Research Article

Synthesis, Antimicrobial, and Antioxidant Activities of N-[(5'-Substituted-2'-phenyl-1H-indol-3'-yl)methylene]-5H-dibenzo[b,f]azepine-5-carbohydrazide Derivatives

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The main aim of the present study was to synthesize new leads with potential antimicrobial and antioxidant activities. As a part of systematic investigation of synthesis and biological activity, some new indole compounds **3a–c** and **4a–c** were prepared and screened for their antimicrobial and antioxidant activities. The antimicrobial evaluation of newly synthesized compounds was carried out by cup-plate method. Antimicrobial activity results revealed that compound **4a** showed promising activity against bacteria *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* and exhibited maximum inhibition against *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus*, and *Aspergillus flavus*. The antioxidant activity was performed by three methods, namely, radical scavenging activity (RSA), ferric ions (Fe^{+3}) reducing antioxidant power (FRAP), and metal chelating activity by using Hatano's, Oyaizu's, and Dinis' methods, respectively. Compound **4a** showed promising RSA, FRAP, and metal chelating activity.

1. Introduction

The emergence and spread of antimicrobial resistance have become one of the most serious public health concerns across the world. In last few years it was reported that indole, its bioisosteres, and derivatives had antimicrobial activity against Gram-negative, Gram-positive bacteria especially against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus epidermidis* and the yeast *Candida albicans* [1], and so forth. On the other hand, indolyl compounds are very efficient antioxidants, protecting both lipids and proteins from peroxidation. It is well known that the indole structure influences the antioxidant efficacy in biological systems [2]. Indole derivatives have been reported to possess a variety of physiological and pharmacological activities like antibacterial [3], antifungal [4, 5], antioxidant [6], anticancer [7, 8], analgesic [9], antiasthmatic [10], and antiviral [11] and to be effective in treatment of sexual dysfunction [12]. An effective anticonvulsant drug, 5H-dibenzo[b,f]azepine-5-

carboxamide, was first synthesized by Schindler [13] in 1960 and since then it has become the most frequently prescribed first-line drug for the treatment of epilepsy. In addition, dibenzo[b,f]azepine derivatives are useful due to their antimicrobial [14], antioxidant [15, 16], and antitubercular [17] activities.

Thiazolidinone, a saturated form of thiazole with carbonyl group on fourth carbon, has been considered as a magic moiety (wonder nucleus) because it gives out novel derivatives with different types of biological activities. This diversity in the biological response profile has attracted the attention of many researchers to explore the potential biological activity (such as antibacterial [18], antifungal [19], and antioxidant [20, 21] activities) of this skeleton.

We have reported earlier the synthesis, antioxidant, and antimicrobial activities of indole analogues containing thiazolidinone derivatives such as 3-[4-(4-substituted)thiazol-2-yl]-2-(2-phenyl-1H-indol-3-yl)thiazolidin-4-ones (**1**) [22],

FIGURE 1: Motivation for synthesis of antimicrobial and antioxidant active compounds.

2-(5-substituted-2-phenyl-1H-indol-3-yl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thiazolidin-4-ones (2) [23], 2-(5-substituted-2-phenyl-1H-indol-3-yl)-3-(4-phenylthiazol-2-yl)thiazolidin-4-ones (3) [24], and 2,5,6-trimethyl-3-[2-(2-phenyl-5-substituted-1H-indol-3-yl)-4-oxothiazolidin-3-yl]thieno[2,3-d]pyrimidin-4(3H)-ones (4) [25] (Figure 1). Prompted by these results, we herein report the synthesis of title compounds and the evaluation of their antimicrobial and antioxidant activities.

2. Result and Discussion

The pathway for the synthesis of title compounds is illustrated in Scheme 1. Cyclocondensation of 5H-dibenzo[b,f]azepine-5-carbohydrazide (1) [26] with 5-chloro-2-phenyl-1H-indol-3-carboxaldehyde (2a) [27] gave the intermediate N-[(5'-chloro-2'-phenyl-1H-indol-3'-yl)methylene]-5H-dibenzo[b,f]azepine-5-carbohydrazide (3a). Further, compound (3a) on refluxing with thioglycolic acid afforded N-[2-(5'-substituted-2'-phenyl-1H-indol-3'-yl)-4-oxothiazolidin-3-yl]-5H-dibenzo[b,f]azepine-5-carboxamide (4a). The structures of all these previously unknown compounds were characterized by spectral studies and elemental analysis.

2.1. Antimicrobial Activities. All the synthesized compounds (3 and 4) were evaluated for their antibacterial activity against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumoniae* (NCTC-13368), and *Pseudomonas aeruginosa* (MTCC-1688) and antifungal activity against *Aspergillus niger* (MTCC-281), *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus terreus* (MTCC-1782), and *Aspergillus flavus* (MTCC-1973) by cup-plate method [28]. The zone of inhibition (in mm) was compared with the inhibition zones obtained using streptomycin and fluconazole as

positive controls for antibacterial and antifungal activities, respectively. The results are tabulated in Tables 1 and 2.

Antibacterial screening revealed that compound **4a** having chloro substitution at C-5 position of indole and dibenzo[b,f]azepine ring along with thiazolidine system showed the maximum inhibition against *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* at all concentrations. Compound **3a** exhibited maximum inhibition against *K. pneumoniae* due to the presence of chloro substitution at C-5 position, whereas compounds **3b** and **4b** showed maximum inhibition against *S. aureus* and *E. coli*, respectively.

Antifungal activity assay revealed that the compounds **3a** and **4a** exhibited maximum inhibition against *A. niger*, *A. oryzae*, and *A. flavus*. This enhanced activity of **3a** and **4a** may be due to the presence of chloro substitution at C-5 position of indole ring. Also compound **4a** showed good activity against *A. terreus*. Compound **3b** exhibited higher activity against *A. niger*, *A. oryzae*, and *A. flavus* at all concentrations. As deduced from above stated results, in general, the presence of the chloro or methyl substitution at C-5 position of indole enhanced the activity of the compounds. Differences in standard deviation were calculated by means of ANOVAs (Tukey) using GraphPad instat software.

2.2. Antioxidant Activities

2.2.1. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA). Antioxidants are intimately involved in the prevention of cellular damage, cancer, aging, and a variety of diseases. DPPH is stable free radical that can accept an electron or hydrogen atom, and its stability originates from delocalization of the unpaired electron over the molecule.

Free radicals are highly reactive species with one or more unpaired electrons in their last orbital. Reactive oxygen species (ROS), important in biological systems, include

SCHEME 1: Synthetic pathways for indole analogues (3 and 4).

superoxide ($O_2^{\bullet-}$), hydroxyl ($\bullet OH$), peroxide ($ROO^\bullet$), alkoxy ($RO^\bullet$), and hydroperoxy ($HOO^\bullet$) radicals, whereas major nonradical reactive species are hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), peroxyxynitrite ($ONOO$), nitric oxide (NO), and singlet oxygen (1O_2). Once formed ROS are highly reactive radicals which can start a chain reaction. Their primary danger comes from the damage they can do when they react with important components such as DNA, RNA, or the cell membrane [29]. Investigation of the RSA of the test compounds was conducted as described by Hatano and colleagues [30], and results were compared with the results obtained using standards 2-tert-butyl-4-methoxy phenol (butylated hydroxyl anisole, BHA), 2-(1,1-dimethylethyl)-1, 4-benzenediol (tertiary butylated hydroquinone, TBHQ) and ascorbic acid (AA) (Figure 2).

The RSA results revealed that the compound **4a** showed highest activity (76.63%), whereas the compounds **3a**, **3b**, and **3c** exhibited good RSA (75.50, 70.11, and 66.85%) at 100 $\mu g/mL$ concentration. Differences in standard deviation were estimated by means of ANOVAs (Tukey) using Graph-Pad instat software and MS-Excel.

2.2.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power (FRAP). Ferric ion (Fe^{3+}) is relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+} depending on the condition, particularly pH [31] and oxidized back through Fenton-type reaction [32] with the production of hydroxyl radical or Haber-Weiss reaction with the generation of superoxide anions. Reducing power is to measure the reductive ability of an antioxidant, and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donation of an electron in the presence of test compounds. Therefore, the concentration of Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Figure 3).

Determination of the reducing power of the compounds was conducted by a method described in [33] using BHA,

FIGURE 2: DPPH RSA of the compounds (3-4).

TBHQ, and AA as standards. The FRAP results revealed that compound **4a** showed considerable high activity at the concentrations of 50, 75, and 100 $\mu g/mL$, whereas compound **3b** exhibited promising activity at the concentration of 100 $\mu g/mL$. Differences in standard deviation were calculated by means of ANOVAs (Tukey) using GraphPad instat software and MS-Excel.

2.2.3. Ferrous (Fe^{2+}) Metal Ion Chelating Activity. Metal chelating capacity reduces the concentration of the catalyzing transition metal in lipid peroxidation. It was reported that chelating agents, which form σ -bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of metal ion [34]. Fenton reaction accelerates peroxidation by

TABLE 1: *In vitro* antibacterial activities of the compounds 3-4.

Compound number	<i>E. coli</i>					<i>S. aureus</i>					<i>K. pneumoniae</i>					<i>P. aeruginosa</i>				
	Conc. (μg/mL)					Conc. (μg/mL)					Conc. (μg/mL)					Conc. (μg/mL)				
	(diameters of inhibition in mm)					(diameters of inhibition in mm)					(diameters of inhibition in mm)					(diameters of inhibition in mm)				
	1000	750	500	1000	750	500	1000	750	500	1000	750	500	1000	750	500	1000	750	500		
3a	10 ± 1.25	9 ± 1.25	5 ± 0.82	9 ± 1.63	9 ± 0.82	9 ± 0.82	12 ± 1.25	12 ± 3.27	12 ± 0.82	12 ± 1.25	12 ± 3.27	12 ± 0.82	5 ± 0.82	8 ± 1.25	10 ± 0.82	5 ± 0.82	8 ± 1.25	10 ± 0.82		
3b	9 ± 0.82	6 ± 0.82	1 ± 0.37	14 ± 1.55	14 ± 0.83	14 ± 1.27	5 ± 1.57	9 ± 1.15	10 ± 0.88	5 ± 1.57	9 ± 1.15	10 ± 0.88	10 ± 1.69	10 ± 1.39	8 ± 0.82	10 ± 1.69	10 ± 1.39	8 ± 0.82		
3c	5 ± 0.49	3 ± 0.41	2 ± 0.45	8 ± 0.86	8 ± 0.99	5 ± 1.27	9 ± 1	5 ± 0.74	5 ± 1.35	9 ± 1	5 ± 0.74	5 ± 1.35	8 ± 0.62	3 ± 1.6	3 ± 0.74	8 ± 0.62	3 ± 1.6	3 ± 0.74		
4a	10 ± 1.43	9 ± 0.71	5 ± 0.9	15 ± 1.43	15 ± 1.43	12 ± 1.7	14 ± 1.4	14 ± 0.8	13 ± 0.93	14 ± 1.4	14 ± 0.8	13 ± 0.93	14 ± 0.8	14 ± 0.74	12 ± 1.43	14 ± 0.8	14 ± 0.74	12 ± 1.43		
4b	14 ± 1.37	14 ± 1.35	13 ± 0.82	10 ± 0.82	3 ± 0.94	4 ± 0.69	4 ± 1.27	8 ± 1.31	8 ± 1.2	4 ± 1.27	8 ± 1.31	8 ± 1.2	10 ± 0.83	10 ± 0.66	2 ± 0.91	10 ± 0.83	10 ± 0.66	2 ± 0.91		
4c	10 ± 0.77	5 ± 1.55	8 ± 1.15	14 ± 0.94	14 ± 0.49	12 ± 1.03	13 ± 1	11 ± 1.06	12 ± 1.96	13 ± 1	11 ± 1.06	12 ± 1.96	15 ± 1.4	13 ± 1.25	12 ± 0.95	15 ± 1.4	13 ± 1.25	12 ± 0.95		
Std.	15 ± 1.59	15 ± 1.79	14 ± 0.74	16 ± 1.69	16 ± 2.05	14 ± 0.61	15 ± 1.01	15 ± 1.35	14 ± 0.95	15 ± 1.01	15 ± 1.35	14 ± 0.95	16 ± 2.95	15 ± 2.16	13 ± 1.31	16 ± 2.95	15 ± 2.16	13 ± 1.31		
Std.: streptomycin.																				

Std.: streptomycin.

TABLE 2: *In vitro* antifungal activities of the compounds 3-4.

Compound number	<i>A. niger</i>				<i>A. oryzae</i>				<i>A. terreus</i>				<i>A. flavus</i>			
	Conc. (µg/mL)				Conc. (µg/mL)				Conc. (µg/mL)				Conc. (µg/mL)			
	1000	750	500	1000	750	500	1000	750	500	1000	750	500	1000	750	500	1000
3a	13 ± 0.82	12 ± 0.97	12 ± 0.24	13 ± 0.83	11 ± 1.41	11 ± 1.63	10 ± 1.51	9 ± 1.27	9 ± 0.65	14 ± 2.08	12 ± 1.15	12 ± 1.36	12 ± 1.15	12 ± 1.15	12 ± 1.36	12 ± 1.36
3b	14 ± 2.2	12 ± 1.78	12 ± 0.82	14 ± 1.85	12 ± 1.57	12 ± 1.05	10 ± 1.63	8 ± 1.39	3 ± 1.31	5 ± 0.9	3 ± 1.39	2 ± 0.54	5 ± 0.9	3 ± 1.39	2 ± 0.54	2 ± 0.54
3c	4 ± 2.36	4 ± 0.96	5 ± 1.11	13 ± 1.19	12 ± 1.05	11 ± 1.27	5 ± 1.53	6 ± 0.97	8 ± 1	10 ± 1.44	10 ± 0.62	9 ± 1.36	10 ± 1.44	10 ± 0.62	9 ± 1.36	9 ± 1.36
4a	14 ± 1.35	13 ± 1.27	12 ± 1.6	14 ± 2.33	11 ± 0.98	12 ± 0.82	14 ± 0.51	13 ± 1.77	12 ± 2.62	13 ± 1.19	13 ± 1.27	12 ± 0.69	13 ± 1.19	13 ± 1.27	12 ± 0.69	12 ± 0.69
4b	5 ± 1.35	2 ± 0.69	2 ± 0.91	5 ± 0.82	5 ± 1.4	5 ± 1.03	15 ± 1.72	13 ± 1.68	11 ± 1.07	6 ± 1.1	2 ± 0.96	2 ± 0.7	6 ± 1.1	2 ± 0.96	2 ± 0.7	2 ± 0.7
4c	3 ± 0.75	4 ± 0.78	3 ± 1.52	13 ± 1.51	12 ± 1.64	12 ± 1.19	3 ± 1.43	6 ± 1.47	6 ± 1.68	3 ± 0.98	4 ± 1.43	4 ± 1.31	3 ± 0.98	4 ± 1.43	4 ± 1.31	4 ± 1.31
Std.	15 ± 0.38	14 ± 0.69	13 ± 0.99	15 ± 1.47	13 ± 1.44	13 ± 1.32	16 ± 0.94	14 ± 1.02	13 ± 1.59	15 ± 1.43	14 ± 1.27	13 ± 0.76	15 ± 1.43	14 ± 1.27	13 ± 0.76	13 ± 0.76

Std.: fluconazole.

FIGURE 3: Ferric (Fe^{3+}) ions reducing capacity of the compounds (3-4).

decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves gain hydrogen and perpetuate the chain reaction of lipid peroxidation [35, 36]:

Determination of ferrous ion (Fe^{2+}) chelating activity of the synthesized compounds was carried out as described in Dinis' method [37] using BHA, TBHQ, and AA as standards (Figure 4). Compounds **3a**, **3b**, **3c**, and **4a** showed promising metal chelating activity (75.21, 74.37, 70.11, and 72.70%) at 75 $\mu\text{g/mL}$ and (78.27, 75.48, 74.09, and 72.42%) at 100 $\mu\text{g/mL}$ concentration, respectively. The compound **3b** showed good activity (72.70%) at 50 $\mu\text{g/mL}$ concentration. Differences in standard deviation were estimated by the help of ANOVAs (Tukey) using GraphPad instat software and MS-Excel.

3. Conclusion

In this study, we have demonstrated the synthesis of some novel indole derivatives incorporating 5H-dibenzo[b,f]azepine and thiazolidine heterocycles in a single structure. Some of the chloro- and methyl-substituted compounds exhibited promising antimicrobial and antioxidant activities.

4. Experimental Protocols

All reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method, and uncorrected melting points are reported in this study. Purity of the compounds was checked by thin layer chromatography (TLC) using silica gel-G coated aluminium plates (Merck), and spots were visualized by exposing the dry plates to iodine vapours. The infrared (IR) (KBr) spectra were recorded with a Perkin-Elmer spectrum one Fourier

FIGURE 4: Metal chelating activity of the compounds (3-4).

transform infrared spectroscopy (FT-IR) spectrometer. The ^1H nuclear magnetic resonance (NMR) (dimethylsulphoxide- d_6) spectra were recorded on Bruker NMR (500 MHz), and the chemical shifts were expressed in parts per millions (ppm) (δ scale) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were recorded with a JEOL GCMATE II GC-MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer.

5H-Dibenzo[b,f]azepine-5-carbohydrazide (1) Was Prepared by the Following Reported Method [26]. A mixture of 5H-dibenzo[b,f]azepine-5-carbonyl chloride (0.01 mol) and hydrazine hydrate (0.01 mol, 80%) in absolute alcohol was stirred for 2 hrs and then refluxed for 30 mins. The product obtained was filtered, washed with cold alcohol, dried, and purified by recrystallization in methanol to give 5H-dibenzo[b,f]azepine-5-carbohydrazide (**1**).

5-Substituted 2-Phenyl Indol-3-carboxyaldehydes (2a-2c) Were Prepared by the Following Literature Procedure [27]. A solution of 5-substituted 2-phenyl-1H-indoles (0.01 mol) in minimum amount of dimethyl formamide was added to a Vilsmeier-Haack complex, prepared from phosphorous oxychloride (1 mL) and dimethyl formamide (3.15 mL), maintaining the temperature between 10 and 20°C. The reaction mixture was kept at 45°C for 30 min and poured into ice water (100 mL) containing sodium hydroxide (20 mL, 10%). This was boiled for 1 min, cooled to room temperature, filtered, washed with water dried, and recrystallized in 1,4-dioxane to give **2a-2c**.

4.1. General Procedure for the Synthesis of N-[(5'-Substituted 2'-Phenyl-1H-indol-3'-yl)methylene]-5H-dibenzo[b,f]azepine-5-carbohydrazides (3a-3c). A solution of **1** (0.01 mol) and 5-substituted 2-phenylindole-3-carboxyaldehydes (**2a-2c**) (0.01 mol) in 1,4-dioxane (40 mL) containing glacial acetic acid (2 mL) was refluxed for 8 hrs. The excess of solvent was

removed under reduced pressure. The reaction mixture was cooled to room temperature and poured into ice-cold water. The separated product was filtered, washed thoroughly with cold water, dried and recrystallized in ethanol to give **3a–3c**.

N-[(5'-Chloro-2'-phenyl-1*H*-indol-3'-yl)methylene]-5*H*-dibenzo[*b,f*]azepine-5-carbohydrazide (**3a**). Yellow crystals, Yield 63%, mp 281–82°C, Rf, 0.75 ethyl acetate : benzene (6 : 4); IR (KBr): ν/cm^{-1} 3419 (indole NH), 3210 (NH), 1627 (C=O), 1574 (C=N); ^1H NMR (DMSO- d_6): δ 12.40 (s, 1H, indole NH), 9.30 (s, 1H, NH), 8.80 (s, 1H, N=CH), 6.70–8.30 (m, 18H, Ar-H); Mass m/z : 488 (M^+), 490 ($M^+ + 2$); Anal. Calcd. for $\text{C}_{30}\text{H}_{21}\text{N}_4\text{OCl}$: C, 73.69; H, 4.33; N, 11.46. Found: C, 73.75; H, 4.28; N, 11.39%.

N-[(5'-Methyl-2'-phenyl-1*H*-indol-3'-yl)methylene]-5*H*-dibenzo[*b,f*]azepine-5-carbohydrazide (**3b**). Colorless needles, Yield 79%, mp 221–22°C, Rf, 0.75 ethyl acetate : benzene (7 : 3); IR (KBr): ν/cm^{-1} 3413 (indole NH), 3200 (NH), 1625 (C=O), 1582 (C=N); ^1H NMR (DMSO- d_6): δ 12.38 (s, 1H, indole NH), 9.20 (s, 1H, NH), 9.00 (s, 1H, N=CH), 7.00–8.10 (m, 18H, Ar-H), 2.30 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{31}\text{H}_{24}\text{N}_4\text{O}$: C, 79.46; H, 5.16; N, 11.96. Found: C, 79.36; H, 5.20; N, 12.00%.

N-[(2'-Phenyl-1*H*-indol-3'-yl)methylene]-5*H*-dibenzo[*b,f*]azepine-5-carbohydrazide (**3c**). White solid, Yield 65%, mp above 300°C, Rf, 0.52 ethyl acetate : benzene (4 : 6); IR (KBr): ν/cm^{-1} 3405 (indole NH), 3219 (NH), 1618 (C=O), 1570 (C=N); ^1H NMR (DMSO- d_6): δ 12.25 (s, 1H, indole NH), 9.30 (s, 1H, NH), 9.00 (s, 1H, N=CH), 7.05–8.20 (m, 19H, Ar-H); Anal. Calcd. for $\text{C}_{30}\text{H}_{22}\text{N}_4\text{O}$: C, 79.27; H, 4.88; N, 12.33. Found: C, 79.32; H, 4.93; N, 12.28%.

4.2. General Procedure for the Synthesis of *N*-[2-(5'-Substituted 2'-Phenyl-1*H*-indol-3'-yl)-4-oxothiazolidin-3-yl]-5*H*-dibenzo[*b,f*]azepine-5-carboxamides (4a–4c**).** A mixture of compounds **3a**, **3b**, and **3c** (0.01 mol) and thioglycolic acid (0.01 mol) containing a pinch of anhydrous zinc chloride in DMF (30 mL) was refluxed for 8 hrs. The mixture was then cooled to room temperature and poured into ice-cold water. The separated product was filtered, washed with saturated sodium carbonate solution to remove unreacted thioglycolic acid followed by cold-water, dried, and recrystallized in ethanol to get pure **4a–4c**.

N-[2-(5'-Chloro-2'-phenyl-1*H*-indol-3'-yl)-4-oxothiazolidin-3-yl]-5*H*-dibenzo[*b,f*]azepine-5-carboxamide (**4a**). Yellow solid, Yield 84%, mp 201–02°C, Rf, 0.65 ethyl acetate : methanol (1 : 2); IR (KBr): ν/cm^{-1} 3410 (indole NH), 3131 (NH), 1732 (C=O), 1626 (C=O); ^1H NMR (DMSO- d_6): δ 12.30 (s, 1H, indole NH), 9.28 (s, 1H, NH), 6.90–8.30 (m, 18H, Ar-H), 4.70 (s, 1H, CHN), 3.90 (s, 2H, CH_2CO); Mass m/z : 562 (M^+), 564 ($M^+ + 2$); Anal. Calcd. for $\text{C}_{32}\text{H}_{23}\text{N}_4\text{O}_2\text{S}$: C, 68.26; H, 4.12; N, 9.95. Found: C, 68.32; H, 4.10; N, 10.00%.

N-[2-(5'-Methyl-2'-phenyl-1*H*-indol-3'-yl)-4-oxothiazolidin-3-yl]-5*H*-dibenzo[*b,f*]azepine-5-carboxamide (**4b**). White solid, Yield 89%, mp above 300°C, Rf, 0.65 ethyl acetate : methanol (1 : 1); IR (KBr): ν/cm^{-1} 3400 (indole NH), 3154 (NH),

1710 (C=O), 1605 (C=O); ^1H NMR (DMSO- d_6): δ 12.40 (s, 1H, indole NH), 9.35 (s, 1H, NH), 7.00–8.00 (m, 18H, Ar-H), 5.00 (s, 1H, CHN), 3.80 (s, 2H, CH_2CO), 2.25 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{33}\text{H}_{26}\text{N}_4\text{O}_2\text{S}$: C, 73.04; H, 4.83; N, 10.32. Found: C, 73.00; H, 4.90; N, 10.25%.

N-[2-(2'-Phenyl-1*H*-indol-3'-yl)-4-oxothiazolidin-3-yl]-5*H*-dibenzo[*b,f*]azepine-5-carboxamide (**4c**). Yellow solid, Yield 65%, mp 264–65°C, Rf, 0.71 ethyl acetate : methanol (6 : 4); IR (KBr): ν/cm^{-1} 3400 (indole NH), 3205 (NH), 1725 (C=O), 1610 (C=O); ^1H NMR (DMSO- d_6): δ 12.10 (s, 1H, indole NH), 9.40 (s, 1H, NH), 7.10–8.25 (m, 19H, Ar-H), 4.70 (s, 1H, CHN), 3.58 (s, 2H, CH_2CO); Anal. Calcd. for $\text{C}_{32}\text{H}_{24}\text{N}_4\text{O}_2\text{S}$: C, 72.71; H, 4.58; N, 10.60. Found: C, 72.85; H, 4.63; N, 10.50%.

5. Biological Activities

5.1. Antimicrobial Activities. The *in vitro* biological screening of the synthesized compounds (**3** and **4**) was carried out against bacterial species *E. coli*, *S. aureus*, *K. pneumonia*, and *P. aeruginosa* and fungal species *A. niger*, *A. oryzae*, *A. terreus*, and *A. flavus* by cup-plate method [28] using nutrient agar and PDA medium for antibacterial and antifungal activities, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer, and these were filled with test solution (1000, 750, and 500 $\mu\text{g/mL}$ in DMF), standard solution (1000, 750, and 500 $\mu\text{g/mL}$ in DMF) and DMF as control. The plates were incubated at 37°C for 24 hours and 72 hours for the evaluation of antibacterial and antifungal activities, respectively. The diameter of the inhibition zones for all the test compounds was measured (in mm), and the results were compared with the results obtained by using streptomycin and fluconazole as positive standard for antibacterial and antifungal activities, respectively.

5.2. Antioxidant Activity Assay

5.2.1. 1,1-Diphenyl-2-picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA). The radical scavenging activity (RSA) of test compounds (**3** and **4**) in methanol at different concentrations (25, 50, 75, and 100 $\mu\text{g/mL}$) containing freshly prepared DPPH in methanol (0.004% w/v) was carried out, and the results were compared with the results obtained by using standards (BHA, TBHQ, and AA) by Hatano's method [30]. All analyses were performed in three replicates, and results were reported as averaged of three replicates. The results in percentage are expressed as the ratio of absorbance of DPPH solutions measured at 517 nm in the presence and the absence of test compounds by using ELICO SL171 mini spec spectrometer. The results are shown in Figure 2. The percentages of DPPH free radical scavenging activity of the samples were determined using the following equation:

$$\% \text{ DPPH radical scavenging} = \frac{A_c - A_s}{A_c} \times 100, \quad (2)$$

where A_c = absorbance of control, A_s = absorbance of test sample.

5.2.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power (FRAP).

The reducing power of the synthesized compounds (**3** and **4**) was determined according to the Oyaizu method [33]. Different concentrations of samples (25, 50, 75, and 100 $\mu\text{g/mL}$) 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 w/v). The mixture was incubated at 50°C for 20 min. A portion of trichloroacetic acid (2.5 mL, 10% w/v) was then added to the mixture, and the mixture was centrifuged for 10 min at 1000 $\times g$. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1 w/v). Absorbance at 700 nm was then measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in Figure 3.

5.2.3. Ferrous Ions (Fe^{2+}) Metal Chelating Activity.

The ferrous ion chelating activities of synthesized compounds (**3** and **4**) and standards were estimated using the method reported by Dinis and colleagues [37]. The test samples (25, 50, 75, and 100 $\mu\text{g/mL}$) in ethanol (0.4 mL) were added to ferrous chloride (0.05 mL, 2 mM) prepared in ethanol. The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM), and the volume was adjusted to 3.5 mL with ethanol and 0.5 mL water so as to make the final total volume 4.0 mL. Ferrozine reacts with the divalent iron to 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 562 nm. All analyses were run in three triplicates, and results are reported as the averages of three replicates. The results are shown in Figure 4. The percent inhibition of the ferrozine- Fe^{2+} complex formation was calculated using the formula

$$\% \text{ Ferrous ion chelating effect} = \frac{A_c - A_s}{A_c} \times 100, \quad (3)$$

where A_c = absorbance of control, A_s = absorbance of test sample.

Conflict of Interests

Since The authors have procured the IR, NMR, and mass spectra of the synthesized compounds from the Sophisticated Analytical Instrument facility, namely, The Indian Institute of Technology, Madras, Chennai, India, as per the condition of institution, authors should acknowledge their services in the research paper while publishing the work, which includes the data provided by them in the research paper. The same has been acknowledged in the acknowledgment section. The authors do not have any agreement, financial assistance, or sponsorship from Perkin-Elmer spectrum, Bruker NMR, and so forth. These names are mentioned in the experimental protocol as these are the instrument models, and it is mandatory for authors to mention the instrument models used to scan the spectra of unknown compounds. Otherwise the corresponding author or coauthors have no direct financial relationship with the commercial identity mentioned in their paper in any form.

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