene), 7.41 (t, 1H, benzene), 8.20 (d, 2H, benzene), 7.45 (t, 2H, benzene), 4.6 (s, 2H, -CH_2), 8.11(t, 1H, -Sec NH), 8.35 (s, 1H, CH), 6.7 – 7.1 (m, 5H, benzene), ES-MS (m/z): 354 [M+1]; Anal. for $C_{22}H_{18}N_4O$: C;74.56, H;5.12, N; 15.81, O; 4.51.

N'-(3-chlorobenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide(JA-3.1): FT-IR (KBr, cm^{-1}): 3159 (N-H Str), 3047 (=C-H str), 2918 (C-H str), 1665 (C=O str), 1699 (C=N str), 1626 and 1464 (C=C str), 1276 (C-N str), 985 (C-Cl bend), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.49 (t, 2H, benzene), 7.42 (t, 1H, benzene), 8.22 (d, 2H, benzene), 7.46 (t, 2H, benzene), 4.7 (s, 2H, -CH_2), 8.11 (t, 1H, -Sec NH), 8.24 (s, 1H, CH), 7.85 (s, 1H, Aromatic CH), 7.55 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.20 (d, 1H, Aromatic CH) ES-MS (m/z): 388 [M+1]; Anal. for $C_{22}H_{17}ClN_4O$: C; 67.95, H; 4.41, Cl; 9.12, N; 14.41, O; 4.11.

N'-(4-chlorobenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.2): FT-IR (KBr, cm^{-1}): 3150 (N-H Str), 3047 (=C-H str), 2922 (C-H str), 1661 (C=O str), 1695 (C=N str), 1623 and 1460 (C=C str), 1275 (C-N str), 990 (C-Cl bend), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.41 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.44 (t, 2H, benzene), 4.8 (s, 2H, -CH_2), 8.11 (t, 1H, -Sec NH), 8.24 (s, 1H, CH), 7.8 (d, 2H, Aromatic CH), 7.52 (d, 2H, Aromatic CH), ES-MS (m/z): 388 [M+1]; Anal. for $C_{22}H_{17}ClN_4O$: C; 67.95, H; 4.41, Cl; 9.12, N; 14.41, O; 4.11.

N'-(2-hydroxybenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.3): FT-IR (KBr, cm^{-1}): 3243 (-OH broad band), 3047 (=C-H str), 2918 (C-H str), 1666 (C=O str), 1699 (C=N str), 1622 and 1462 (C=C str), 1274 (C-N str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.49 (t, 2H, benzene), 7.41 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.43 (t, 2H, benzene), 4.75 (s, 2H, -CH_2), 8.11 (s, 1H, -Sec NH), 8.24 (s, 1H, CH), 10.8 (s, 1H, OH), 7.55 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.18 (t, 1H, Aromatic CH), 7.58 (d, 1H, Aromatic CH), ES-MS (m/z): 370 [M+1]; Anal. for $C_{22}H_{18}N_4O_2$: C; 71.74, H; 4.90, N; 15.13, O; 8.64.

N'-(3-hydroxybenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.4): FT-IR (KBr, cm^{-1}): 3248 (-OH broad band), 3045 (=C-H str), 2920 (C-H str), 1652 (C=O str), 1696 (C=N str), 1620 and 1460 (C=C str), 1270 (C-N str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.42 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.45 (t, 2H, benzene), 4.71 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 8.24 (s, 1H, CH), 10.8 (s, 1H, OH), 7.85 (s, 1H, Aromatic CH), 7.54 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.20 (d, 1H, Aromatic CH), ES-MS (m/z): 370 [M+1]; Anal. for $C_{22}H_{18}N_4O_2$: C; 71.74, H; 4.90, N; 15.13, O; 8.64.

N'-(4-hydroxybenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.5): FT-IR (KBr, cm^{-1}): 3252 (-OH broad band), 3045 (=C-H str), 2917 (C-H str), 1655 (C=O str), 1698 (C=N str), 1624 and 1464 (C=C str), 1271 (C-N str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.43 (t, 2H, benzene), 4.8 (s, 2H, -CH_2), 8.12 (s, 1H, -Sec NH), 8.23 (s, 1H, CH), 10.81 (s, 1H, OH), 7.81 (d, 2H, Aromatic CH), 7.52 (d, 2H, Aromatic CH), ES-MS (m/z): 370 [M+1]; Anal. for $C_{22}H_{18}N_4O_2$: C; 71.74, H;4.90, N; 15.13, O; 8.64.

N'-(3-Nitrobenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.6): FT-IR (KBr, cm^{-1}): 3140 (N-H Str), 3030 (=C-H str), 2925 (C-H str), 1664 (C=O str), 1694 (C=N str), 1590 and 1465 (C=C str), 1270 (C-N str), 1545 and 1348 (N=O bend), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.43 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.54 (t, 2H, benzene), 4.7 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 8.24 (s, 1H, CH), 7.85 (d, 1H, Aromatic CH), 7.54 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.2 (d, 1H, Aromatic CH), ES-MS (m/z): 399 [M+1]; Anal. for $C_{22}H_{17}N_5O_3$: C; 66.16, H; 4.29, N; 17.53, O; 12.02.

N'-(4-nitrobenzylidine)-2-(2-phenyl-1H-benzo[d]imidazole-1-yl)acetohydrazide (JA-3.7): FT-IR (KBr, cm^{-1}): 3142 (N-H Str), 3028 (=C-H str), 2928 (C-H str), 1667 (C=O str), 1695 (C=N str), 1592 and 1468 (C=C str), 1275 (C-N str), 1542 and 1350 (N=O bend), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.47 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.44 (t, 2H, benzene), 4.80 (s, 2H, -CH_2), 8.11 (s, 1H, -Sec NH), 8.23 (s, 1H, CH), 7.82 (d, 2H, Aromatic CH), 7.52 (d, 2H, Aromatic CH), ES-MS (m/z): 399 [M+1]; Anal. for $C_{22}H_{17}N_5O_3$: C; 66.16, H; 4.29, N; 17.53, O; 12.02.

***N*-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl) acetoamide (JA-4):** FT-IR (KBr, cm^{-1}): 3275 (N-H Str), 3050 (=C-H str), 2918 (C-H str), 1668 (C=O amide str), 1742 (C=O cyclic amide str), 1622 (C=N str), 1591 and 1460 (C=C str), 1545 (C-N cyclic amide str), 1274 (C-N str), 740 (C-Cl str) , ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.42 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.44 (t, 2H, benzene), 4.6 (s, 2H, -CH_2), 8.1 (s, 1H, -Sec NH), 5.1 (d, 1H, -CH), 5.4 (d, 1H, -CH), 6.75 – 6.96 (m, 5H, Aromatic CH), ES-MS (m/z): 430 [M+1]; Anal. for $C_{24}H_{19}ClN_4O_2$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(3-chlorophenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.1):** FT-IR (KBr, cm^{-1}): 3270 (N-H Str), 3052 (=C-H str), 2918 (C-H str), 1665 (C=O amide str), 1740 (C=O cyclic amide str), 1622 (C=N

str), 1591 and 1460 (C=C str), 1542 (C-N cyclic amide str), 1275 (C-N str), 980 (aromatic C-Cl str) , 740 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.61 (d, 2H, benzene), 7.47 (t, 2H, benzene), 7.41 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.45 (t, 2H, benzene), 4.8 (s, 2H, -CH_2), 8.11 (s, 1H, -Sec NH), 5.12 (d, 1H, -CH), 5.45 (d, 1H, -CH), 7.86 (s, 1H, Aromatic CH), 7.54 (d, 1H, Aromatic CH), 7.33 (t, 1H, Aromatic CH),7.2 (d, 1H, Aromatic CH), ES-MS (m/z): 464 [M+1]; Anal. for $C_{24}H_{18}Cl_2N_4O_2$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.2):** FT-IR (KBr, cm^{-1}): 3271 (N-H Str), 3050 (=C-H str), 2919 (C-H str), 1662 (C=O amide str), 1738 (C=O cyclic amide str), 1621 (C=N str), 1590 and 1461 (C=C str), 1540 (C-N cyclic amide str), 1278 (C-N str), 982 (aromatic C-Cl str) , 745 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.49 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.45 (t, 2H, benzene), 4.8 (s, 2H, -CH_2), 8.11 (s, 1H, -Sec NH), 5.1 (d, 1H, -CH), 5.44 (d, 1H, -CH), 7.81 (d, 2H, Aromatic CH), 7.52 (d, 2H, Aromatic CH), ES-MS (m/z): 464 [M+1]; Anal. for $C_{24}H_{18}Cl_2N_4O_2$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.3):** FT-IR (KBr, cm^{-1}): 3360 (-OH Broad band), 3050 (=C-H str), 2920 (C-H str), 1663 (C=O amide str), 1738 (C=O cyclic amide str), 1624 (C=N str), 1592 and 1460 (C=C str), 1542 (C-N cyclic amide str), 1280 (C-N str), 745 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.42 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.43 (t, 2H, benzene), 4.76 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 5.1 (d, 1H, -CH), 5.42 (d, 1H, -CH), 10.8 (s,1H, -OH) 7.54 (d, 1H, Aromatic CH), 7.33 (t, 1H, Aromatic CH), 7.18 (t, 1H, Aromatic CH), 7.57 (d, 1H, Aromatic CH) ES-MS (m/z): 446 [M+1]; Anal. for $C_{24}H_{19}ClN_4O_3$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.4):** FT-IR (KBr, cm^{-1}): 3364 (-OH Broad band), 3035 (=C-H str), 2922 (C-H str), 1663 (C=O amide str), 1735 (C=O cyclic amide str), 1625 (C=N str), 1592 and 1461 (C=C str), 1544 (C-N cyclic amide str), 1281 (C-N str), 743 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.47 (t, 2H, benzene), 7.41 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.44 (t, 2H, benzene), 4.70 (s, 2H, -CH_2), 8.1 (s, 1H, -Sec NH), 5.1 (d, 1H, -CH), 5.4 (d, 1H, -CH), 10.7 (s,1H, -OH) 7.86 (s, 1H, Aromatic CH), 7.5 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.21 (d, 1H, Aromatic CH) ES-MS (m/z): 446 [M+1]; Anal. for $C_{24}H_{19}ClN_4O_3$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.5):** FT-IR (KBr, cm^{-1}): 3365 (-OH Broad band), 3049 (=C-H str), 2921 (C-H str), 1665 (C=O amide str), 1737 (C=O cyclic amide str), 1628 (C=N str), 1590 and 1460 (C=C str), 1545 (C-N cyclic amide str), 1280 (C-N str), 745 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.47 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.43 (t, 2H, benzene), 4.75 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 5.1 (d, 1H, -CH), 5.4 (d, 1H, -CH), 10.8 (s,1H, -OH) 7.82 (d, 2H, Aromatic CH), 7.52 (d, 2H, Aromatic CH), ES-MS (m/z): 446 [M+1]; Anal. for $C_{24}H_{19}ClN_4O_3$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(3-nitroyphenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.6):** FT-IR (KBr, cm^{-1}): 3150 (-NH str), 3045 (=C-H str), 2920 (C-H str), 1738 (C=O cyclic amide str), 1667 (C=O amide str), 1627 (C=N str), 1593 and 1465 (C=C str), 1545 and 1345 (N=O str), 1280 (C-N str), 746 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.62 (d, 2H, benzene), 7.48 (t, 2H, benzene), 7.42 (t, 1H, benzene), 8.2 (d, 2H, benzene), 7.4 (t, 2H, benzene), 4.70 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 5.10 (d, 1H, -CH), 5.40 (d, 1H, -CH), 7.86 (s, 1H, Aromatic CH) 7.55 (d, 1H, Aromatic CH), 7.32 (t, 1H, Aromatic CH), 7.21 (d, 1H, Aromatic CH), ES-MS (m/z): 475 [M+1]; Anal. for $C_{24}H_{18}ClN_5O_4$: C, 60.50; H, 4.23; N, 35.27.

***N*-(3-chloro-2-(4-nitroyphenyl)-4-oxoazetidin-1-yl)-2-(2-phenyl-1*H*-benzo[d]imidazole-1-yl)acetoamide (JA-4.7):** FT-IR (KBr, cm^{-1}): 3148 (-NH str), 3048 (=C-H str), 2923 (C-H str), 1736 (C=O cyclic amide str), 1665 (C=O amide str), 1628 (C=N str), 1590 and 1464 (C=C str), 1543 and 1346 (N=O str), 1278 (C-N str), 746 (C-Cl str), ^{1}H-NMR (400MHz, $CDCl_3$ δ ppm): 7.6 (d, 2H, benzene), 7.46 (t, 2H, benzene), 7.4 (t, 1H, benzene), 8.21 (d, 2H, benzene), 7.43 (t, 2H, benzene), 4.75 (s, 2H, -CH_2), 8.10 (s, 1H, -Sec NH), 5.10 (d, 1H, -CH), 5.4 (d, 1H, -CH), 7.82 (d, 2H, Aromatic CH) 7.51 (d, 2H, Aromatic CH), ES-MS (m/z): 475 [M+1]; Anal. for $C_{24}H_{18}ClN_5O_4$: C, 60.50; H, 4.23; N, 35.27.

The physiochemical characterization of synthesized derivatives is given in table 1.

Table 1: Physiochemical characteristics of synthesized compounds

Compound Number	R	Molecular Formula	Molecular weight	M.P. [°C]	R_f [value][a]	Yield (%)
JA-4	-H	$C_{24}H_{19}ClN_4O_2$	430	291-293	0.82	82.4
JA-4.1	-3-Cl	$C_{24}H_{18}Cl_2N_4O_2$	464	297-298	0.80	80.2
JA-4.2	-4-Cl	$C_{24}H_{18}Cl_2N_4O_2$	464	304-305	0.89	79.2
JA-4.3	-2-OH	$C_{24}H_{19}ClN_4O_3$	446	311-312	0.81	79.5
JA-4.4	-3-OH	$C_{24}H_{19}ClN_4O_3$	446	306-308	0.83	81.4
JA-4.5	-4-OH	$C_{24}H_{19}ClN_4O_3$	446	309-310	0.82	82.6
JA-4.6	-3-NO_2	$C_{24}H_{18}ClN_5O_4$	475	320-322	0.75	87.3
JA-4.7	-4-NO_2	$C_{24}H_{18}ClN_5O_4$	475	328-329	0.86	81.5

[a]*TLC mobile phase: hexane: Ethyl acetate (7.5:2.5)*

Table 2: Evaluation of anti-bacterial activity of acetamide linked azetidinone-benzimidazoles

Bacterial strain	*B. subtilis* (736)			*S. aureus* (9542)			*E. coli* (1698)			*P. aeruginosa* (6458)		
Conc. (µg/ml)	25	50	100	25	50	100	25	50	100	25	50	100
Compound Number	**Zone of inhibition (mm)**											
JA-4	**22**	**29**	**33**	**20**	**27**	**31**	**19**	**25**	**29**	**20**	**26**	**30**
JA-4.1	**21**	**27**	**30**	**19**	**25**	**29**	**20**	**25**	**28**	**20**	**25**	**30**
JA-4.2	17	19	22	15	20	22	16	20	23	17	20	23
JA-4.3	16	19	23	16	18	23	17	21	25	15	19	21
JA-4.4	15	19	22	16	20	22	19	23	25	15	19	22
JA-4.5	16	19	23	18	20	22	17	21	24	19	21	24
JA-4.6	16	18	21	16	19	21	15	17	20	15	19	22
JA-4.7	**22**	**28**	**29**	**19**	**23**	**27**	**20**	**24**	**28**	**20**	**23**	**28**
Ciprofloxacin	17	19	22	21	24	26	21	26	29	22	26	28
DMSO	0	0	0	0	0	0	0	0	0	0	0	0

Anti-microbial activity

Gram negative *(Escherichia coli, Pseudomonas aeruginosa)* and gram positive (*Staphylococcus aureus, Bacillus subtilis)* bacteria were used to check the *in vitro* anti-bacterial activity of synthesized derivatives using cup and plate method[30]. Three different concentrations (25, 50 and 100 µg/ml) were used to check the activity of synthesized benzimidazoles against selected strains. For the comparison of antibacterial activity of synthesized compounds, ciprofloxacin was used as the standard drug. The results obtained by using cup and plate method has been summarized in table 2.

RESULTS AND DISCUSSION

2-(Substituted phenyl)-1H-benzimidazoles were synthesized in 5 steps by the reaction of *o*-phenylenediamine with benzoic acid using polyphosphoric acid. The products of first step were further reacted with ethylchloroacetate in dry acetone to give ethyl-2-[2-(substituted phenyl)-*1H*- benzimidazol-1-yl]acetate which was treated with hydrazine hydrate to form 2-[2-(substituted phenyl)-*1H*- benzimidazol-1-yl]acetohydrazide. Finally, the end substituted aceto-hydrazides were reacted with substituted aldehydes to yield Schiff base of benzimidazole. The resultant Schiff bases were treated with triethylamine,1,4-dioxane and chloroacetyl chloride to produce acetamide linked azetidinone-benzimidazole hybrid molecule derivatives.

Elemental analysis was done to confirm the formula of the synthesized molecules. Structures were confirmed with the help of IR, ^{1}H NMR and ES-MS spectra. In the IR spectra, all the vibrational bands appeared in the predictable regions. The appearance of a single band in the region of 3045-3404 cm^{-1} confirmed the presence of sec. N-H functional group. For ester and amide, C=O str. vibrations appeared at 1735-1749 and 1610-1620 cm^{-1} respectively. ^{1}H NMR spectrum was used to identify different types of protons in the synthesized derivatives. M+1 peaks of the synthesized compounds were found to be in agreement with their molecular formula.

The synthesized benzimidazole azetidinone derivatives showed good antibacterial potential *in vitro* against both Gram-positive and Gram-negative bacteria. Among these, JA-4, JA-4.1 and JA-4.7 were found to possess better antibacterial activity as compared to standard drug ciprofloxacin. At a concentration of 25µg/ml, compound JA-4 and JA-4.7 showed maximum activity against *B. subtilis* (zone of inhibition- 22 mm for each) which was better as compared to standard drug (Ciprofloxacin). JA-4 showed good activity against *B. subtilis* (zone of inhibition- 29 mm) at 50µg/ml concentration. JA-4 and JA- 4.1 exhibited the maximum antibacterial activity against all strains of bacteria at a concentration of 100µg/ml.

CONCLUSION

A novel series of acetamide linked azetidinone-benzimidazole hybrid molecule derivatives was synthesized. Mass, IR, NMR, and elemental analysis were used to elucidate the structures of all the synthesized molecules. Cup and plate method were used to evaluate the *in vitro* anti-bacterial activity of synthesized azetidinone-benzimidazole hybrids against both Gram negative and Gram positive strains of bacteria. All the benzimidazole derivatives exhibited good antibacterial activity and compounds JA-4, JA-4.1, and JA-4.7 were observed to possess better antibacterial activity as compared to standard drug ciprofloxacin.

ACKNOWLEDGMENT

The author(s) would like to acknowledge IKG Punjab Technical University, Kapurthala, Punjab, India and Panjab University, Chandigarh for providing spectral and analytical data of synthesized compounds.

REFERENCES

1. He BY, Yang JW, Robinson D, Risen L, Ranken R, Blyn L, et al., 2- Piperidin-4-yl benzimidazoles with Broad Spectrum Antibacterial Activities. Bioorg. Med. Chem. Lett 2003;13:3253-3256.
2. Pathak D, Siddiqui N, Bhrigu B, Ahsan W, Alam, SM. Benzimidazoles: A New Profile of Biological Activities. Sch. Res. Lib 2010;2:27-34.
3. Zhou R, Skibo EB. Chemistry of the Pyrrolo[1,2-a]benzimidazole Antitumor Agents: Influence of the 7-Substituent on the Ability To Alkylate DNA and Inhibit Topoisomerase II. J. Med. Chem 1996;39 (21):4321–4331.
4. Srivastava RP, Sharma S. Synthesis of 2,5-disubstituted benzimidazoles, 1,3,4-thiadiazoles and 3,5-diiodosalicylanilides as structural congeners of rafoxanide and closantel. Die Pharmazie 1990;45:34.
5. Kubo K, et al., Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazoles. J. Med. Chem 1993;36:1772.
6. Kubo K, et al., Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazole carboxylic acids. J. Med. Chem 1993;36:2182.
7. Kubo K, et al., Nonpeptide angiotensin II receptor antagonists. Synthesis and biological activity of benzimidazole carboxylic acids. J. Med. Chem 1993; 36:2343.
8. Jafar AA, Kaliapillai NVK, Venkatraman BR, Venkatesh G. Synthesis, Characterization and Biological Activity of some New Benzimidazole Derivative. Orbital 2009;1(4):306-309
9. Gurer HH, Orhan H, Suzen S, Puskullu MO, Buyukbingol E. Synthesis and Evaluation of In vitro Antioxidant Capacities

of Some Benzimidazole Derivatives. J. Enz Inhib Med Chem 2006;21(2):241-247.
10. Zienab MN, et al., Novel Benzimidazole Derivatives as Expected Anticancer Agents. Acta Poloniae Pharmaceutica-Drug Res 2011;68(4): 519-534.
11. Sreena K, Ratheesh R, Rachana M, Poornima M, Shyni C. Synthesis and Anthelmintic Activity of Benzimidazole Derivatives. Hygeia 2009;1(1): 21-22
12. Lazer ES, Matteo MR, Possanza GJ. Benzimidazole derivatives with atypical ntiinflammatory activity. J Med Chem 1987;30(4):726-729.
13. Jat RK, Jat J, Pathak DP. Synthesis of Benzimidazole Derivatives: As Anti-hypertensive Agents. E-J Chem 2006;3(4):278-285.
14. Katiyar SK, Gordon VR, Mclaughlin GL, Edlind TD. Antiprotozoal Activities of Benzimidazoles and Correlations with 13-Tubulin Sequence. Antimicrob. Age and Chemotherap 1994;38(9):2086-2090
15. Eswayah A, et al., Synthesis and Analgesic Activity Evaluation of Some New Benzimidazole Derivatives. American Journal of Chemistry and Application 2017;4(5): 30-35.
16. Cho SY, Kang SK, Kim SS, Cheon HG, Choi JK, Yum EK. Synthesis and SAR of Benzimidazole Derivatives Containing Oxycyclic Pyridine as a Gastric H+/K+-ATPase Inhibitors. Bull. Korean Chem. Soc 2011; 22(11):1217-1223.
17. Shingalapur RV, Hosamani KM, Keri RS, Hugar MH. Derivatives of Benzimidazole Pharmacophore: Synthesis, Anticonvulsant, Antidiabetic and DNA Cleavage Studies. Eur. J. Med. Chem 2010;4(5):1753-1759.
18. Li YF, et al., Synthesis and Anti-Hepatitis B Virus Activity of Novel Benzimidazole Derivatives. J Med Chem 2006;49(15):4790-4794.
19. Sanja O, Kuzmanovi P, Dijana JB, Dragoljub DC. Quantitative Structure-Activity Relationship of Some 1-Benzyl benzimidazole Derivatives as Antifungal Agents. APTEFF 2007;38:139-147.
20. Tonelli M, et al., Antiviral activity of benzimidazole derivatives. I. Antiviral activity of 1-substituted-2-[(benzotriazol-1/2-yl)methyl]benzimidazole.Chem Biodivers 2008;5(11):2386-2401.
21. Alasmary FAS, Snelling AM, Zain ME, Alafeefy AM, Awaad AS, Karodia N. Synthesis and Evaluation of Selected Benzimidazole Derivatives as Potential Antimicrobial Agents. Molecules 2015;20:15206-15223.
22. Kavitha CS, Kallappa A, Hosamani M, Seetharamareddy HR. In vivo Analgesic and Anti-inflammatory Activities of Newly Synthesized Benzimidazole Derivatives. Eur. J. Med. Chem 2010;45:2048-2054.
23. Deep A, Kumar P, Narasimhan B, Lim SM, Ramasamy K, Mishra RK, Mani V. 2-Azetidinone Derivatives: Synthesis, Antimicrobial, Anticancer Evaluation and QSAR Studies. Acta Pol Pharm 2016;73(1):65-78.
24. Patel RB, Desai PS, Desai KS, Chikhalia KH. Synthesis of Pyrimidine based Thiazolidinones and Azetidinones: Antimicrobial and Antitubercular agents. Indian J. Chem 2006;45B:773.
25. Freddy HH, Kumar S, Mishra J. Synthesis of some azetidi-2-ones and thiazolidin-4-ones as potential antimicrobial Agents. Indian J. Heterocy. Chem 2004;13:197-200.
26. Kumar VMMJ, Nagaraja TS, Shameer H, Jayachandran E, Sreenivasa GM. N-Substituted-3-chloro-2-azetidinones: Synthesis and Characterization of New Novel Anti-inflammatory agents. J. Pharm. Sci. & Res 2009;1(2):83-92.
27. Kumar VMMJ, Yogananda R, Snehalatha, Shameer H, Jayachandran E, Sreenivasa GM. Synthesis and Characterization of Novel N-Substituted-3-chloro-2-Azetidinones as Potential Anticonvulsant Agents. Biomed Sci and Res 2009;1(1):1-10
28. Shanmugapandiyan P, Denshing KS, Ilavarasan R, Anbalagan N, Nirmal R. Synthesis and Biological Activity of 2-(Thiazolidin-4-one) phenyl]-1H-phenyl benzimidazoles and 2-[4-(Azetidin-2-one)-3-chloro-4-phenyl]-1H-phenyl benzimidazoles. Int. J. Pharma. Sci. and Drug Res 2010;2(2):115-119
29. Gurupadayya BM, Gopal M, Padmashali B, Manohara YN. Synthesis and Pharmacological Evaluation of Azetidin-2-ones and Thiazolidin-4-ones encompassing Benzothiazole. Indian J. Pharm. Sci 2008; 70(5):572-577.
30. Alemam MA, Abdelkarim AM, Karrar E. Comparative evaluation of antimicrobial activity of sudanese honey samples collected from different regions. World Journal of Pharmacy and Pharmaceutical Sciences 2017;12 (6):225-232.

Cite this article as:

Gurvinder Singh *et al*. Acetamide linked azetidinone-benzimidazole derivatives: Synthesis and antibacterial activity. Int. Res. J. Pharm. 2019;10(3):148-153 http://dx.doi.org/10.7897/2230-8407.100394

Source of support: Nil, Conflict of interest: None Declared