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ORIGINAL ARTICLE

Synthesis of novel Indolyl benzo[*b*][1,4]diazepins as a potent antimicrobial and antioxidant agents



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KEYWORDS

2,5-Disubstituted indole-3-carboxaldehydes;
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Vilsmeier Haack formylation;
Claisen–Schmidt condensation;
Indolylbenzo[*b*][1,4]diazepins;
Antimicrobial activity;
Antioxidant activity

Abstract A new series of novel indolyl benzo[*b*][1,4]diazepins bearing a 2,5-dichlorothiophene moiety are reported. Claisen–Schmidt condensation of 2,5-disubstituted indole-3-carboxaldehydes with 2,5-dichloro-3-acetylthiophene will produce (*E*)-3-(2,5-disubstituted-1*H*-indol-3-yl)-1-(2,5-dichlorothiophene-3-yl)prop-2-en-1-one. The acid catalysed cyclocondensation of preformed chalcones with substituted ortho-phenylenediamine has produced the titled compounds in good yields. All the newly synthesized compounds are characterised by IR, ¹H NMR, ¹³C NMR, elemental analysis and mass spectroscopic data. Compounds **4b**, **4c** and **4f** have emerged as most potent analogues in antimicrobial and antioxidant evaluations.

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1. Introduction

Benzodiazepines have attracted greater attention as an important class of heterocyclic compounds in the field of drugs and pharmaceuticals. These compounds are widely used as anti-convulsants (Landquist, 1984), as antianxiety drugs (Schutz, 1982; Randall and Kapple, 1977), analgesics (Fryer, 1991), sedatives (Randall and Kapple, 1977), anti-depressives (Schutz, 1982), hypnotics (Randall and Kapple, 1977) and anti-inflammatory agents (De Baun et al., 1977). Since the discovery of flurazepam, flunitrazepam, quazepam, halazepam and trifluazepam the chemistry of [1,4] and [1,5] benzodiazepines and allied compounds have assumed considerable importance due to their use as drugs controlling mild to moderate CNS depression (Oikkola and Ahonen, 2008), mental tension depression (Oikkola and Ahonen, 2008), as hypnotics, anxiolytic agents and for controlling chronic insomnia (Stembach, 1971; Oikkola and Ahonen, 2008). In particular [1,4]benzodiazepines have been demonstrated to be non-nucleoside reverse transcriptase inhibitors, acquire a significant place in the treatment of the infections by the HIV (Darnag et al., 2010). The same analogues containing conjugated acrylyl C2-substituents possess significant cytotoxicity according to the NCI 60-cell line screen surpassing anthracycline in potency (Chen et al., 2004). Geigy, 1965, has described the [1,4]benzodiazepine analogues as anticonvulsants, muscle relaxants, blood pressure lowering and CNS depressant agents (Geigy, 1965).

Alongside, indoles and its biheterocycles are featured widely in a wide variety of biologically and pharmacologically

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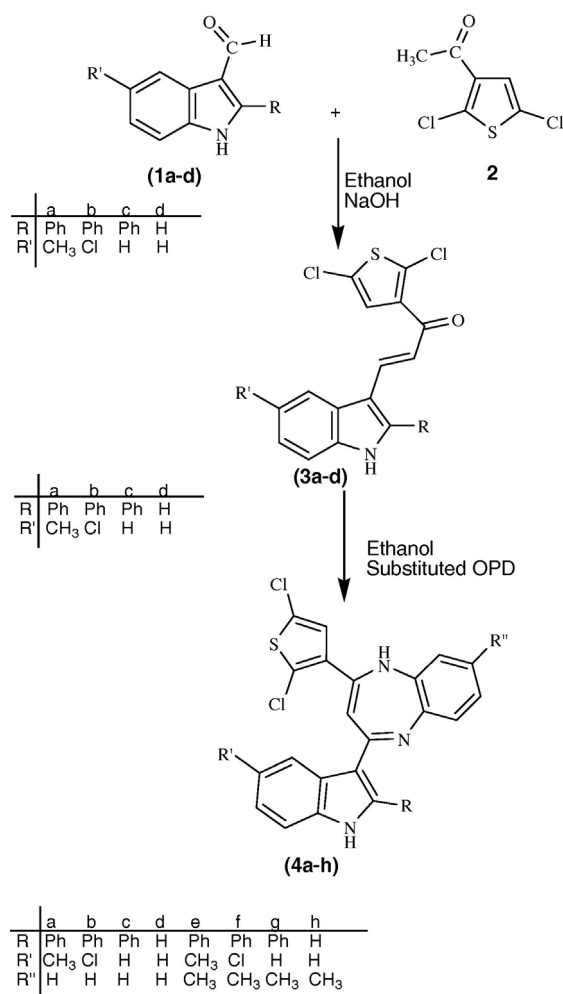
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active compounds (Sundberg, 1996). The indole derivatives are known to possess anticancer (Wu et al., 2009; Pojarova et al., 2007; Biradar and Sasidhar, 2011), antioxidant (Biradar et al., 2010a; Biradar et al., 2010b; Biradar and Sasidhar, 2011), anti-rheumatoid and anti-HIV (Buyukbingol et al., 1994; Suzen and Buyukbingol, 1998) activities. Many indole derivatives are considered as the most potent scavenger of free radicals (Chyan et al., 1999). Indolyl[1,4]benzodiazepines are reported to possess inhibitory activity of central and motor nervous system, depression of blood pressure and dilation of pulmonary vessels (Reynolds and Carson, 1972). In our previous approach, we have reported the indolyl[1,4]benzodiazepine systems as antimicrobial, DNA cleavage (Biradar et al., 2010a; Biradar et al., 2010b), analgesic, CNS depressant and anti-inflammatory agents (Biradar and Manjunath, 2004).

In continuation of our enduring efforts on synthesis of 'Drug-Like' molecules (Biradar et al., 2008; Biradar and Sasidhar, 2011; Biradar and Manjunath, 2004a, 2004b), It was envisaged that the two pharmacophores if linked together (Scheme 1) would generate novel molecular templates which are likely to exhibit interesting biological properties.



Scheme 1 Schematic representation for the synthesis of novel Indolylbenzodiazepines.

2. Results and discussion

2.1. Chemistry

Herein, we report the synthesis of novel indolyl benzo[*b*][1,4]diazepines bearing thiophenes via two step methodology utilising 2,5-disubstituted indole-3-carboxaldehydes. (*E*)-3-(2,5-disubstituted-1*H*-indol-3-yl)-1-(2,5-dichlorothiophen-3-yl)prop-2-en-1-one (**3a-d**) are obtained by the Claisen-Schmidt condensation of 2,5-dichloro-3-acetylthiophene (**2**) with various 2,5-disubstituted indole-3-carboxaldehydes (**1a-d**) (Scheme 1). The IR spectrum of (*E*)-1-(2,5-dichlorothiophen-3-yl)-3-(5-methyl-2-phenyl-1*H*-indol-3-yl)prop-2-en-1-one (**3a**) has shown a strong absorption at 3258 cm⁻¹ corresponding to indole NH, absorptions at 2936 and 1717 cm⁻¹ correspond to C-H (Aromatic) and C=O stretching, respectively. The ¹H NMR spectrum of **3a** has exhibited a singlet at δ 11.2 (s, 1H, indole NH) integrating for single proton due to deshielded indole NH which is D₂O-exchangeable. A singlet at δ 2.0 (s, CH₃, 3H) for methyl protons and a multiplet between δ 6.9–8.0 (m, 9Ar-H, 2H, –CH=CH–) integrating for eleven protons in the molecule. This spectrum has also exhibited the absence of aldehydic proton confirming the formation of product. The ¹³C NMR spectrum of **3a** has displayed highly deshielded signal at δ 180 for carbonyl carbon, up field signal at δ 30 for methyl carbon. In the mass spectrum of compound **3a**, molecular ion peak is observed at *m/z* 412(100%) which is also the base peak, corresponding to the molecular weight of the compound. These spectral data support the formation of **3a**.

The acid catalysed cyclocondensation of preformed chalcones (**3a-d**) and substituted ortho-phenylenediamine in ethanol has produced the titled compounds (**4a-h**) (Scheme 1). All the newly synthesized compounds were characterised by IR, ¹H NMR, ¹³C NMR, elemental analysis and mass spectroscopic data.

The IR spectrum of (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-4-(5-methyl-2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[*b*][1,4]diazepine (**4a**) has shown a strong absorption at 3131 cm⁻¹ corresponding to indole NH and absorption at 3063 cm⁻¹ is for diazepine NH. Absorptions at 2910 and 1626 cm⁻¹ correspond to the C–H and C=N stretching respectively. The ¹H NMR spectrum of **4a** has exhibited a singlet at δ 11.80 due to deshielded indole NH, which is also D₂O exchangeable. A singlet at δ 2.3 (s, 3H, CH₃) for methyl protons and a multiplet between δ 6.8–7.6 ppm (m, 15H, 14Ar-H and Diazepine NH) integrating for fifteen protons. The ¹³C NMR spectrum of **4a** has displayed a downfield signal at δ 165 integrated for the C=N of diazepine ring. Up field signal at δ 30 corresponds to the methyl carbon.

Mass spectrum of compound **4a** has shown a peak at *m/z* 499 (90%), 501 (30) and 503 (10%) corresponding to the molecular weight and isotopic chlorine in the required ratio. Molecular ion has undergone into fragmentation by two routes. In the first route it has lost chlorine from thiophene ring to generate **m**₁ at *m/z* 464(25%) and 466 (8%), fragment **m**₁ has lost thiophene ring (C₄HClS) to generate **m**₂ at *m/z* 348 (55%), the radical cation **m**₂ has lost C₆H₅N to generate the cation **m**₃ at *m/z* 257 (35%). Cation **m**₃ has lost azitidinyl fragment to generate **m**₄ at *m/z* 206 (55%) and **m**₄ has generated **m**₅ at *m/z* 90 (27%) by losing the fragment C₈H₆N. In another route,

molecular ion on fragmentation loses $C_6H_5N_2$ radical from the diazepine ring and displayed peaks at **m**₆ m/z 394 (15%), 396 (5%) and 398 (1%) corresponding to the isotopic chlorine in the required ratio. **M**₆ has lost the fragment $C_7H_3Cl_2S$ to generate **m**₇ at m/z 206 (55%) and **m**₇ has lost the fragment C_8H_6N to generate **m**₈ at m/z 90 (27%). This fragmentation pattern supports the proposed structure of compound **4a**. Spectral data of compounds **4b–h** support their structures.

2.2. Biological evaluations

2.2.1. Antimicrobial activity

Results of antimicrobial activity are summarised in Table 1. Applying the agar plate diffusion technique (Verma and Imam, 1973) all of the newly synthesized compounds were screened *in vitro* for antibacterial activity against *Escherichia coli* (*E. coli*), (Gram-negative) and *Bacillus subtilis* (Gram-positive) at 25, 50, and 100 µg/ml concentrations, respectively. Under identical conditions, Streptomycin sulfate was used as the standard. The zone of inhibition was measured in mm for each concentration. All of the screened compounds were found to have moderate to significant antibacterial activity. Compound **4a** and **4e** have shown very interesting results against both the strains. Compound **4b** against *E. coli* and **4f** against *B. subtilis* have exhibited maximum inhibition.

Similarly, the antifungal screening of the compounds was carried out *in vitro* against two fungi *Aspergillus niger* and *Aspergillus flavus* at 25, 50, and 100 µg/ml concentrations respectively, by using griseofulvin as the positive reference. Of all the tested indolylbenzodiazepine systems, majority of the compounds exhibited moderate to significant antifungal activity. Compounds **4a** and **4e** have shown very significant activity against both the strains and **4f** against *A. flavus* has shown promising results by maximum inhibition than that of the standard Griseofulvin.

Hence, results clearly signify, the antimicrobial activity was increased with the methyl and chloro substitutions at the fifth position of the indole ring. Further, significant improvement in the activity was observed with the incorporation of methyl substituted benzodiazepine ring.

2.2.2. Antioxidant activity

2.2.2.1. Free radical scavenging activity. The newly synthesized compounds were screened for free radical scavenging activity by DPPH method (Singh et al., 2002). Samples were prepared at concentrations of 10, 50, and 100 µg/100 µl and Butylated hydroxy anisole (BHA) is taken as the standard. Among the tested compounds, **4b** and **4f** have shown promising scavenging activity. The increased activity may be due to the existence of 'Cl' substitution at the fifth position of the indole ring. Whereas, compounds **4c**, **4g** and **4h** with 'H' substitution at the fifth position of the indole ring have shown moderate activity. In contrast, 'CH₃' substituted indolylbenzodiazepine derivatives **4a** and **4e** have shown least activity compared with the standard. Bar graph representation of percentage free radical scavenging activity is shown in Fig. 1.

2.2.2.2. Total antioxidant capacity. In total antioxidant activity (Mruthunjaya and Hukkeri, 2008), antioxidant capacities are expressed as equivalents of ascorbic acid. Among the compounds tested, **4b** and **4f** which are 'Cl' substituted indolylbenzodiazepine analogues have shown strong antioxidant capacity and compounds **4e**, **4g** and **4h** with CH₃ and H substitution at the fifth position of the indole ring have shown moderate activity. It is also observed that, indolylbenzodiazepine system has improved the activity significantly than that of the chalcones Fig. 2.

2.2.2.3. Ferric reducing antioxidant power activity. The compounds are also screened for ferric reducing antioxidant activity (Barreira et al., 2008). Butylated hydroxy anisole (BHA) was used as the standard. All the tested compounds have shown positive tendency toward the ferric reducing activity. As in the previous cases, indolylbenzodiazepine analogues (**4a–h**) have shown improved activity than the chalcones (**3a–d**). Further, compounds **4a**, **4e** and **4g** with 'CH₃' substitution have shown excellent ferric reducing antioxidant activity and other derivatives of indole have shown moderate to high activity. The presence of benzodiazepine ring in addition to the alkyl group may play an important role to act as a better

Table 1 Zone of inhibition in mm at 25, 50 and 100 µg/ml concentrations.

Compd	Antibacterial activity						Antifungal activity					
	<i>E. coli</i>			<i>B. subtilis</i>			<i>A. niger</i>			<i>A. flavus</i>		
	25	50	100	25	50	100	25	50	100	25	50	100
3a	8	9	11	10	10	11	8	9	11	9	10	12
3b	8	9	10	7	8	9	8	9	10	8	8	9
3c	6	6	7	4	5	7	5	6	6	6	6	7
3d	4	5	6	6	6	7	4	6	6	6	6	7
4a	11	12	13	10	12	14	10	11	13	9	11	14
4b	9	10	12	9	9	11	10	10	11	8	9	11
4c	7	7	9	8	8	9	8	8	9	7	8	8
4d	7	8	8	7	7	7	7	8	10	8	8	9
4e	10	12	14	14	15	17	11	12	14	11	12	14
4f	9	11	12	12	12	14	11	11	13	12	13	15
4g	6	8	10	8	8	9	7	7	8	9	9	10
4h	6	7	7	9	9	9	9	10	10	7	8	9
Std. 1	10	12	13	12	13	15	—	—	—	—	—	—
Std. 2	—	—	—	—	—	—	12	13	15	11	13	14

Std. 1, Streptomycin; Std. 2, Griseofulvin.

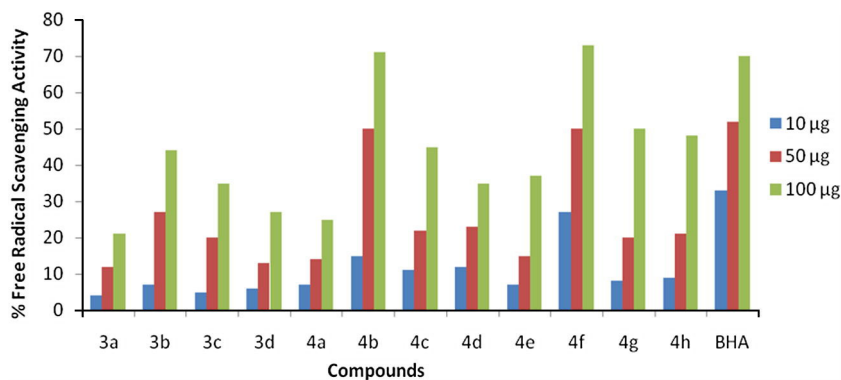


Figure 1 Free radical scavenging activity of novel Indolylbenzodiazepines.

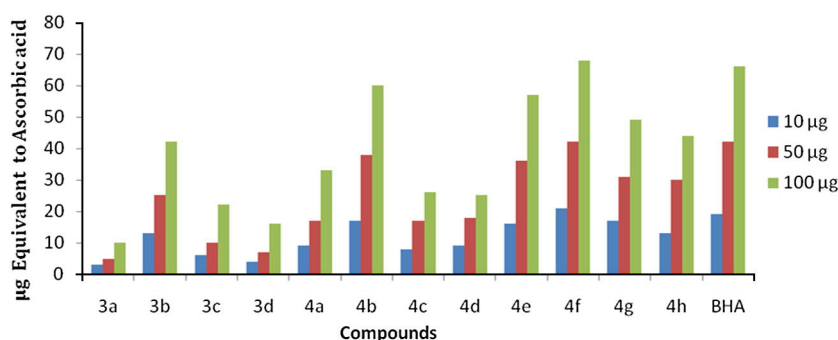


Figure 2 Total antioxidant capacity of novel Indolylbenzodiazepines.

electron donor which may enhance reducing power ability of **4a**, **4e** and **4g**. The results are presented in Fig. 3.

3. Experimental

3.1. Chemistry

3.1.1. General considerations

All the chemicals and reagents were purchased from MERCK, Himedia and SD fine chemical companies and are used

without further purification. Melting points of the synthesized compounds are determined in open capillaries and are uncorrected. Reactions are monitored by thin-layer chromatography (TLC) on Silica Gel 60 F₂₅₄ aluminium sheets (MERCK) and detection was made using UV light and iodine. IR spectra are recorded in KBr on Perkin–Elmer and FTIR Spectrophotometer (ν_{max} in cm^{-1}). ^1H NMR and ^{13}C NMR spectra on BRUKER AVENE II 400 MHz NMR Spectrometer (Chemical shift in δ ppm down field from TMS as an internal reference). The Mass spectra are recorded on LC-MSD-Trap-

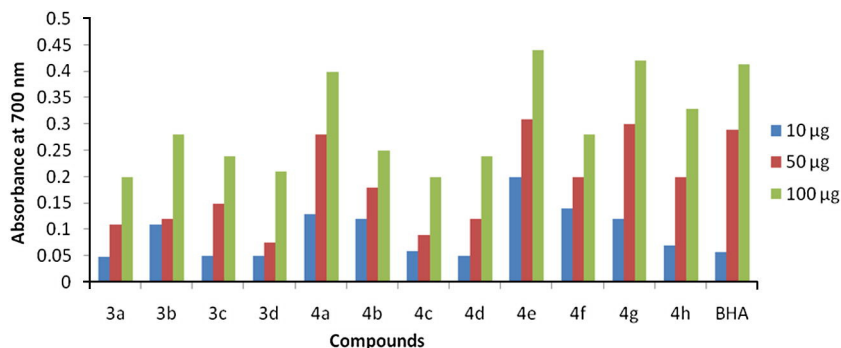


Figure 3 Ferric reducing antioxidant activity of novel Indolylbenzodiazepines.

SL instruments. The elemental analysis was determined on FLASH EA 1112 SERIES instrument. All the compounds gave C, H and N analysis within $\pm 0.5\%$ of the theoretical values.

3.1.2. Typical experimental procedure for the synthesis of 2,5-disubstituted indole-3-carboxaldehydes (**1a–d**)

The precursors 2,5-disubstituted indole-3-carboxaldehydes (**1a–d**) were obtained from the Vilsmeier Haack formylation reaction of 2,5-disubstituted indoles (Biradar, 1982).

3.1.3. General procedure for the synthesis of (*E*)-3-(2,5-disubstituted-1*H*-indol-3-yl)-1-(2,5-dichlorothiophene-3-yl)prop-2-en-1-one (**3a–d**)

Claisen–Schmidt condensation of an equimolar mixture of 2,5-dichloro-3-acetylthiophene (**2**) (0.01 mol) and various 2,5-disubstituted indole-3-carboxaldehydes (**1a–d**) (0.01 mol) were refluxed (3–4 h) in ethanol (15–20 ml) with 1 ml of (20%) sodium hydroxide solution (Scheme 1). The completion of reaction was monitored by TLC in chloroform and benzene (1:1). After the completion of reaction, excess of solvent was removed under reduced pressure and kept at room temperature for few hours for the formation of crystals. The product obtained was filtered and purified with ethanol. All the newly synthesized compounds were characterised by IR, ^1H NMR, ^{13}C NMR and mass spectroscopic data. Yield 70–85%.

3.1.3.1. (*E*)-1-(2,5-dichlorothiophen-3-yl)-3-(5-methyl-2-phenyl-1*H*-indol-3-yl)prop-2-en-1-one (**3a**). Yield 85% (ethanol); mp 244–245 °C; IR (KBr) (ν_{max} in cm^{-1}): 3258, 2936, 1717, 1545; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.20 (s, 1H, indole NH), 6.9–8.0 (m, 11H, 9Ar-H, $\text{CH}=\text{CH}$), 2.0 (s, 3H, CH_3); ^{13}C NMR (DMSO- d_6 + CDCl_3) δ (ppm): 185 (C=O), 30 (CH_3) and 152, 150, 146, 144, 142, 141, 139, 137, 133, 129, 124, 105, 104, 100 and 30; MS: m/z = 413 [$\text{M} + 1$] $^+$. Anal. Calcd for $\text{C}_{22}\text{H}_{15}\text{Cl}_2\text{NOS}$ (412): C, 64.08; H, 3.67; N, 03.40. Found: C, 64.10; H, 3.62; N, 03.45.

3.1.3.2. (*E*)-3-(5-chloro-2-phenyl-1*H*-indol-3-yl)-1-(2,5-dichlorothiophen-3-yl)prop-2-en-1-one (**3b**). Yield 80% (ethanol); mp 275–277 °C; IR (KBr) (ν_{max} in cm^{-1}): 3309, 2923, 1725, 1581; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.60 (s, 1H, indole NH), 7.1–8.2 (m, 11H, 9Ar-H, $\text{CH}=\text{CH}$); MS: m/z = 433 [$\text{M} + 1$] $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{12}\text{Cl}_3\text{NOS}$ (432): C, 58.28; H, 2.79; N, 3.24. Found: C, 58.30; H, 2.76; N, 03.28.

3.1.3.3. (*E*)-1-(2,5-dichlorothiophen-3-yl)-3-(2-phenyl-1*H*-indol-3-yl)prop-2-en-1-one (**3c**). Yield 77% (ethanol); mp 236–237 °C; IR (KBr) (ν_{max} in cm^{-1}): 3228, 2946, 1730, 1550; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 10.60 (s, 1H, indole NH), 6.8–8.2 (m, 12H, 10Ar-H, $\text{CH}=\text{CH}$); MS: m/z = 400 [$\text{M} + 2$] $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{13}\text{Cl}_2\text{NOS}$ (398): C, 63.32; H, 3.29; N, 3.52. Found: C, 63.38; H, 3.26; N, 3.50.

3.1.3.4. (*E*)-1-(2,5-dichlorothiophen-3-yl)-3-(1*H*-indol-3-yl)prop-2-en-1-one (**3d**). Yield 70% (ethanol); mp 220–221 °C; IR (KBr) (ν_{max} in cm^{-1}): 3211, 2930, 1717, 1595; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.00 (s, 1H, indole NH), 7.0–8.3 (m, 8H, 6Ar-H, $\text{CH}=\text{CH}$); MS: m/z = 323 [$\text{M} + 1$] $^+$. Anal. Calcd for $\text{C}_{15}\text{H}_9\text{Cl}_2\text{NOS}$ (322): C, 55.91; H, 2.82; N, 04.35. Found: C, 55.97; H, 3.85; N, 04.24.

3.1.4. General procedure for the synthesis of (2*Z*,4*E*)-8-substituted-2-(2,5-dichlorothiophen-3-yl)-4-(2,5-disubstituted-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepine (**4a–h**)

A mixture of appropriate (*E*)-3-(2,5-disubstituted-1*H*-indol-3-yl)-1-(2,5-dichlorothiophene-3-yl)prop-2-en-1-one (**3a–d**) (0.01 mol) and substituted orthophenylenediamine (0.01 mol) in ethanol (20 ml) were refluxed with catalytic amounts of acetic acid for 5–6 h. The completion of reaction was monitored by TLC in chloroform and ethylacetate (3:1). The excess of solvent was removed; reaction mixture was cooled and poured into crushed ice with constant stirring. The solid mass thus obtained was filtered and washed with water and recrystallized from ethanol to get the title compounds (**4a–h**) (Scheme 1). All the newly synthesized compounds were characterised by IR, ^1H NMR, ^{13}C NMR and mass spectroscopic data. Yield 67–75%.

3.1.4.1. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-4-(5-methyl-2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepine (**4a**). Yield 75% (ethanol); mp 180–181 °C; IR (KBr) (ν_{max} in cm^{-1}): 3131, 3063, 2910, 1626, 1573; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.80 (s, 1H, indole NH), 6.8–7.6 (m, 15H, 14Ar-H, diazepine NH), 2.3 (s, 3H, CH_3); ^{13}C NMR (DMSO- d_6 + CDCl_3) δ (ppm): 165 (C=N), 30 (CH_3) and 148, 138, 134, 132, 131, 130, 129, 128, 127, 124, 120, 117, 115, 114, 113 and 108; MS: m/z = 499 [$\text{M} - 1$] $^+$. Anal. Calcd for $\text{C}_{28}\text{H}_{19}\text{Cl}_2\text{N}_3\text{S}$ (500): C, 67.20; H, 3.83; N, 08.40. Found: C, 67.17; H, 3.88; N, 08.32.

3.1.4.2. (2*Z*,4*E*)-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-2-(2,5-dichlorothiophen-3-yl)-1*H*-benzo[b][1,4]diazepine (**4b**). Yield 70% (ethanol); mp 260–261 °C; IR (KBr) (ν_{max} in cm^{-1}): 3156, 3003, 2947, 1635, 1581; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 12.09 (s, 1H, indole NH), 7.2–7.8 (m, 15H, 14Ar-H, diazepine NH); MS: m/z = 521 [$\text{M} + 1$] $^+$. Anal. Calcd for $\text{C}_{27}\text{H}_{16}\text{Cl}_3\text{N}_3\text{S}$ (520): C, 62.26; H, 3.10; N, 08.07. Found: C, 62.22; H, 3.04; N, 08.05.

3.1.4.3. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-4-(2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepine (**4c**). Yield 67% (ethanol); mp 255–257 °C; IR (KBr) (ν_{max} in cm^{-1}): 3201, 3011, 2990, 1646, 1553; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.20 (s, 1H, indole NH), 6.6–7.3 (m, 16H, 15Ar-H, diazepine NH); MS: m/z = 488 [$\text{M} + 2$] $^+$. Anal. Calcd for $\text{C}_{27}\text{H}_{17}\text{Cl}_2\text{N}_3\text{S}$ (486): C, 66.67; H, 3.52; N, 8.64. Found: C, 66.58; H, 3.26; N, 8.50.

3.1.4.4. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-4-(1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepine (**4d**). Yield 67% (ethanol); mp 225–227 °C; IR (KBr) (ν_{max} in cm^{-1}): 3109, 3070, 2923, 1630, 1581; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 11.40 (s, 1H, indole NH), 6.9–7.6 (m, 12H, 11Ar-H, diazepine NH); MS: m/z = 409 [$\text{M} - 1$] $^+$. Anal. Calcd for $\text{C}_{21}\text{H}_{13}\text{Cl}_2\text{N}_3\text{S}$ (410): C, 61.47; H, 3.19; N, 10.24. Found: C, 61.42; H, 3.23; N, 10.21.

3.1.4.5. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-8-methyl-4-(5-methyl-2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[b][1,4]diazepine (**4e**). Yield 71% (ethanol); mp 187–188 °C; IR (KBr) (ν_{max} in cm^{-1}): 3211, 3105, 2915, 1650, 1543; ^1H NMR (DMSO- d_6 + CDCl_3) δ (ppm): 9.90 (s, 1H, indole NH), 7.4–7.8 (m, 14H, 13Ar-H, diazepine NH), 2.6 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); MS: m/z = 515 [$\text{M} + 1$] $^+$. Anal. Calcd for

C₂₉H₂₁Cl₂N₃S (514): C, 67.70; H, 4.11; N, 08.17. Found: C, 67.76; H, 4.14; N, 08.13.

3.1.4.6. (2*Z*,4*E*)-4-(5-chloro-2-phenyl-1*H*-indol-3-yl)-2-(2,5-dichlorothiophen-3-yl)-8-methyl-1*H*-benzo[*b*][1,4]diazepine (**4f**). Yield 70% (ethanol); mp 220–221 °C; IR (KBr) (ν_{\max} in cm⁻¹): 3305, 3150, 2918, 1641, 1601; ¹H NMR (DMSO-*d*₆ + CDCl₃) δ (ppm): 11.70 (s, 1H, indole NH), 6.8–7.5 (m, 14H, 13Ar-H, diazepine NH), 2.2 (s, 3H, CH₃); MS: m/z = 535 [M+1]⁺. Anal. Calcd for C₂₈H₁₈Cl₃N₃S (534): C, 62.87; H, 3.39; N, 07.86. Found: C, 62.58; H, 3.41; N, 07.83.

3.1.4.7. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-8-methyl-4-(2-phenyl-1*H*-indol-3-yl)-1*H*-benzo[*b*][1,4]diazepine (**4g**). Yield 68% (ethanol); mp 267–268 °C; IR (KBr) (ν_{\max} in cm⁻¹): 3233, 3115, 2895, 1630, 1531; ¹H NMR (DMSO-*d*₆ + CDCl₃) δ (ppm): 10.10 (s, 1H, indole NH), 7.1–7.7 (m, 15H, 14Ar-H, diazepine NH), 2.5 (s, 3H, CH₃); MS: m/z = 347 [M+2]⁺. Anal. Calcd for C₂₈H₁₉Cl₂N₃S (345): C, 67.20; H, 3.83; N, 8.40. Found: C, 67.28; H, 3.76; N, 8.20.

3.1.4.8. (2*Z*,4*E*)-2-(2,5-dichlorothiophen-3-yl)-4-(1*H*-indol-3-yl)-8-methyl-1*H*-benzo[*b*][1,4]diazepine (**4h**). Yield 67% (ethanol); mp 177–178 °C; IR (KBr) (ν_{\max} in cm⁻¹): 3218, 3180, 2900, 1646, 1542; ¹H NMR (DMSO-*d*₆ + CDCl₃) δ (ppm): 12.30 (s, 1H, indole NH), 7.3–8.0 (m, 11H, 10Ar-H, diazepine NH), 2.4 (s, 3H, CH₃); MS: m/z = 425 [M+1]⁺. Anal. Calcd for C₂₂H₁₅Cl₂N₃S (424): C, 62.27; H, 3.56; N, 09.90. Found: C, 62.20; H, 3.52; N, 09.96.

4. Conclusions

In conclusion, a new series of novel indolylbenzo[*b*][1,4]diazepines are designed and synthesized in good yields. In biological evaluations; it was observed that, the antimicrobial activity was increased with the methyl (**4a** and **4e**) and chloro (**4b** and **4f**) substitutions at the fifth position of the indole ring. Compounds **4b** and **4f** have shown promising results for free radical scavenging and total antioxidant capacity activities. In ferric reducing antioxidant power, compounds **4a**, **4e** and **4g** have shown maximum reducing power. Therefore, our findings will provide a great impact on chemists and biochemists for further investigations in the indole field in search of molecules possessing potent antioxidant and antimicrobial activities. Based on these results, selected novel compounds are being screened *in vivo* which will be reported in due course.

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