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Research Article

DESIGN AND SYNTHESIS OF BENZODIAZEPINES BEARING BENZIMIDAZOLE/BENZOTHIAZOLE AND INDOLE MOIETIES AS A POTENT ANTIMICROBIAL AND ANTIOXIDANT AGENTS

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ABSTRACT

Objective: The present work deals with the synthesis and characterization of biologically active new indole derivatives, namely, 2-((1*H*-benzo[d] imidazol-2-yl) thio)-*N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide 3a-d, *N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide 4a-d and *N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide 5a-d.

Methods: All these newly synthesized compounds were screened for their *in vitro* antimicrobial activity by an agar plate diffusion method, antioxidant activities such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), radical scavenging activity (RSA), ferric ions (Fe^{3+}) reducing antioxidant power (FRAP), and ferrous (Fe^{2+}) metal ion chelating activity.

Result: The structures of all the newly synthesized compounds were characterized by their infrared, ^{1}H nuclear magnetic resonance, mass spectral studies, and elemental analysis. Compounds 7a and 7b exhibited good RSA at a concentration 100 µg/ml, compounds 6d, 7a-d and 8a-c displayed good FRAP at a concentration 100 µg/ml, compounds 7b-d and 8b-d showed good Fe^{2+} ion metal chelating activity. Compounds 6b, 6d, 7a-d, and 8a-d exhibited good activity against all the screened bacteria and fungi.

Conclusion: Some of the compounds have shown potent antimicrobial activity against all the screened bacteria and fungi, and some have exhibited very good antioxidant activity.

Keywords: Benzodiazepine, Indole, Benzimidazole/Benzothiazole, Antimicrobial, Antioxidant.

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INTRODUCTION

Benzodiazepines and their derivatives are a very important class of bioactive compounds because of their diverse pharmacological properties. They are widely used as antidepressants, anticonvulsant, analgesic, hypnotic, and sedative [1]. This compound possesses anti-inflammatory [2], antimicrobial, antioxidant [3], and anticancer activity [4]. It acts as an inhibitor of respiratory syncytial virus [5]. 1, 4-benzodiazepine analogs have been demonstrated as anticonvulsants, muscle relaxants, blood pressure lowering, and CNS depressant agents [6].

Alongside, indole and its biheterocycles are featured widely in a wide variety of biological and pharmacologically active compounds [7]. The indole derivatives are known to possess anticancer, antioxidant, antitumor, and anti-HIV [8-11, 28] activities.

Benzimidazole is an essential pharmacophore and a privileged structure in medicinal chemistry. It has been found to possess antioxidant, anti-inflammatory, diuretic, antiviral, anticonvulsant, and antidiabetic [12-17] activities.

Benzothiazole is also a heterocyclic compound, with various biological activities. This heterocycle possess diverse biological activities such as antitumor, anticancer, antifungal and antibacterial, and antidiabetic [18-22] activities.

In the view of above-mentioned facts, we describe herein the design, synthesis, and characterization of some benzodiazepines bearing benzimidazole/benzothiazole and indole moieties as a potent antimicrobial and antioxidant agents.

MATERIALS AND METHODS

Materials

All chemicals and solvents were of commercial reagent grade and used as received from Sigma-Aldrich and Spectrochem Pvt., Ltd. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC using silica gel-G coated aluminum plates (Merck), and spots were visualized by exposing the dry plates to iodine vapors. The infrared (IR) (KBr) spectra were recorded on a Perkin-Elmer spectrum one FT-IR spectrometer. The ^{1}H nuclear magnetic resonance (NMR) dimethyl sulfoxide (DMSO-d_6) spectra recorded on a Bruker NMR (400 MHz) and the chemical shifts were expressed in ppm (δ scale) downfield from TMS. Mass spectral data were recorded by electron impact method on JEOL GCMATE II GC-MS mass spectrometer. Elemental analysis was performed using Flash EA 1112 series elemental analyzer. All the compounds gave C, H, and N analysis within ±0.5% of the theoretical values.

General procedure for the synthesis of *N*-(4-acetylphenyl)-2-chloroacetamide (2) was prepared by following the literature method [23].

General procedure for the synthesis of 2-(1*H*-benzo[d]imidazole-2-ylthio) *N*-(4-acetylphenyl) acetamide, *N*-(4-acetylphenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl)thio) acetamide and *N*-(4-acetylphenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (3, 4, and 5) was prepared by following the literature method [23].

N-(4-acetylphenyl)-2-chloroacetamide (2) (0.01 mol) obtained was further reacted with 2-mercatobenzimidazole, 2-mercapto-5-methoxy benzimidazole and 2-mercapto benzothiazole (0.01 mol). The reaction was stirred for 4 h at room temperature in the presence of K_2CO_3 (0.02 mol) and acetone (20 ml) was used as the reaction medium. After the completion of the reaction, it was monitored on TLC using Toluene: Acetone (8:2) as mobile phase, the product obtained was poured into water and stirred vigorously for 1 h. The separated precipitate was collected and dried. The product was recrystallized from ethanol.

2-(1*H*-benzo[d]imidazole-2-ylthio) *N*-(4-acetylphenyl) acetamide (3)
Yield 86% (Ethanol); M.P 210°C; IR (KBr) (λ_{max} in cm^{-1}): 1409, 1650, 2850, 3110, 3285, 3400. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.50 (S,3H, -CH_3), 4.32 (S, 2H, -CH_2), 7.10-7.96 (m, 8H, Ar-H), 10.85 (S, 1H, -NH), 12.86 (S, 1H, benzimidazole -NH). Anal. Calcd for $C_{17}H_{15}N_3O_2S$ (325): C, 62.75; H, 4.65; N, 12.91. Found: C, 62.74; H, 4.67; N, 12.90.

***N*-(4-acetylphenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (4)**
Yield 80% (Ethanol); M.P 152°C; IR (KBr) (λ_{max} in cm^{-1}): 1406, 1625, 2862, 2992, 3285, 3379. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.50 (S, 3H, -CH_3), 3.76 (S, 3H, OCH_3), 4.32 (S, 2H, -CH_2), 6.72-8.22 (m, 7H, Ar-H), 10.88 (S, 1H, -NH), 12.48 (S, 1H, benzimidazole -NH). Anal. Calcd for $C_{18}H_{17}N_3O_3S$ (355): C, 60.83; H, 4.82; N, 11.82. Found: C, 60.81; H, 4.85; N, 11.81.

***N*-(4-acetylphenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (5)**
Yield 77% (Ethanol); M.P 130°C; IR (KBr) (λ_{max} in cm^{-1}): 1457, 1625, 2850, 2992, 3276, 3380. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.50 (S, 3H, -CH_3), 4.40 (S, 2H, -CH_2), 7.29-7.92 (m, 8H, Ar-H), 10.71 (S, 1H, -NH). Anal. Calcd for $C_{17}H_{14}N_2O_2S_2$ (342): C, 59.63; H, 4.12; N, 8.18. Found: C, 59.61; H, 4.15; N, 8.19.

2-((1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (6a-d)
The Claisen–Schmidt condensation of an equimolar mixture of 2-(1*H*-benzo[d]imidazol-2-ylthio)-*N*-(4-acetylphenyl) acetamide (0.01 mol) and various 2,5-disubstituted indole-3-carboxaldehydes (0.01 mol) were refluxed (3–4 h) in ethanol (15–20 ml) in the presence of piperidine. After 4 h substituted ortho-phenylenediamine and a catalytic amount of acetic acid was added to the reaction mixture and was further refluxed (7-8 h). The completion of the reaction was monitored by TLC. The product was poured in ice cold water. The product obtained was filtered and purified by ethanol.

2-((1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (6a)
Yield 70% (Ethanol); M.P 176-178°C; IR (KBr) (λ_{max} in cm^{-1}): 1673, 2838, 3098, 3110, 3318, 3409; ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.4 (S, 3H, -CH_3), 4.4 (S, 2H, -CH_2), 7.8-8.2 (m, 18H, 17Ar-H, diazepine -NH), 10.1 (S, 1H, indole -NH), 11.2 (S, 1H, -NH), 12.27 (S, 1H, benzimidazole -NH). MS: m/z = 554 $[M]^+$. Anal. Calcd for $C_{33}H_{26}N_6OS$ (554): C, 71.46; H, 4.72; N, 15.15. Found: C, 71.43; H, 4.69; N, 15.12.

2-((1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (6b)
Yield 72% (Ethanol); M.P 144-146°C; IR (KBr) (λ_{max} in cm^{-1}): 1685, 2819, 3112, 3153, 3301, 3397, 769. ^{1}H NMR (DMSO-d_6 + $CDCl_3$) $^\delta$(ppm): 2.3 (S, 3H, -CH_3), 4.3 (S, 2H, -CH_2), 7.6-8.00 (m, 21H, 20Ar-H, diazepine -NH), 10.3 (S, 1H, indole -NH), 11.4 (S, 1H, -NH), 12.4 (S, 1H, benzimidazole -NH). MS: m/z = 664 $[M]^+$, 666 $[M+2]^+$ (3:1). Anal. Calcd for $C_{39}H_{29}ClN_6OS$ (664): C, 70.42; H, 4.39; N, 12.63. Found: C, 70.46; H, 4.36; N, 12.61.

2-((1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(8-methyl-4-(5-methyl-2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (6c)
Yield 69% (Ethanol); M.P 154-156°C; IR (KBr) (λ_{max} in cm^{-1}): 1659, 2810, 3026, 3099, 3298, 3393. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.42 (S, 3H, -CH_3), 2.6 (S, 3H, -CH_3), 4.41 (S, 2H, -CH_2), 7.4-8.3 (m, 21H, 20Ar-H, diazepine -NH), 9.97 (S, 1H, indole -NH), 10.86 (S, 1H, -NH), 12.10 (S, 1H, benzimidazole -NH). MS: m/z = 644 $[M]^+$. Anal. Calcd for $C_{40}H_{32}N_6OS$ (644): C, 74.51; H, 5.00; N, 13.03. Found: C, 74.48; H, 5.03; N, 13.05.

2-((1*H*-benzo[d]imidazol-2-yl)thio)-*N*-(4-(4-(5-bromo-1*H*-indol-3-yl)-8-methyl-1*H*-benzo [b][1,4]diazepin-2-yl) phenyl) acetamide (6d)
Yield 64% (Ethanol); M.P 210-212°C; (λ_{max} in cm^{-1}): 1668, 2899, 3079, 3099, 3327, 3417, 783. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.39 (S, 3H, -CH_3), 4.26 (S, 2H, -CH_2), 7.7-8.5 (m, 17H, 16Ar-H, diazepine -NH), 10.4 (S, 1H, indole -NH), 11.1 (S, 1H, -NH), 12.2 (S, 1H, benzimidazole -NH). MS: m/z = 632 $[M]^+$, 634 $[M+2]^+$ (1:1). Anal. Calcd for $C_{33}H_{25}BrN_6OS$ (632): C, 62.56; H, 3.98; N, 13.26; Found: C, 62.58; H, 3.96; N, 13.25.

***N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (7a-d)**
The Claisen–Schmidt condensation of an equimolar mixture of *N*-(4-acetylphenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (0.01 mol) and various 2,5-disubstituted indole-3-carboxaldehydes (0.01 mol) were refluxed (3–4 h) in ethanol (15–20 ml) in the presence of piperidine. After 4 h substituted ortho-phenylenediamine and a catalytic amount of acetic acid was added to the reaction mixture and was further refluxed (7-8 h). The completion of the reaction was monitored by TLC. The product was poured in ice cold water. The product obtained was filtered and purified by ethanol.

***N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (7a)**
Yield 67% (Ethanol); M.P 160-162°C; IR (KBr) (λ_{max} in cm^{-1}):1663, 2829, 3091, 3107, 3297, 3384. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.42 (S, 3H, -CH_3), 3.86 (S, 3H, -OCH_3), 4.2 (S, 2H, -CH_2), 7.5-8.4 (m, 17H, 16Ar-H, diazepine -NH), 10.4 (S, 1H, indole -NH), 11.3 (S, 1H, -NH), 11.99 (S, 1H, benzimidazole -NH). MS: m/z = 584 $[M]^+$. Anal. Calcd for $C_{34}H_{28}N_6O_2S$ (584): C, 69.84; H, 4.83; N, 14.37. Found: C, 69.86; H, 4.80; N, 14.35.

***N*-(4-(4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (7b)**
Yield 72% (Ethanol); M.P 188-190°C; IR (KBr) (λ_{max} in cm^{-1}):1693, 2831, 3108, 3164, 3341, 3405,781. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.42 (S, 3H, -CH_3), 3.91 (S, 3H, -OCH_3), 4.46 (S, 2H, -CH_2), 7.1-8.3 (m, 20H, 19Ar-H, diazepine -NH), 10.5 (S, 1H, indole -NH), 11.6 (S, 1H, -NH), 12.2 (S, 1H, benzimidazole -NH). MS: m/z = 694 $[M]^+$, 696 $[M+2]^+$ (3:1). Anal. Calcd for $C_{40}H_{31}ClN_6O_2S$ (694): C, 69.10; H, 4.49; N, 12.09. Found: C, 69.13; H, 4.45; N, 12.11.

2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(8-methyl-4-(5-methyl-2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (7c)
Yield 76% (Ethanol); M.P 148-150°C; (KBr) (λ_{max} in cm^{-1}):1641, 2798, 3031, 3317, 3291, 3389. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.41 (S, 3H, -CH_3), 2.61 (S, 3H, -CH_3), 3.89 (S, 3H, -OCH_3), 4.13 (S, 2H, -CH_2), 7.1-8.5 (m, 20H, 19Ar-H, diazepine -NH), 10.12 (S, 1H, indole -NH), 10.97 (S, 1H, -NH), 12.6 (S, 1H, benzimidazole -NH). MS: m/z = 674 $[M]^+$. Anal. Calcd for $C_{41}H_{34}N_6O_2S$ (674): C, 72.97; H, 5.08; N, 12.45. Found: C, 72.95; H, 5.02; N, 12.44.

***N*-(4-(4-(5-bromo-1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4] diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (7d)**
Yield 65% (Ethanol); M.P 188-190°C; (λ_{max} in cm^{-1}): 1670, 2877, 3085, 3121, 3340, 3427, 774. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.44 (S, 3H, -CH_3), 3.89 (S, 3H, -OCH_3), 4.61 (S, 2H, -CH_2), 7.2-8.1 (m, 16H, 15Ar-H, diazepine -NH), 10.55 (S, 1H, indole -NH), 11.32 (S, 1H, -NH), 12.4 (S, 1H, benzimidazole -NH). MS: m/z = 662 $[M]^+$, 664 $[M+2]^+$ (1:1). Anal.

Calcd for $C_{34}H_{27}BrN_6O_2S$ (662): C, 61.54; H, 4.10; N, 12.66. Found: C, 61.51; H, 4.15; N, 12.64.

***N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-(benzo[d]thiazol -2-ylthio) acetamide (8a-d)**

The Claisen–Schmidt condensation of an equimolar mixture of *N*-(4-acetylphenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (0.01 mol) and various 2,5-disubstituted indole-3-carboxaldehydes (0.01 mol) were refluxed (3–4 h) in ethanol (15–20 ml) in the presence of piperidine. After 4 h substituted ortho-phenylenediamine and a catalytic amount of acetic acid was added to the reaction mixture and was further refluxed (7-8 h). The completion of the reaction was monitored by TLC. The product was poured in ice cold water. The product obtained was filtered and purified by ethanol.

***N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (8a)**

Yield 70% (Ethanol); M.P 134-136°CIR (KBr) (λ_{max} in cm^{-1}):1668, 2813, 3099, 3128, 3398. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.41 (S, 3H, -CH_3), 4.12 (S, 2H, -CH_2), 7.3-8.3 (m, 18H, 17Ar-H, diazepine -NH), 10.2 (S, 1H, indole -NH), 11.4 (S, 1H, -NH). MS: m/z = 571 $[M]^+$. Anal. Calcd for $C_{33}H_{25}N_5OS_2$ (571): C, 69.33; H, 4.41; N, 12.25. Found: C, 69.35; H, 4.44; N, 12.28.

2-(benzo[d]thiazol-2-ylthio)-*N*-(4-(4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (8b)

Yield 78% (Ethanol); M.P 178-180°C; IR (KBr) (λ_{max} in cm^{-1}): 1671, 2809, 3102, 3164, 3412, 773. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.44 (S, 3H, -CH_3), 4.18 (S, 2H, -CH_2), 7.1-8.1 (m, 21H, 20Ar-H, diazepine -NH), 10.4 (S, 1H, indole -NH), 11.2 (S, 1H, -NH). MS: m/z = 681 $[M]^+$, 683 $[M+2]^+$ (3:1). Anal. Calcd for $C_{39}H_{28}ClN_5OS_2$ (681): C, 68.66; H, 4.14; N, 10.26. Found: C, 68.61; H, 4.17; N, 10.28.

2-(benzo[d]thiazol-2-ylthio)-*N*-(4-(8-methyl-4-(5-methyl-1*H*-indol-3-yl)-1*H*-benzo [b][1,4]diazepin-2-yl) phenyl) acetamide (8c)

Yield 73% (Ethanol); M.P 146-148°C; IR (KBr) (λ_{max} in cm^{-1}):1652, 2808, 3031, 3089, 3399. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.45 (S, 3H, -CH_3), 2.62 (S, 3H, -CH_3), 4.43 (S, 2H, -CH_2), 7.5-8.2 (m, 21H, 20Ar-H, diazepine -NH), 10.14 (S, 1H, indole -NH), 11.35 (S, 1H, -NH). MS: m/z = 585 $[M]^+$. Anal. Calcd for $C_{34}H_{27}N_5OS_2$ (585): C, 69.72; H, 4.65; N, 11.96. Found: C, 69.71; H, 4.68; N, 11.99.

2-(benzo[d]thiazol-2-ylthio)-*N*-(4-(4-(5-bromo-1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide (8d)

Yield 64% (Ethanol); M.P 196-198°C; IR (KBr) (λ_{max} in cm^{-1}): 1671, 2913, 3094, 3121, 3441, 786. ^{1}H NMR (DMSO-d_6 + $CDCl_3$)$^\delta$(ppm): 2.4 (S, 3H, -CH_3), 4.27 (S, 2H, -CH_2), 7.6-8.6 (m, 17H, 16Ar-H, diazepine -NH), 10.6 (S, 1H, indole -NH), 11.56 (S, 1H, -NH). MS: m/z = 649 $[M]^+$, 651 $[M+2]^+$ (1:1). Anal. Calcd for $C_{33}H_{24}BrN_5OS_2$ (649): C, 60.92; H, 3.72; N, 10.76. Found: C, 60.95; H, 3.76; N, 10.75.

BIOLOGICAL ACTIVITIES

Antimicrobial activity [24]

The *in vitro* antimicrobial activity of the synthesized compounds 6a-d, 7a-d, 8a-d was carried out against bacterial strains *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), and *Pseudomonas aeruginosa* (MTCC-1688) and fungal species, *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973), and *Aspergillus oryzae* (MTCC-3567^T) by cup plate method [25] using nutrient agar and PDA as medium, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer, and these were filled with test solution (1000 µg/ml in DMF) and DMF used as a control. The plates were incubated at 37°C for 24 and 72 h in case antibacterial and antifungal activity, respectively. The zones of inhibition around the wells were determined, and the averages based on triplicate measurements were recorded.

Antioxidant activity assay

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA)

The free RSA of all the compounds at concentrations of 25, 50, 75, and 100 µg/ml was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hatano's method [25] using 2-tert-butyl-4-methoxyphenol butylated hydroxy anisole, 2-(1,1-dimethylethyl) -1,4-benzenediol 2-*tert*-butyl hydroquinone and Ascorbic acid as standards. All the test analyses were performed on three replicates, and the results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence of test compounds and absorption of DPPH in the absence of test compounds at λ 517 nm on ELICO SL 171 Mini Spec, spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

$$\%\ \text{of DPPH RSA} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results are shown in Fig. 1.

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The FRAP of the synthesized compounds were determined according to literature method [26]. Different concentrations of samples (25, 50, 75, and 100 µg/ml) in DMSO (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. After which a portion of trichloroacetic acid (2.5 ml, 10%) was added to the mixture and centrifuged for 10 min, at 1000 Xg. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). Then, absorbance at λ 700 nm was measured in a spectrophotometer. The higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in Fig. 2.

Ferrous (Fe^{2+}) metal ion chelating activity

The chelating activity of Fe^{2+} ion of synthesized compounds was estimated by following reported method [27]. The test samples (25, 50, 75, and 100 µg/ml) in ethanolic solution (0.4 ml) were added to a solution of $FeCl_2$ (0.05 ml, 2 mmol). The reaction was initiated by the addition of ferrozine (0.2 ml, 5 mmol) and the total volume was adjusted to 4 ml with ethanol. Ferrozine reacted with the divalent iron form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then, the absorbance of the solution was measured spectrophotometrically at λ 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine Fe^{2+} complex formations was calculated using the following formula:

$$\%\ \text{of ferrousion chelating} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The results are shown in Fig. 3.

RESULTS AND DISCUSSION

Chemistry

In the present investigation, 4-aminoacetophenone (1) was reacted with chloroacetyl chloride to form an intermediate *N*-(4-acetylphenyl)-2-chloroacetamide (2), which on reaction with 2-mercatobenzimidazole, 2-mercapto-5-methoxy benzimidazole and 2-mercapto benzothiazole, resulted in the formation of 2-(1*H*-benzo[d]imidazole-2-ylthio) *N*-(4-acetylphenyl) acetamide (3), *N*-(4-acetylphenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide (4) and *N*-(4-acetylphenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (5), respectively, by following the literature method [22].

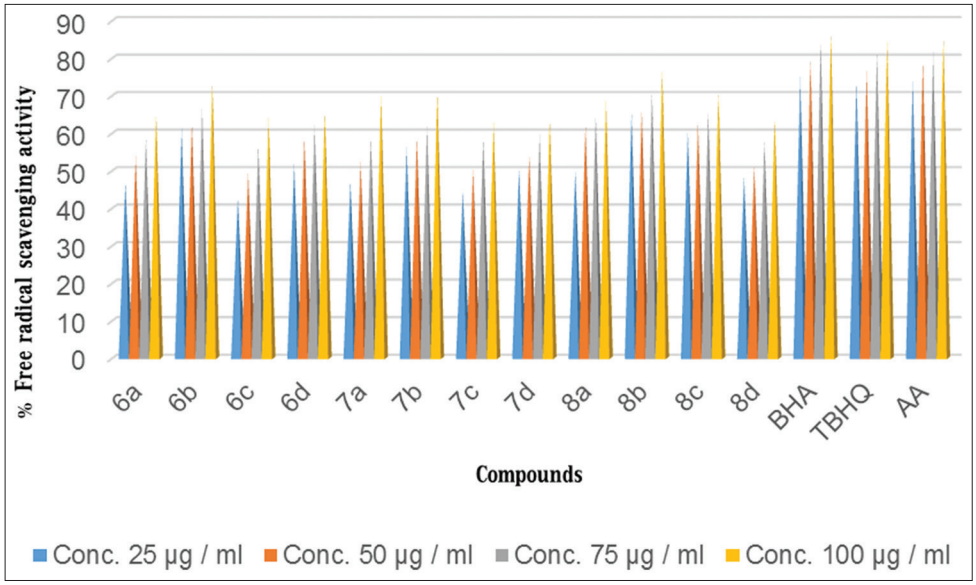


Fig. 1 DPPH radical scavenging activity of compounds 6a-d, 7a-d and 8a-d.

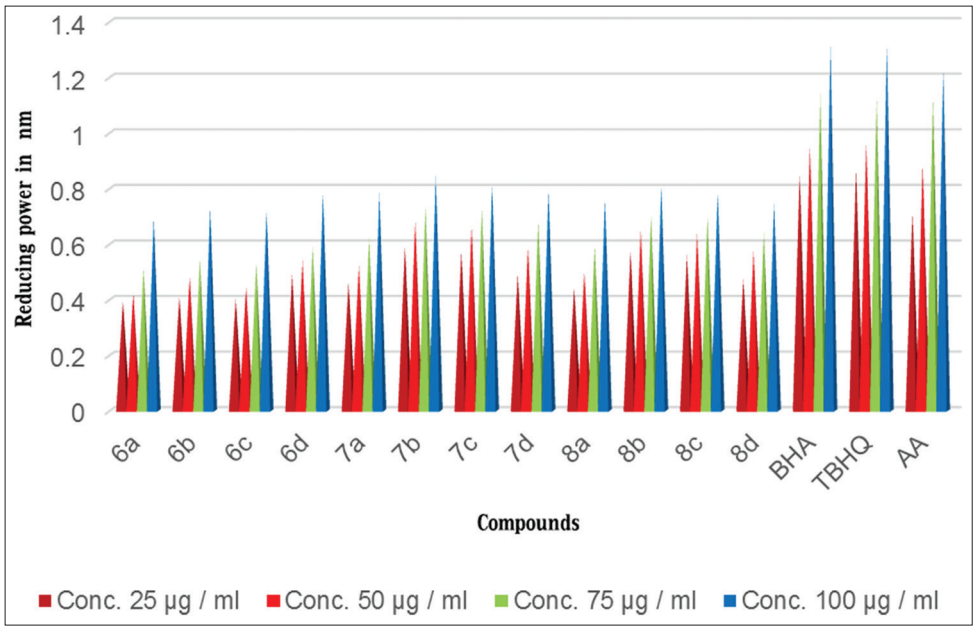


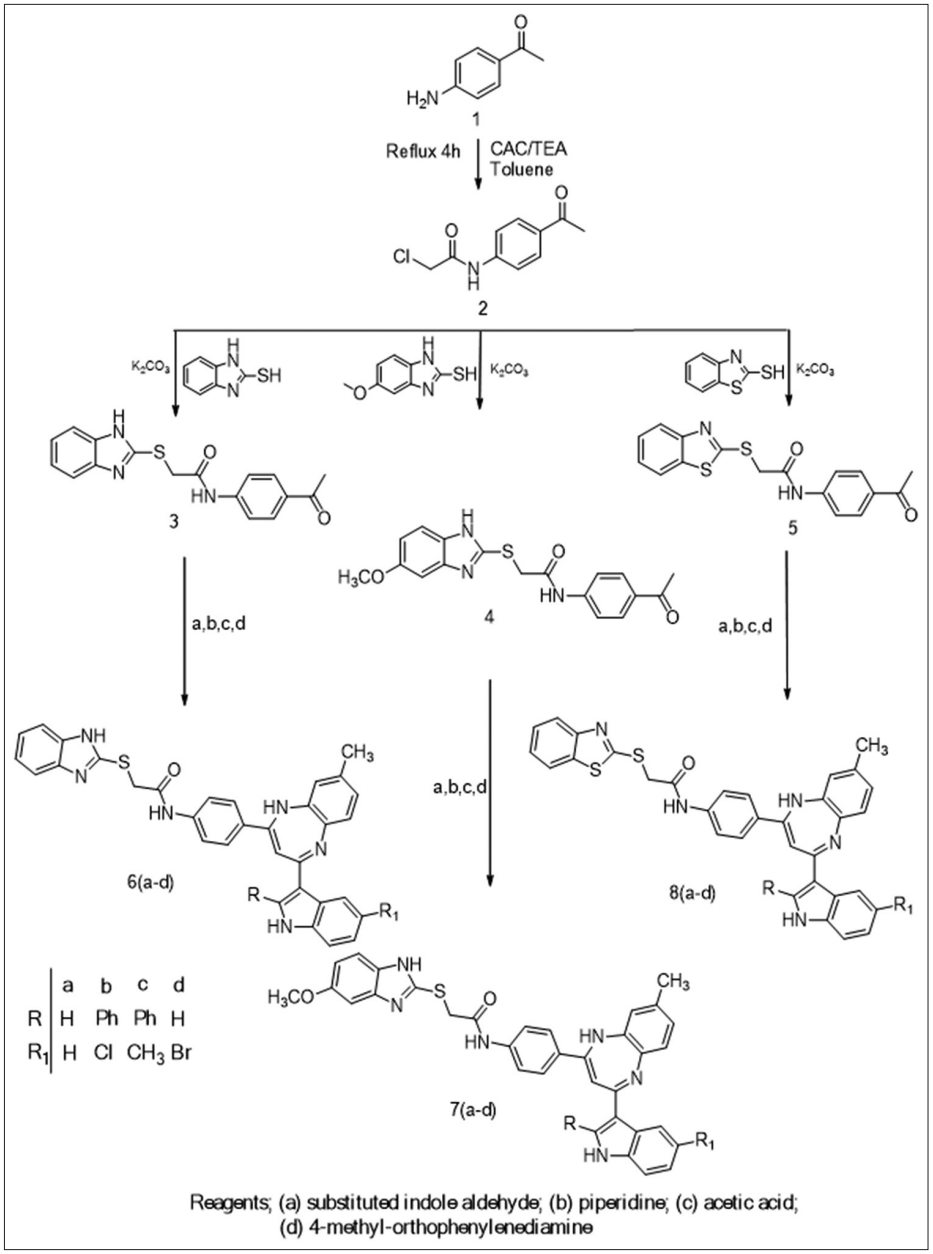
Fig. 2 Reducing Power activity of compounds 6a-d, 7a-d and 8a-d

Compounds 3, 4, and 5 on Claisen–Schmidt condensation with various 2,5-disubstituted indole-3-carboxaldehydes were refluxed in ethanol in the presence of piperidine for 4 h. After 4 h substituted ortho-phenylenediamine and catalytic amount of acetic acid was added to reaction mixture and was further refluxed (7-8 h) to yield the products 2-((1*H*-benzo[d]imidazol-2-yl) thio)-*N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl) acetamide 6a-d, *N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-((5-methoxy-1*H*-benzo[d]imidazol-2-yl) thio) acetamide 7a-d and *N*-(4-(4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[b][1,4]diazepin-2-yl) phenyl)-2-(benzo[d]thiazol -2-ylthio) acetamide 8a-d, respectively. The structures of all the novel compounds were confirmed by IR, ^{1}H NMR, and mass spectral studies and elemental data. The synthetic approach is outlined in Scheme 1.

The physical data of the compounds are presented in Table 1. The structures of the compounds were confirmed by IR, ^{1}H NMR, mass spectral studies and elemental data. The IR spectrum of 6a exhibited absorption band at 3409 cm^{-1}, 3318 cm^{-1}, 3110 cm^{-1}, and 3098 cm^{-1} for NH stretching frequency of indole, benzimidazole, amide, and diazepine, respectively. The absorption band at 1673 cm^{-1} corresponds to C=O stretching of amide. In the ^{1}HNMR spectrum, the compound 6a showed a singlet peaks at 12.27, 11.2, and 10.1 ppm ascribed to NH protons of benzimidazole, amide, and indole, respectively. In addition to this, 17 aromatic protons and one proton of diazepine NH resonated as a multiplet in the region 7.8-8.2 ppm. The singlet at 4.4 ppm and 2.4 ppm is due to the two protons of methylene group and three protons of methyl group, respectively. Further, the mass spectrum of 6a showed a molecular ion peak M$^+$ at m/z 554, which confirms its molecular weight and is in good agreement with nitrogen rule.

Antimicrobial activity

The analysis of antibacterial screening (Table 2) revealed that all compounds tested have moderate to high antibacterial activity as compared to the standard drug streptomycin. Compounds 6d, 7b-d, and 8b-d have showed excellent antibacterial activity against the tested microorganism *S. aureus* (ATCC-29513). Compounds 6b, 6d, 7a-d, and 8a-d have exhibited good activity against *E. coli* (MTCC-723) whereas



Scheme 1: Synthesis of compounds 3, 4, 5, 6a-d, 7a-d, and 8a-d

Table 1: Physical constant of all the synthesized compounds 3, 4, 5, and 6a-d, 7a-d, 8a-d

Sample code	R	R^1	M. For.	M. Wt.	M. Pt. °C
3	-	-	$C_{17}H_{15}N_3O_2S$	325	210
4	-	-	$C_{18}H_{17}N_3O_3S$	355	152
5	-	-	$C_{17}H_{14}N_2O_2S_2$	342	130
6a	H	H	$C_{33}H_{26}N_6O_S$	554	176
6b	Ph	Cl	$C_{39}H_{29}ClN_6OS$	664	144
6c	Ph	CH_3	$C_{40}H_{32}N_6OS$	644	154
6d	H	Br	$C_{33}H_{25}BrN_6OS$	632	210
7a	H	H	$C_{34}H_{28}N_6O_2S$	584	160
7b	Ph	Cl	$C_{40}H_{31}ClN_6O_2S$	694	188
7c	Ph	CH_3	$C_{41}H_{34}N_6O_2S$	674	148
7d	H	Br	$C_{34}H_{27}BrN_6O_2S$	662	188
8a	H	H	$C_{33}H_{25}N_5OS_2$	571	134
8b	Ph	Cl	$C_{39}H_{28}ClN_5OS_2$	681	178
8c	Ph	CH_3	$C_{34}H_{27}N_5OS_2$	585	146
8d	H	Br	$C_{33}H_{24}BrN_5OS_2$	649	196

M. for.: Molecular formula, M. wt.: Molecular weight, M. pt.: Melting point

the compounds 6b, 6d, 7a-d, and 8a-d displayed good activity against *P. aeruginosa* (MTCC-1688).

The antifungal activity results (Table 3) discovered that all the synthesized compounds have moderate to high antifungal activity as compared to the standard drug fluconazole. Compounds 6c-d, 7b-c, and 8b-d have revealed good activity against *A. niger* (MTCC-281). Compound 6a-b, 7a-c, and 8b-d showed good activity against *A. flavus* (MTCC-1782) whereas the compounds 6a, 7b-c, and 8b-c have profound activity against *A. oryzae* (MTCC-3567^T).

The rest of the compounds was either less or moderately active against the bacterial or fungal strains.

Antioxidant activities

DPPH RSA

In vitro method of scavenging of the stable DPPH radical is extensively used to evaluate the antioxidant activity in less time than other methods. DPPH is a stable free radical that can accept hydrogen radical

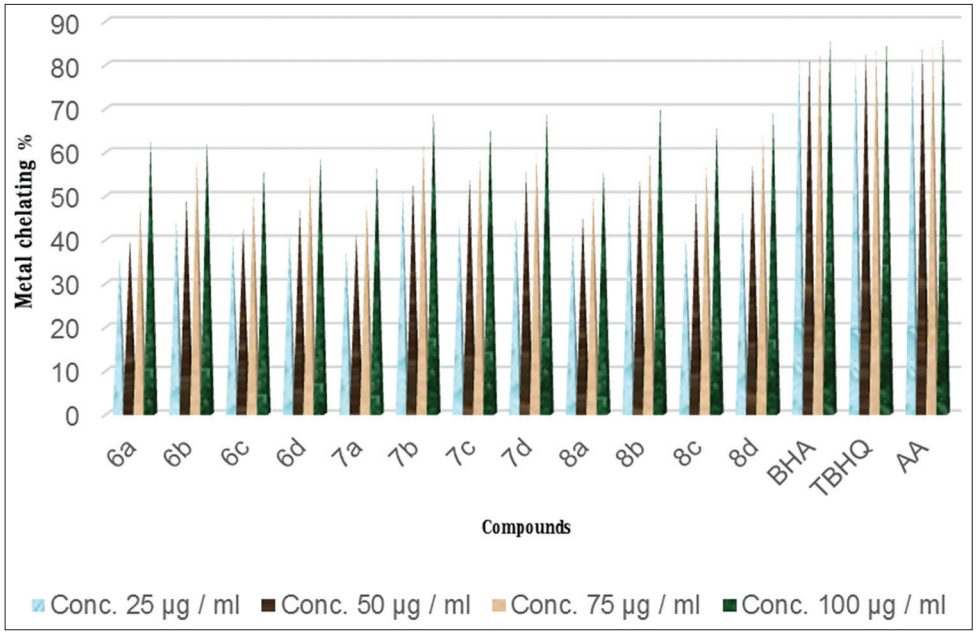


Fig. 3 Metal chelating activity of compounds 6a-d, 7a-d and 8a-d

Table 2: Antibacterial activity, size of inhibition zone (mm) formed at different concentrations (1000, 500, 250, and 125 µg/ml) of synthesized compounds 6a-d, 7a-d, and 8a-d

Compound	Zone of inhibition in mm											
	S. aureus				*E. coli*				*P. aeruginosa*			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
6a	11	13	13	12	11	12	12	13	11	12	13	12
6b	15	15	14	15	16	16	15	16	15	15	15	14
6c	13	13	12	11	11	12	13	13	12	13	13	12
6d	14	15	16	16	15	15	16	15	15	12	14	15
7a	15	13	14	15	15	14	15	15	13	14	15	14
7b	17	17	16	16	16	16	17	17	17	17	16	15
7c	16	15	15	14	14	15	14	15	15	14	14	14
7d	16	15	16	16	16	16	15	15	15	15	15	14
8a	14	14	15	15	14	14	15	15	15	15	14	14
8b	17	17	17	16	16	16	15	15	16	16	16	16
8c	15	15	16	16	15	15	14	14	14	14	14	13
8d	16	16	16	16	15	15	16	15	15	15	14	14
Streptomycin	17	17	17	17	17	16	16	16	15	16	16	15

Value are expressed as mean (n=3). *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa*

Table 3: Antifungal activity, size of inhibition zone (mm) formed at different concentrations (1000, 500, 250, and 125 µg/ml) of synthesized compounds 6a-d, 7a-d, and 8a-d

Compounds	Zone of inhibition in mm											
	A. niger				*A. flavus*				*A. oryzae*			
	1000	500	250	125	1000	500	250	125	1000	500	250	125
6a	15	14	13	13	15	12	13	14	15	16	15	14
6b	14	13	11	12	14	13	12	14	15	13	14	13
6c	12	11	14	12	13	12	12	13	14	12	13	14
6d	13	14	14	13	13	13	14	13	14	11	11	12
7a	13	12	11	13	12	12	11	14	11	12	12	14
7b	16	16	15	15	17	15	15	15	16	16	16	17
7c	14	14	14	14	16	14	13	14	13	12	14	15
7d	14	12	13	13	13	14	14	13	13	12	12	13
8a	13	13	12	11	11	14	13	12	12	12	11	13
8b	17	16	15	16	16	15	16	16	16	16	16	17
8c	16	16	14	15	13	14	14	15	13	15	14	16
8d	12	14	14	14	14	13	14	15	12	11	13	14
Fluconazole	17	16	15	15	17	16	16	15	17	17	16	16

Values are expressed as mean (n=3). *A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, A. oryzae: Aspergillus oryzae*

or an electron and must thus be converted to a stable diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms are taken up. The DPPH antioxidant assay measures the hydrogen donating capacity of the molecules under study. When the free radical DPPH is reduced by the sample its color changes from violet to yellow. The results (Fig. 1) suggested that compounds 6b, 8b, and 8c showed promising RSA at all concentrations. Compounds 6d, 7b, 7d, and 8a were found to enhance the RSA 51.77, 56.21, 50.59, and 50.00%, respectively, at Conc. 25 µg/ml. Compound 8a showed good activity, i.e., 62.13% at conc. 50 µg/ml and 64.79 at Conc. 75 µg/ml. Compounds 7a and 7b showed promising activity, i.e., 69.82 and 71.00%, respectively, at conc. 100 µg/ml. The rest of the compounds was found to possess less to moderate activity.

FRAP

The FRAP results (Fig. 2) suggested that, the compounds 6d, 7a-d, and 8a-c showed good absorbance 0.782, 0.783, 0.841, 0.811, 0.793, 0.761, 0.809, and 0.786 nm, respectively, at concentration 100 µg/ml, indicating that these compounds have good FRAP at concentrations of 100 µg/ml. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The rest of the compounds showed lower absorbance as related to the standards. The higher the absorbance of the compounds indicated greater reducing power.

Fe^{2+} metal ion chelating activity

Fe^{2+} metal ion chelating activity results (Fig. 3) revealed that synthesized compounds obstructed the formation of Fe^{2+} and ferrozine complex. Compounds 7b-d and 8b-d exhibited (69.15, 66.04, 69.15, 71.02, 66.04, and 68.84%, respectively) good metal chelating activity at concentration of 100 µg/ml. Highest metal chelating activity of these compounds indicates that these compounds are able to capture Fe^{2+} ion before ferrozine.

This might be the reason for the higher metal chelating activity. The rest of the compounds showed reasonable to less activity when compared with the standard drugs.

CONCLUSION

The title compound of benzodiazepine derivatives attached with benzimidazole/benzothiazole and indole moieties is synthesized and characterized using spectral and analytical data. All the compounds have been subjected for antimicrobial, antifungal, and antioxidant screening. We have found that compounds are active toward antibacterial and antifungal strains. Few compounds gave better antioxidant activity compared to the standard. These studies may promote further extension of the benzodiazepine derivatives bearing benzimidazole/benzothiazole and indole moieties, which may lead to compounds with effective antioxidant and antimicrobial activities.

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