FABAD J. Pharm. Sci., 46, 3, 0-XX, 2021

RESEARCH ARTICLE

Synthesis, Characterization and *In Vitro* Evaluation for Antimicrobial and Anthelmintic Activity of Novel Benzimidazole Substituted 1,3,4-Thiadiazole Schiff's Bases

Saravanan KALIYAPERUMAL[*], Priyabrata PATTANAYAK[**,°]

Synthesis, Characterization and In Vitro Evaluation for Antimicrobial and Anthelmintic Activity of Novel Benzimidazole Substituted 1,3,4-Thiadiazole Schiff's Bases

SUMMARY

Benzimidazoles, 1,3,4-Thiadiazoles, and Schiff bases have shown many properties against different types of diseases, including bacterial infection and helminthiasis. Because of the need for new antimicrobial and anthelmintic agents, novel benzimidazole substituted 1,3,4-thiadiazole Schiff's bases were designed and synthesized. The synergy arising from the successful incorporation of benzimidazole ring, thiadiazole ring, and Schiff's base in one pharmacophore was exploited in this work. Eleven such derivatives were synthesized and investigated for their in vitro antimicrobial and anthelmintic properties.1H-benzo[d]imidazole-2-carboxylic acid was first prepared by the oxidation of 2-methyl-1H-benzo[d] imidazole with alkaline potassium permanganate. 1H-benzo[d] imidazole-2-carboxylic acid was then converted to N-arylidene-5-(1H-benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-amine by reacting with an aqueous solution of thiosemicarbazide in the presence of few drops of concentrated sulphuric acid. Finally, different benzimidazole substituted 1,3,4-thiadiazole Schiff's bases were prepared by reacting thiadiazole substituted benzimidazole with suitable aryl aldehyde. Compound PP-4 was found to be more potent than the standard drug in causing the death of nematodes, which took an average time of 13.22 and 19.00 min against Perionyx excavatus and Pheretima posthuma, respectively. Compounds PP-4, PP-6, and PP-8 containing electron-withdrawing groups (4-nitro, 2-bromo, 4-chloro) exhibited antimicrobial activity with the zone of inhibition ranging from 8-27 mm comparable to Ampicillin with the value ranging from 22-27 mm for all the tested strains.

Key Words: *Schiff base, Benzimidazole, 1,3,4-Thiadiazole, Anthelmintic activity, Helminthiasis, Antibacterial*

Yeni Benzimidazol Sübstitüe 1,3,4-Tiyadiazol Schiff Bazlarının Sentezi, Karakterizasyonu ve Antimikrobiyal ve Anthelmintik Aktivitesinin In Vitro Değerlendirmesi

ÖZ

Benzimidazoller, 1,3,4-Tiyadiazoller ve Schiff bazları çeşitli bakteriyel enfeksiyonlar ve helmintiyazis gibi hastalıklara karşı farklı özellikler göstermektedir. Yeni antimikrobiyal ve antihelmintik bileşiklere duyulan ihtiyaç göz önüne alınarak yeni benzimidazol-sübstitüe 1,3,4-tiyadiazol Schiff bazları tasarlanmış ve sentezlenmiştir. Bu araştırmada benzimidazol halkası, tiyadiazol halkası ve Schiff bazı farmakoforlarının başarılı bir şekilde bir araya gelmesiyle elde edilecek sinerjiden yararlanılması planlanmıştır. Bu amaçla, 11 türev sentezlenip, in vitro antimikrobiyal ve antihelmintik özellikleri açısından araştırılmıştır. Öncelikle, 1H-benzo[d]imidazol-2-karboksilik asit, 2-metil-1H-benzo[d]imidazolün alkali potasyum permanganat ile oksidasyonu ile hazırlanmıştır. Daha sonra, 1H-benzo[d]imidazol-2-karboksilik asit birkaç damla konsantre sülfürik asit varlığında tiyosemikarbazidin sulu çözeltisi ile muamele edilerek N-ariliden-5-(1H-benzo[d]imidazol-2-yl)-1,3,4-tiyadiazol-2-amin'e dönüştürülmüştür. Son olarak, farklı benzimidazol-sübstitüe Schiff bazları uygun arilaldehit ile tiyadiazol-sübstitüe benzimidazol halkasının reaksiyonu ile hazırlanmıştır. Bileşik PP-4'ün Perionyx excavatus and Perionyx posthuma'ya karşı nematodları öldürme etkisinin, sırasıyla 13.22 ve 19.00 dakika süreler ile standart ilaçtan daha güçlü olduğu bulunmuştur. Elektron çekici gruplar (4-nitro, 2-bromo, 4-kloro) içeren PP-4, PP-6 ve PP-8 bileşikleri tüm suşlarda 8-27 mm inhibisyon alanı ile 22-27 mm inhibisyon değeri gösteren Ampisilin ile karşılaştırılabilir antimikrobiyal aktivite sergilemiştir.

Anahtar Kelimeler: *Schiff bazı, Benzimidazol, 1,3,4-Tiyadiazol, Antihelmintik aktivite, Helmintiyazis, Antibakteriyel*

Received: XX.XX.XXXX
Revised: XX.XX.XXXX
Accepted: XX.XX.XXXX

[*] ORCID: 0000-0002-8859-8099, Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India, 305004.
[**] ORCID: 0000-0003-4035-1182, Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer, Rajasthan, India, 305004.

° Corresponding Author; Priyabrata Pattanayak
Phone: +91 9438269361, e-mail: Priyabrata2005@gmail.com

INTRODUCTION

Antimicrobial resistance (AMR) to antibiotics is one of the global threats to public health standards, and it has reduced the efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. This raises the need to search for new potent antimicrobial agents with reduced resistance to pathogens and having a broad spectrum of biological activity. On the other side, infections with parasitic helminths are important causes of morbidity and mortality globally. Anthelmintics are anti-parasitic drugs that expel parasitic worms from the human body without causing significant damage to the host. Resistance to benzimidazoles used to treat helminthiasis, has been reported. There are genetic factors in parasitic helminths that favor the development of anthelmintic resistance (Ahn et al., 1993). Frequent usage of the same group of anthelmintic drugs, using anthelmintics in sub-optimal doses, prophylactic mass treatment of domestic animals, and continuous use of a single drug has contributed to the overall development of anthelmintic resistance (El-Zemity et al., 2006).

In the last few decades, the chemistry of five-membered heterocyclics like 1, 3, 4 - thiadiazole and five - membered fused heterocyclics like benzimidazoles are reported to show a wide spectrum of biological activity. Benzimidazoles like albendazole, thiabendazole, and flubendazole (anthelmintic) (Figure 1), omeprazole, and lansoprazole (anti ulcerative), and astemizole (antihistaminic) are in use. The chemistry and pharmacology of benzimidazole have been of great interest to medicinal chemist because its derivatives possessed various biological activities such as antioxidant (Gurer-Orhan et al.,2006), antimicrobial (Ozkay et al., 2010), anticancer (Zienab et al., 2011), antihypertensive (Kumar et al., 2006), anti-inflammatory (Lazer et al., 1987), analgesic (Achar et al., 2010), antiprotozoal (Katiya et al., 1994), anti-hepatitis (Li et al., 2006), antiulcer (Cho et al., 2001), antifungal (Sanja et al., 2007) and anticonvulsant activity (Shingalpur et al., 2010). Apart from the above activities, benzimidazole derivatives also have reported anthelmintic activities (Mahama et al., 2011; Beatriz et al., 2013; Faruk et al., 2014; Katti et al., 2019).

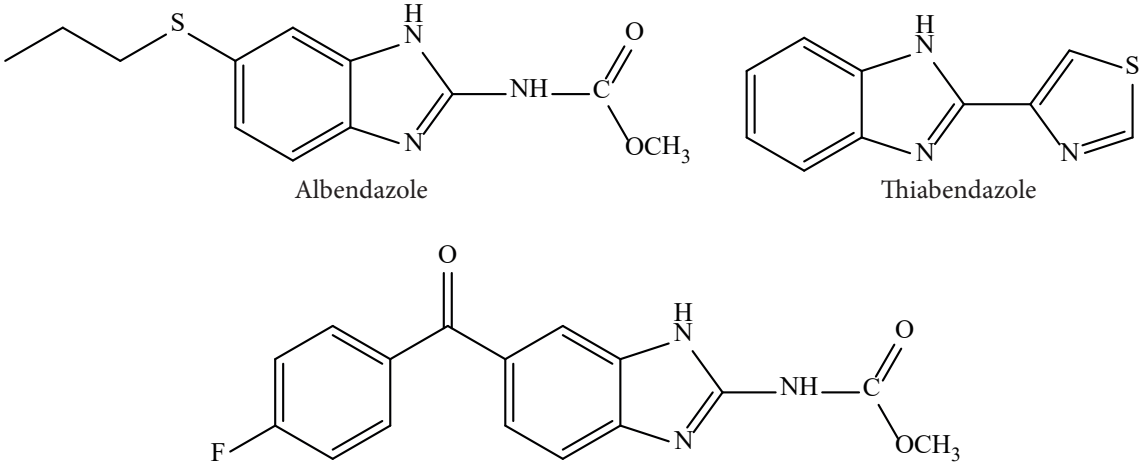

Figure 1. The structure of some marketed benzimidazole anthelmintics

The 1,3,4-thiadiazole pharmacophore has extensive pharmacological significance. The derivatives of 1,3,4- thiadiazole possess an array of biological activities due to azomethine linkage. A variety of 1,3,4-thiadiazole is in use, like Acetazolamide (diuretic), Cefazolin, Cefazedone (antibiotics), Megazol (antiprotozoal), Timolol maleate (NSAIDs), Methazolamide (carbonic anhydrase inhibitor), and Sulphamethizole (antibacterial). 1,3,4-thiadiazole derivatives also have reported anthelmintic activities (Marin et al., 1992; Somnath et al., 2015).

Schiff's bases are an important class of organic compounds with imine or azomethine (–C=N–)

functional group. Schiff's bases are reported to possess a wide variety of pharmacological actions, including potential anti-inflammatory, antibacterial, antitubercular, antiviral, anticonvulsant, and anthelmintic activities. It was proposed that a wide spectrum of biological activities of Schiff's bases could be due to interaction of nitrogen atom of azomethine with the active centers of cell constituents by forming a hydrogen bond, and thus, it interferes in normal cell processes (Venugopala et al., 2003). Schiff's bases also reported as a promising anthelmintic (Rao et al., 2014; Varshney et al., 2014; Reddy and Kumar 2014; Husaina et al., 2018; Satyajit, 2011; Balaji et al., 2017) with some derivatives possessing an activity better than that of albendazole and piperazine citrate.

Because of the need for new antibacterial and anthelmintic agents, novel benzimidazole substituted 1,3,4-thiadiazole Schiff's bases were designed and synthesized. The synergy arising from the successful incorporation of benzimidazole ring, thiadiazole ring, and Schiff's base pharmacophore was exploited in this research. Since the individual pharmacophores have antibacterial and anthelmintic activity, it was expected that, their successful incorporation in one molecule would improve the antibacterial and anthelmintic activity of the compound.

MATERIALS AND METHODS

General

All the chemicals and reagents used in this study are purchased from Himedia, Fischer & Merck chemicals and are self-funded. The melting points for the compounds were determined in an open glass capillary using a Kjeldahl flask containing paraffin and are uncorrected. All the synthesized compounds were characterized by CHN (Carbon, Hydrogen, and Nitrogen) analysis, *IR* spectral data, ^{1}H NMR, and some selected compounds were characterized by mass spectroscopy. The *IR* spectra were recorded using analytical technologies FT-IR spectrophotometer 2202. ^{1}H-NMR spectra was recorded on Bruker 300 MHz in DMSO. Mass spectra were scanned on Brucker MICR QTOF-QII, ESI mass spectrophotometer. C, H, N elemental analyses were recorded on Heraeus CHN rapid analyzer, and the values were found within ± 0.4% of the theoretical values. The Purity of the compounds was checked by TLC (thin layer chromatography) on Merck silica gel 60 F_{254} pre-coated sheets in a chloroform/methanol mixture solvent system, and spots were located using an iodine chamber or a *UV* chamber.

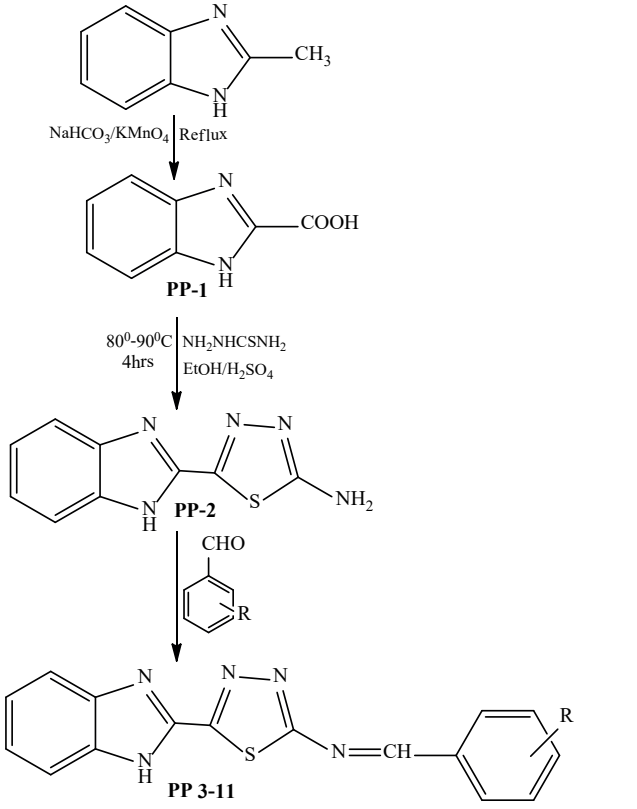

Figure 2. The scheme of Protocol for synthesis of benzimidazole substituted 1,3,4-thiadiazole Schiff's bases.

Chemistry

Synthesis of 1*H*-benzo[d]imidazole-2-carboxylic acid (PP-1): 2-methyl-1*H*-benzo[d]imidazole (0.01mole) was added to a solution of sodium bicarbonate (0.01mole) and potassium permanganate (0.01mole) in water (Agrawal et al., 1982; Norris, 1924), then the reaction mixture was refluxed for 15 hrs (Figure 2). The reaction mixture was cooled and acidified with conc. HCl and the product was collected and recrystallized from ethanol. Yield: 82%; m.p. 176-180^{0}C; IR(cm^{-1}): 3426(-NH), 3080 (C-H aromatic), 2971(OH acid) 1742, 1631(C=N),1721(C=O acid); ^{1}H NMR(DMSO-d6, δ, ppm): 12.34 (1H, -COOH), 7.49-7.11 (4H, Ar-H), 4.7 (1H, NH); MS (EI, *m/z*): 162(M$^+$), 118 (100%).

Synthesis of 5-(1*H*-benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-amine (PP-2): An ethanolic solution of 1*H*-benzo[d]imidazole-2-carboxylic acid (Compound PP-1, 0.01 mole), was added to aqueous solution of thiosemicarbazide (0.02 moles) with stirring, few drops of conc. sulphuric acid (Hussain et al., 2005) was added and heated for 4 hrs at 80-90^{0}C. With completion of the reaction (TLC), reaction mixture was cooled and poured into ice-cold water, basified with 10% Na_2CO_3 solution, filtered, dried and recrystallized from ethanol. Yield: 74%; m.p. 184-185^{0}C; IR(cm^{-1}): 3426 (-NH), 3250 (-NH_2), 1742, 1631 (C=N), 1020 (C-S); ^{1}H NMR(DMSO-d6,δ,ppm): 7.60-7.20 (4H, Ar-H), 7.51 (2H, NH_2) 4.7 (1H, Benzimidazole); MS (EI, *m/z*): 218(M$^+$); Anal. % Calc/found for $C_9H_7N_5S$ (M.W. 217.25): C, 49.76/49.87; H, 3.25/3.71; N, 32.24/32.97.

General synthesis of *N*-Arylidene-5-(1*H*-benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-amine (PP 3-11): The corresponding aryl aldehyde (0.1 moles) was added to a solution of the thiadiazole substituted benzimidazole derivative (compound PP-2, 0.1 moles) in absolute ethanol (30 ml) and the mixture was refluxed for 2 hrs (Varshney et al., 2014; Husaina et al., 2018). The reaction mixture was cooled and kept for 24 hrs. The crystals found were filtered, dried, and recrystallized from ethanol.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-benzylidene-1,3,4-thiadiazol-2-amine (PP-3): Yield: 92%; m.p. 174-176^{0}C; IR(cm^{-1}): 3378(-NH), 3078(Ar-H), 2970-2855(C-H aliphatic), 1604(C=C), 1632(CH=N), 690.47 (C-S-C); ^{1}H NMR (DMSO-d6, δ, ppm): 4.69 (1H,NH-Benzimdazole),7.2-7.8(9H,Ar),8.24 (1H,-N=CH); MS (EI, *m/z*): 305 (M$^+$); Anal. % Calc/found for $C_{16}H_{11}N_5S$ (M.W. 305.36): C,62.93/62.87; H, 3.63/3.71; N, 22.93/22.97.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-(4-nitrobenzylidene)-1,3,4-thiadiazol-2-amine (PP-4): Yield: 82% m.p. 168-169^{0}C; IR(cm^{-1}): 3379(-NH), 3024 (Ar-H), 2923(C-H aliphatic), 1517, 1313 (NO_2); ^{1}H NMR (DMSO-d6, δ, ppm): 4.7 (1H,NH-benzimidazole), 7.9-7.4(8H, Ar), 8.67 (1H, -N=CH); MS (EI, *m/z*): 349.5 (M$^+$); Anal. % Calc/found for $C_{16}H_{10}N_6O_2S$ (M.W. 350.35): C,54.85/54.88; H, 2.88/2.85; N, 23.99/24.04.

2-(((5-(1*H*-Benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol (PP-5): Yield: 88% m.p. 171-173^{0}C; IR(cm^{-1}): 3307(-NH), 3263(-OH), 3036(Ar-H); ^{1}H NMR (DMSO-d6, δ, ppm): 4.7 (1H, NH-benzimidazole), 7.4-7.0(8H, Ar), 4.51 (1H, OH), 8.39 (1H, -N=CH); MS (EI, *m/z*): 321 (M$^+$); Anal. % Calc/found for $C_{16}H_{11}N_5OS$ (M.W. 321.36): C,59.80/59.88; H, 3.45/3.38; N, 21.79/21.74.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-(2-bromobenzylidene)-1,3,4-thiadiazol-2-amine (PP-6): Yield: 76%; m.p. 181-183^{0}C; IR(cm^{-1}): 3065 (C-H_{arom}), 2900-2960 (C-$H_{aliph.}$), 1620(C=N), 686-515(C-Br); ^{1}H NMR (DMSO-d6, δ, ppm): 4.7 (1H,NH-benzimidazole), 7.2-7.7(8H, Ar),9.33 (1H, -N=CH); MS (EI, *m/z*): 384 (M$^+$); Anal. % Calc/found for $C_{16}H_{10}N_5BrS$ (M.W. 384.25): C,50.01/49.88; H, 2.62/2.68; N, 18.23/18.28.

4-(((5-(1*H*-Benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-yl)imino)methyl)phenol (PP-7): Yield: 83%; m.p. 174-175^{0}C; IR(cm^{-1}): 3379 (-NH), 3211 (-OH), 3078 (C-H_{arom}), 2854 (C-$H_{aliph.}$), 1631 (C=N); ^{1}H NMR (DMSO-d6, δ, ppm): 4.69 (1H, NH-benzimidazole), 7.78 (2H, Ar), 7.61 (2H, Ar), 7.20 (2H, Ar), 6.85 (2H, Ar), 4.51 (1H, OH), 9.54 (1H, -N=CH); Anal. % Calc/found for $C_{16}H_{11}N_5OS$ (M.W. 321.36): C,59.80/59.86; H, 3.45/3.43; N, 21.79/21.83.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-(4-chlorobenzylidene)-1,3,4-thiadiazol-2-amine (PP-8): Yield: 74%; m.p. 189-192^{0}C; IR(cm^{-1}): 3380(-NH), 3065 (C-H_{arom}), 1701(C=N), 815(C-Cl); ^{1}H NMR (DMSO-d6, δ, ppm): 5.0 (1H,NH-benzimidazole), 7.2-7.7(8H, Ar), 7.90 (1H, -N=CH); MS (EI, *m/z*): 341 (M$^+$); Anal. % Calc/found for $C_{16}H_{10}N_5ClS$(M.W. 339.80): C,56.55/56.49; H, 2.97/2.98; N, 20.61/20.68.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-(4-methylbenzylidene)-1,3,4-thiadiazol-2-amine (PP-9): Yield: 81%; m.p. 187-189^{0}C; IR(cm^{-1}): 3024 (C-H_{arom}), 2923(-CH_3), 2855(=CH), 1699 (C=N); ^{1}H NMR (DMSO-d6, δ, ppm): 4.79 (1H, NH-benzimidazole), 7.22-7.7 (8H, Ar), 8.02 (1H, -N=CH-), 2.35(3H, CH_3); MS (EI, *m/z*): 320 (M$^+$); Anal. % Calc/found for $C_{17}H_{13}N_5S$ (M.W. 319.38): C,63.93/63.87; H, 4.10/4.16; N, 21.93/22.00.

5-(1*H*-Benzo[d]imidazol-2-yl)-*N*-(4-methoxybenzylidene)-1,3,4-thiadiazol-2-amine (PP-10): Yield: 70%; m.p. 173-174^{0}C; IR(cm^{-1}): 3070 (C-H_{arom}), 1699(-C=N), 1234(-OCH_3); ^{1}H NMR (DMSO-d6, δ,

ppm): 4.79 (1H, NH-benzimidazole), 7.0-7.8(8H, Ar), 9.32(1H,-N=CH-), 3.99(3H, -OCH_3); MS (EI, *m/z*): 336 (M^+); Anal. % Calc/found for $C_{17}H_{13}N_5OS$ (M.W. 335.38): C,60.88/60.87; H, 3.91/4.02; N, 20.88/20.80.

4-(((5-(1*H*-Benzo[d]imidazol-2-yl)-1,3,4-thiadiazol-2-yl)imino)methyl)-3-methoxyphenol (PP-11): Yield: 68%; m.p. 166-168^{0}C; IR(cm^{-1}): 3434(-OH), 3037, 2882 (C-H_{arom}), 2853(C-$H_{aliph.}$), 1148(-OCH_3), 687(C-S) ; ^{1}H NMR (DMSO-d6, δ, ppm): 4.79 (1H, NH-benzimidazole), 7.2-7.7(5H, Ar-H), 6.41-6.48(2H, Ar), 5.34(1H,-OH), 9.33(1H,-N=CH-) 3.83 (3H,-OCH_3); MS (EI, *m/z*): 352 (M^+); Anal. % Calc/found for $C_{17}H_{13}N_5O_2S$ (M.W. 351.38): C, 58.11/58.17; H, 3.73/3.68; N, 19.93/19.86.

In vitro Antimicrobial Activity

Antibacterial activity of the newly synthesized compounds was carried out using three gram-positive bacterial strains; *S. aureus* and *B. cereus* and *S. epidermidis*, and three gram-negative bacterial strains; E. coli, *S. typhi*, and *K. pneumonia*, by standard disc diffusion method (Cruickshank et al., 1975; Sahoo et al., 2010). Standard inoculums (1/100 mL of medium) with suspension (105 CFU/mL) were introduced into the surface of sterile agar plates, and an even distribution of the inoculum was achieved by using a sterile bent glass spreader. The paper disks prepared from Whatman paper (grade no. 1), measuring 6 mm in diameter and 2 mm thickness, were sterilized by dry heat for 1 h. Three paper discs impregnated with each test samples (PP 1-11) in a concentration of 25 µg/mL in DMF (dimethyl formamide), one standard disc of drug- Ampicillin (20 µg/disc) and one negative control disc impregnated with solvent- DMF were placed at different places in a nutrient agar plate medium having a pH (7.2±0.2). All the Petri dishes were then inverted and kept in an incubator for a period of 24 h at 37±2^{o}C. Inhibition zones (in mm) were measured and the average zone diameter of test samples was obtained in triplicate sets. Inhibition zones of the test samples were compared with the inhibition zone of the standard drug.

Anthelmintic Studies

The newly synthesized benzimidazole substituted 1,3,4-thiadiazole Schiff's bases were tested for anthelmintic activity against two different worms species; *Pheretima posthuma* and *Perionyx excavatus*, at a 2 mg/mL concentration (Dahiya et al., 2007). Earthworms collected from local marshy areas were washed with normal saline water to remove adhering soil and fecal matter. Suspensions of the synthesized compounds (100 mg) were prepared by triturating with Tween 80 (0.5%) and normal saline solution and stirring the resulting mixtures for 30 min. These suspensions were suitably diluted to obtain conc. of 0.2% w/v of the test samples. The suspension (0.2% w/v) of the standard drug albendazole was prepared in the same manner. Three sets of five earthworms of almost similar sizes (approx. 2 inches in length) were placed in Petri dishes of 4 inches diameter containing 50 mL of a suspension of prepared test samples and albendazole. Another petri dish containing 50 mL suspension of distilled water and tween 80 (0.5%) was kept as control and a set of five earthworms was placed in it. The paralyzing and death times for each synthesized compound and standard drug were noted, and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50^{0}C) which stimulated the movement, if the worm was alive.

Statistical Evaluation

Antibacterial activity of the titled compounds were analyzed by mean ± Standart deviation (n=3) and compared with the standard. Similarly, anthelmintic activity of the test compounds was analyzed by mean ± SD (n=5) and compared with reference drug Albendazole.

RESULT AND DISCUSSION

Chemistry

A novel series of benzimidazole heterocyclic compounds incorporated with thiadiazole Schiff bases were prepared as per literature with little modification. The compounds were obtained in moderate to good yield ranging from 68-92%. Elemental analysis data of the synthesized compounds are within ±0.4% of the theoretical values. In general, IR spectra of compounds PP 3-11 exhibited the presence of absorption bands for C=N stretching between 1630-1700 cm^{-1} and a C-S-C linkage in thiadiazole at around 690cm^{-1}. The ^{1}H NMR also confirms the presence of shift value at 8.24-9.37 for CH=N groups. The reported spectral data have given sufficient evidence for the successful synthesis of desired compounds.

Biological Studies

All the newly synthesized benzimidazole derivatives showed moderate to good antibacterial activity against all the tested strains. The results of antibacterial studies are presented in "Table 1", and a comparison of their activity in "Figure 3". While performing the antimicrobial studies by disc diffusion method, it was noticeable that compounds (PP 3-11) having varied substitutions at *ortho-* and *para-* position of the aryl ring have different antimicrobial activity spectrum. Compounds PP-4, PP-6 and PP-8 having electron-withdrawing groups (4-nitro, 2-bromo, 4-chloro) exhibited inhibitory effect comparable to Ampicillin. Compound PP-2 containing a free $-NH_2$ group on the thiadiazole ring also showed significant antibacterial activity compared to the standard.

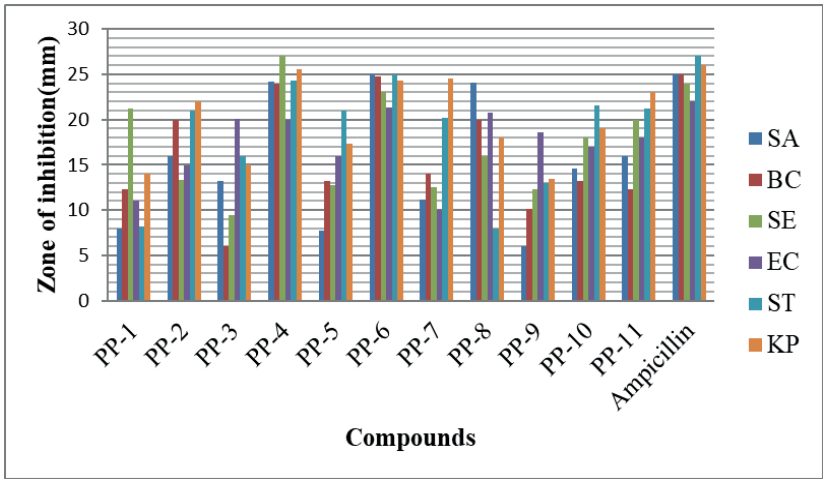

Figure 3. Comparison of antimicrobial activity of the compounds.

SA = *Staphylococcus aureus* (ATCC 11633), **BC** = *Bacillus cereus* (ATCC 11778), **SE** = *Staphylococcus epidermidis* (ATCC 155), **EC** = *Escherichia coli* (ATCC10536), **ST** = *Salmonella typhi* (MTCC 733), **KP** = *Klebsiella pneumonia* (ATCC 10031).

Table 1. Antibacterial activity of the synthesized compounds

Compounds	Antibacterial activity (Zone of inhibition in mm)[a]					
	Gram (+) bacteria			Gram (-) bacteria		
	SA	BC	SE	EC	ST	KP
PP-1	8±0.00	12.32±0.23	21.21±0.32	11±0.00	8.2 ±0.24	14 ± 0.00
PP-2	16±0.00	20±0.00	13.32±0.32	14.9±0.15	21±0.00	22 ± 0.00
PP-3	13.2±0.26	6 ±0.00	9.42±0.25	20.1±0.41	16±0.00	15±0.00
PP-4	24.16±0.12	24±0.00	27±0.00	20±0.32	24.36±0.28	25.6±0.17
PP-5	7.77±0.22	13.24±0.32	12.76±0.27	16±0.00	21±0.00	17.3±0.20
PP-6	25 ±0.00	24.76±0.2	23.1±0.43	21.29±0.41	25±0.00	24.34±0.12
PP-7	11.2±0.26	14±0.00	12.5±0.31	10±0.00	20.2±0.26	24.52±0.3
PP-8	24.1 ±0.32	20±0.00	16±0.00	20.8±0.24	8±0.00	18.0±0.00
PP-9	6±0.00	10.1±0.52	12.3±0.43	18.6±0.4	13±0.00	13.48±0.30
PP-10	14.6±0.10	13.22±0.42	18±0.00	17±0.00	21.6±0.47	19±0.00
PP-11	16±0.00	12.33±0.2	20±0.00	18±0.00	21.25±0.38	23±0.00
Ampicillin	25±0.00	25±0.00	24±0.00	22±0.00	27±0.00	26±0.00
DMF	-	-	-	-	-	-

[a]Data are given as mean ± S.D (n = 3); SA = *Staphylococcus aureus* (ATCC 11633); BC = *Bacillus cereus* (ATCC 11778); SE = *Staphylococcus epidermidis* (ATCC 155); EC = *Escherichia coli* (ATCC10536);ST = *Salmonella typhi* (MTCC 733); KP = *Klebsiella pneumonia* (ATCC 10031).

However, the pattern of the result of anthelmintic activity of the test compounds was quite different from their antibacterial activity. Some of the compounds were found to show anthelmintic activity comparable to the standard drug albendazole. The results of anthelmintic studies are reported in "Table 2", and a comparison of their activity in "Figure 4". The mean paralyzing time (min) of tested compounds against *P. excavatus* and *P. posthuma,* was observed to be 9.10-18.23 and 13.11-22.31 min in comparison to 9.70 and 12.20 min shown by albendazole. The mean death time (min) of tested compounds against *P. excavatus* and *P. posthuma*, ranged from 13.22-28.36 and 19.00-28.32 min in comparison to 14.80 and 20.70 min shown by albendazole. The most and the least potent anthelmintic compound in terms of mean paralyzing time against *P. excavatus* was noted to be PP-4 (9.10 min) and PP-9 (18.23 min), while against *P. posthuma*, PP-4 (11.10 min) and PP-1 (28.32 min) had the similar spectrum of activity. Compound PP-4 was found to be more potent than the standard drug in causing the death of nematodes, which took an average time of 13.22 and 19.00 min against *P. excavatus* and *P. posthuma*, respectively. Apart from compound PP-4, other compounds like PP-6 and PP-11 had comparable anthelmintic activity to that of albendazole. Compounds with electron-withdrawing substituent at *ortho* position led to a considerable increase in activity. Again, the compound with hydroxyl substituent in *para* position and methoxy substituent in *ortho* position also shows significant increase in the anthelmintic activities. Hence, there is a significant role of electron-withdrawing group on the anthelmintic activity.

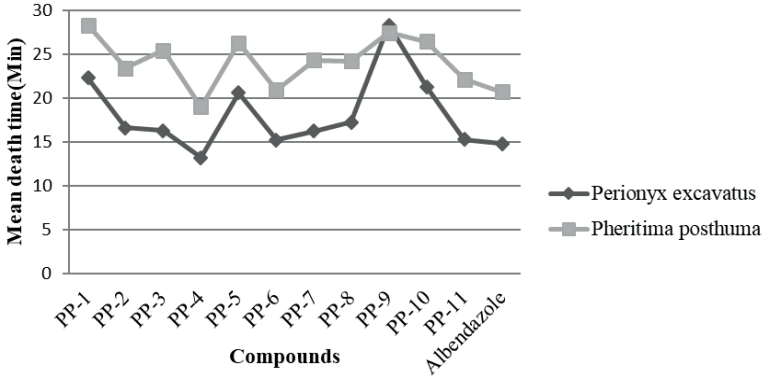

Figure 4. Comparison of anthelmintic activity of the compounds

Table 2. Anthelmintic activity of the title compounds PP1-11

Compounds	Earthworm Species			
	Perionyx excavates		*Pheritima posthuma*	
	Mean paralyzing time (min)[a]	Mean death time (min)[a]	Mean paralyzing time (min)[a]	Mean death time (min)[a]
PP-1	17.43±0.28	22.32±0.13	21.11±0.92	28.32±0.30
PP-2	11.86±0.30	16.62±0.11	14.87±0.68	23.42±0.42
PP-3	11.5±0.28	16.30±0.35	15.67±0.21	25.48±0.53
PP-4	9.10±0.32	13.22±0.25	11.10±0.72	19.00±0.10
PP-5	15.12±0.36	20.62±0.15	21.23±0.12	26.30±0.22
PP-6	10.24±0.47	15.20±0.32	13.42±0.56	20.91±0.15
PP-7	12.21±0.11	16.22±0.30	15.78±0.56	24.36±0.13
PP-8	12.31±0.16	17.22±0.41	16.11±0.75	24.23±0.28
PP-9	18.23±0.19	28.36±0.25	19.67±0.81	27.48±0.91
PP-10	16.23±0.32	21.32±0.71	22.31±0.43	26.47±0.18
PP-11	10.12±0.33	15.30±0.24	13.15±0.53	22.16±0.63
Albendazole	9.70±0.19	14.80±0.42	12.20±0.35	20.70±0.21
Control	No paralysis	No death	No paralysis	No death

[a] Data are given as mean ± S.D (n = 5)

CONCLUSION

Various benzimidazole substituted 1,3,4-thiadiazole Schiff base derivatives were prepared with the objective of developing better antibacterial and anthelmintic moiety. The procedure reported herein is simple, economical, efficient, and environmentally friendly. Additionally, the derivatives were precipitated in their analytical grade without the necessity for chromatographic purification. Three of the eleven new compounds had a comparable antibacterial and anthelmintic activity to that of standard and, as such, could be further developed as alternative antimicrobial and anthelmintic agents for the future.

ACKNOWLEDGEMENTS

The authors are thankful to SAIF, Punjab University, and Dr. Ganesh P. Mishra, KSCP, Swami Vivekananda Subharti University, Meerut, for providing and interpreting spectral data.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTION STATEMENT

Initial litrature survey, experimental design, antimicrobial and anthelmintic assay, statistical analysis,-data acquisition, writting the manuscript (Pattanayak, P.), data interpretation, spectra analysis, approval of the final manuscript (Savanan, K.).

REFERENCES

Achar, K. C., & Hosamani, K. M. (2010). *In-vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. *European Journal of Medicinal Chem*istry, *45*, 2048-2054. doi: 10.1016/j.bioorg.2016.11.014

Agrawal, R. K., & Sharma, S. (1982). A new synthesis of substituted benzimidazole-2-one. *Indian Journal of Chemistry, 21(b),* 967-968.

Ahn, Y. J., Kwon, M., Yoo, J. K., Byun, S. J. (1993). Toxicity of flufenoxuron alone and in mixture with alphacypermethrin or fenbutanin oxide to Tetranychus urticae and Panonychus ulmi. *Journal of Economic Entomology, 86*, 1334-1338. doi: /10.1093/jee/86.5.1334.

Balaji, P. N., Ranganayakulu, D., Reddy, G. V. (2017). Synthesis, in vitro evaluation for anthelmintic and antimicrobial activity for the novel thiazolidine-4-one incorporate substituted chloro-quinoline. *Asian Journal of Pharmacy and Pharmacology, 3*, 9-15.

Beatriz, M., Pablo, M., Romina, E., Andrea L. (2013). Synthesis and Anthelmintic Evaluation of Novel Valerolactam-Benzimidazole Hybrids. *Letters in Drug Design & Discovery, 10*, 1007-1014. doi: 10.2174/15701808113109990028.

Bhinge, S. D, Chature, V., Sonawane, L. V. (2015). Synthesis of some novel 1,3,4-thiadiazole derivatives and biological screening for anti-microbial, antifungal and anthelmintic activity, *Pharmaceutical Chemistry Journal, 49*, 367-372. doi: /10.1007/s11094-015-1287-8

Cho, S. Y., Kang, S. K., Kim, S. S., Yum, E. M. (2001). Synthesis and SAR of benzimidazole derivatives containing oxycyclic pyridine as a gastric ATPase inhibitors. *Bulletin of the Korean Chemical Society, 22*, 1217-1223.

Cruickshank, R., Duguid, J.P., Marion, B.P., Swain, R.H.A. (1975). Medicinal microbiology, 12th ed.; Churchill Livingstone: London, Vol. 2, 196-202.

Dahiya, R., Pathak, D. (2007). Synthetic studies on novel benzimidazole peptides with antimicrobial, cytotoxic and anthelmintic potential. *European Journal of Medicinal Chemistry, 42*, 772–98. doi: /10.1016/j.ejmech.2006.11.015.

El-Zemity, S., Badawy, M. E., Khattab M. M., Marei, A. E. (2006). Structure and Acaricidal Activity Relationship of Some Sulfonamide Derivatives Against the Two-spotted Spider Mite, etranychus urticae (Koch). *International Journal of Agricultural Biology, 8*, 661-665.

Faruk, A., Biplab, K. D., Sharma, K. (2014). Synthesis, antimicrobial and anthelmintic activity of some novel benzimidazole derivatives. *International Journal of Drug Research and Technology, 4*, 31-38.

Gurer-Orhan, H., Suzen, S., Buyukbingol, E., (2006). Synthesis and evaluation of *in vitro* antioxidant capacities of some benzimidazole derivatives. *Journal of Enzyme Inhibition and Medicinal Chemistry, 21*, 241-247. doi: 10.1080/14756360600586031

Husaina, A., Munendra, M. V., Versha, P. (2018). Nalidixic Acid Schiff Bases: Synthesis and Biological Evaluation, *Letters in Drug Design & Discovery, 15*, 103-111. doi: 10.2174/1570180814666170710160751

Hussain, A., Sharba, K., Al-Bayati, R., Rezki, N., Aouad, M. R. (2005). Synthesis of Thiadiazoles and 1,2,4-Triazoles derived from cyclopropane dicarboxylic acid, *Molecules*, *10,* 1153-1160. doi: 10.3390/10091153

Katiya, S. K., Gordon, V. R., Edlind, T. D. (1994). Antiprotozoal activities of benzimidazoles and correlations with beta-Tubulin sequence. *Antimicrobial Agents and Chemotherapy*, *38*, 2086-2090. doi: 10.1128/aac.38.9.2086

Katti, S. A., Desai, K. S., Loharkar, S. V. (2019). Synthesis, molecular docking & evaluation of anthelmintic activity of 2-(2-amino ethyl)-1hbenzimidazole derivatives. *World Journal of Pharmaceutical Research, 8*, 1141-1151. doi: 10.20959/wjpr201911-15922

Kumar, J. R., Jawharand, J., Pathak, D. P. (2006). Synthesis of benzimidazole derivatives as anti-hypertensive agents. *Journal of Chemistry*, *3*, 278-285. doi: 10.1155/2006/765712

Lazer, E. S., Matteo, M. R., Possanza, G. J. (1987). Benzimidazole derivatives with atypical anti-inflammatory activity. *Journal of Medicinal Chemistry*, *30*, 726-729. doi: 10.1021/jm00387a026

Li, Y. F., Wang, G. F., Zhu, F. H. (2006). Synthesis and anti-Hepatitis-B virus activity of novel benzimidazole derivatives. *Journal of Medicinal Chem*istry, *49*, 4790-4794. doi: 10.1021/jm060330f

Mahama, O., Drissa, S., Mamidou W. K. (2011). Synthesis and anthelmintic activity of some hybrid Benzimidazolyl-chalcone derivatives. *Tropical Journal of Pharmaceutical Research, 10*, 767-775. doi: 10.4314/tjpr.v10i6.10

Marin, A., Valls, N., Berenguer, F. J., Alonso, M. T., Martinez, A. R. (1992). Synthesis and anthelmintic activity of carbamates derived from imidazo[2,1-b][1,3,4]thiadiazole and imidazo[2,1-b]thiazole. *Farmaco, 47*, 63-75. PMID: 1616578.

Norris, J. F. (1924). Preparation of Terepthalic acid from p- Toluic Acid. In Experimental Organic Chemistry, 2nd ed.; Mc Graw-Hill Book Company, Inc.: New York, NY, USA.

Ozkay, Y., Tunai, Y., Karaka, H., Isikdag, I. (2010). Antimicrobial activity and a SAR study of some novel benzimidazole bearing hydrazone moity. *European Journal of Medicinal Chemistry, 45*, 3293-3298. doi: 10.1016/j.ejmech.2010.04.012

Rao, R., Reddy, K.R., Mahendra, K.N. (2014). Synthesis, characterization, antibacterial, antifungal and anthelmintic activities of a new 5 - nitroisatin Schiff base and its metal complexes. *Bulgarian Chemical Communications, 46,* 11–17.

Reddy, D. R. S., Kumar, K. H. (2014). N-substituted fluoro benzothiazolo Schiff 's bases: Synthesis and characterisation of new novel anthelmintic agents. *International Journal of Pharmaceutical and Clinical Research*, *6*, 71-75.

Sahoo, P.K., Sharma, R., Pattanayak, P. (2010). Synthesis and evaluation of 4-amino-5-phenyl-4H-[1,2,4]-triazole-3-thiol derivatives as antimicrobial agents. *Medicinal Chemistry Research. 19*, 127–135. doi: 10.1007/s00044-009-9178-8

Sanja, O., Dijana, J. B., Dragaljub, D. (2007). QSAR of some 1-benzyl benzimidazole derivatives as antifungal agent. *APTEFF*, *38*, 139-147. doi:10.2298/APT0738139P

Satyajit, D. (2011). Studies on synthesis and anthelmintic activities of some N-Benzylidenepyridin-2-amines. *FABAD Journal of Pharmaceutical Sciences, 36*, 189-195.

Shingalpur, R. V., Keri, R. S., Hugar, M. H. (2010). Derivatives of benzimidazole pharmacophore: synthesis, anticonvulsant, antidiabetic studies. *European Journal of Medicinal Chemistry, 4*, 1735-1759. doi: 10.1016/j.ejmech.2010.01.007

Varshney, M. M., Husain, A., Parcha, V. (2014). Synthesis, characterization, *in vitro* antimicrobial, and anthelmintic evaluations of 2-(4-chloro-3-methylphenoxy)-N′-[{5′-(substituted aryl)-furan-2′-yl}-methylidene]-acetohydrazides. *Medicinal Chemistry Research, 23*, 4034–4041. doi: /10.1007/s00044-014-0982-4

Venugopala, K. N., & Jayashree, B. S. (2003). Synthesis of carboxamides of 2'-amino-4'-(6-bromo-3-coumarinyl) thiazole as analgesic and antiinflammatory agents. *Indian Journal of Heterocyclic Chemistry, 12*, 307–310.

Zienab, M., Nofal, A., Soliman S. S., Sroor S.S. (2011). Novel benzimidazole derivatives as expected anticancer agents. *Acta Polonica Pharmaceutica, 68*, 519-534. PMID: 21796934.