Research Article

Synthesis Antioxidant and Antimicrobial Activities of N-[(5'-Substituted 2'-phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amines

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The main aim of the present study was to develop antimicrobial and antioxidant compounds. As a part of systematic investigation, several new compounds **3a–c**, **4a–c**, and **5a–f** were synthesized and screened for their biological activities. Compound **5f** showed good zone of inhibition versus *E. coli*, *S. aureus*, and *K. pneumoniae*, whereas the compound **3a** showed promising radical scavenging activity, ferric ions (Fe^{3+}) reducing antioxidant power, and ferrous ions (Fe^{2+}) metal chelating activity.

1. Introduction

Antimicrobials are one of the most significant weapons in fighting bacterial infections. They have extremely benefited the health-related quality of human life. Indole and its derivatives have been reported to possess a variety of physiological and pharmacological activities like antibacterial [1], antifungal [2, 3], antitumor [4, 5], antiviral [6, 7], antioxidant [8], and so forth. In addition, oxadiazole derivatives are useful in antibacterial [9–11], anti-inflammatory [12, 13], antitubercular [14] and anticonvulsant [15] activities.

As part of interest in the synthesis of heterocyclic compounds that have been explored for developing pharmaceutically important molecules, 4-thiazolidinones [16–18] and 2-azetidinones [19–23] have played an important role in medicinal chemistry. Moreover, several derivatives of 4-thiazolidinone and 2-azetidinone have been studied extensively because of their ready accessibility, diverse chemical reactivity, and broad spectrum of biological activities.

In continuation of our research [24–26] and in view of the therapeutic importance of indole, oxadiazole, thiazolidinone, and azetidinone, which prompted us to construct several analogues of indole-containing oxadiazole, thiazolidinone,

and azetidinone systems so as to get biologically more potent molecules.

2. Results and Discussion

The title compounds were synthesized as outlined in Scheme 1. The starting compound, 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**2**), was prepared using isoniazide (**1**) by following the literature procedure [27]. The precursor N-[(5'-substituted 2'-phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amines (**3a–c**) was synthesized by cyclocondensation of 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**2**) with 5-substituted 2-phenyl-1*H*-indol-3-carboxaldehydes [28] in 1,4-dioxane at reflux temperature. Compounds (**3a–c**) on refluxing with thioglycolic acid in N,N-dimethyl formamide afforded 2-(5'-substituted 2'-phenyl-1*H*-indol-3'-yl)-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-ones (**4a–c**). Similarly, compounds (**3a–c**) on cyclocondensation with acetyl chloride or phenyl acetyl chloride afforded 4-(5'-substituted 2'-phenyl-1*H*-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-ones (**5a–c**)

SCHEME 1: Synthesis of indole analogues (3–5).

or 4-(5'-substituted 2'-phenyl-1*H*-indol-3'-yl)-3-phenyl-1-azetidin-2-ones (**5d–f**), respectively. The structures of all these previously unknown compounds were confirmed by their spectral studies and elemental analysis.

2.1. Antimicrobial Activity. The investigation of antibacterial screening (Table 1) revealed that compounds **5d** and **5e** exhibited maximum zone of inhibition against *E. coli* and *P. aeruginosa* at all concentrations. Compound **5f** showed good zone of inhibition versus *E. coli*, *S. aureus*, and *K. pneumoniae* at all concentration, whereas the compounds **4a** and **5b** showed maximum zone of inhibition against *E. coli* and *P. aeruginosa* at all concentrations.

Antifungal activity results (Table 2) indicated that compound **3a** showed potent activity against *A. niger* and *A. terreus*, whereas compound **3b** exhibited high activity against *A. oryzae* and *A. flavus*. Compound **3c** showed good activity against *A. terreus* and *A. flavus*, whereas the compound **5a** showed good activity against *A. flavus* at all concentration.

In general from the above results, it can be concluded that compounds having chloro- or methyl-substitution at C-5 position of indole enhanced the activity due to electron-accepting or electron-donating nature of the groups.

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, the common pathway for cancer, aging, and a variety of diseases. DPPH is a stable free radical that can accept an electron or hydrogen atom, and stability originates from delocalization of the unpaired electron over the molecule. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, RNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants. The scavenging of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical model is widely used method to evaluate antioxidant activities in relatively short time compared with other methods. DPPH has an odd electron and so has a strong absorption band at 517 nm.

The radical scavenging activity of test compounds (**3–5**) in presence of DPPH radical was carried out, and the results were compared with the 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)

TABLE 1: Antibacterial activity of compounds 3–5.

Comp. number	Conc. ($\mu\text{g/mL}$) (zone of inhibition in mm)											
	<i>E. coli</i>			<i>S. aureus</i>			<i>K. pneumoniae</i>			<i>P. aeruginosa</i>		
	1000	750	500	1000	750	500	1000	750	500	1000	750	500
3a	10	10	11	12	12	12	05	05	08	09	09	09
3b	05	05	08	09	09	10	02	02	05	05	05	08
3c	08	08	10	05	05	07	10	10	11	11	11	10
4a	13	13	14	09	09	10	09	10	10	02	02	05
4b	09	09	10	05	05	08	10	05	06	10	10	11
4c	10	10	11	02	02	05	09	09	10	11	11	10
5a	09	11	11	07	07	09	09	09	10	09	10	11
5b	11	03	05	00	00	00	05	05	06	14	13	12
5c	09	09	09	04	04	06	05	09	10	02	02	05
5d	14	14	14	12	12	12	10	10	12	13	13	13
5e	14	14	14	09	09	12	09	10	10	13	13	13
5f	13	13	14	16	14	13	13	13	13	05	06	08
Std.	16	16	15	18	16	15	15	15	14	15	14	14

Std.: Streptomycin (Standard).

TABLE 2: Antifungal activity of compounds 3–5.

Comp. number	Conc. ($\mu\text{g/mL}$) (zone of inhibition in mm)											
	<i>A. niger</i>			<i>A. oryzae</i>			<i>A. terreus</i>			<i>A. flavus</i>		
	1000	750	500	1000	750	500	1000	750	500	1000	750	500
3a	12	12	12	10	10	10	13	12	12	02	02	02
3b	05	05	08	14	12	12	10	10	09	13	13	12
3c	10	09	09	10	10	10	14	13	12	13	13	12
4a	02	02	05	05	05	08	—	—	—	06	06	06
4b	10	08	04	09	09	09	—	—	—	09	09	09
4c	09	09	10	03	03	05	—	—	—	05	05	05
5a	09	10	10	02	02	02	06	06	08	13	12	12
5b	—	—	—	02	02	02	07	09	09	00	00	00
5c	02	09	09	05	05	08	05	05	08	09	09	09
5d	10	10	10	09	09	11	05	05	09	01	01	02
5e	10	09	09	09	09	09	02	02	03	03	03	03
5f	05	05	09	02	02	05	09	10	10	05	05	08
Std.	14	14	14	15	14	14	15	14	14	14	14	13

Std.: Fluconazole (Standard).

(Figures 1 and 2). The results of test compounds indicated that compounds 3a, 4a, 5a, and 5f exhibited good RSA at 75 and 100 $\mu\text{g/mL}$ concentrations. Compound 5a showed promising activity at 50 $\mu\text{g/mL}$ concentration. However, none of the compounds exhibited RSA better than the standards. The higher activity of compounds 4a and 5a may be due to the presence of labile hydrogen of indole-NH and/or chlorine atom in their structure.

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

The ferric ion (Fe^{3+}) is a relatively biologically inactive form of iron. However, it can be reduced to the active Fe^{2+} depending on the condition, particularly pH [29], and oxidized back through Fenton-type reaction [30] with the

production of hydroxyl radical or the Haber-Weiss reaction with 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 Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

The reducing power activity of the test compounds was carried out at different concentrations, and the results were compared with the standards BHA, TBHQ, and AA (Figures 3 and 4). The analysis of the results indicated that compound 5a exhibited promising reducing power activity at 25 $\mu\text{g/mL}$ concentration. Compounds 3a and 5a showed higher reducing power activity at 50 $\mu\text{g/mL}$ concentration, whereas the

FIGURE 1: Radical scavenging activity of compounds (3-4).

FIGURE 3: FRAP of compounds (3-4).

FIGURE 2: Radical scavenging activity of compounds (5).

FIGURE 4: FRAP of compounds (5).

compounds **3a**, **5a**, and **5e** exhibited good reducing activity at 75 µg/mL concentration. Compounds **3a**, **3b**, **5a**, and **5e** showed higher reducing power activity at 100 µg/mL concentration.

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 therapy stabilizing the oxidized form of metal ion [31]. Fenton's reaction accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy

radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [32, 33].

Fe^{3+} ion also produces radical from peroxides, although the rate is tenfold less than that of Fe^{2+} ion, which is the most powerful pro-oxidant among the various types of metal ions.

The chelating effect of ferrous ion (Fe^{2+}) with the test compounds was determined, and the results were compared with standards BHA, TBHQ, and AA (Figures 5 and 6). Compound **3a** showed promising chelating activity at 50 and 75 µg/mL concentrations. Whereas, the compounds **3a**, **3c**,

FIGURE 5: Metal-chelating activity of compounds (3-4).

and **5b** showed good chelating activity at 100 µg/mL concentration. Compound **3a** showed higher chelating activity which may be accounted for the presence of azomethine group in the structure of compound **3a**.

3. Conclusion

In this study, we have demonstrated the synthesis of some novel indole derivatives, incorporating potential chemotherapeutic units, namely, isoniazide, oxadiazole, thiazolidinone and azetidinone. Some of the chloro- and methyl-substituted compounds have exhibited promising antimicrobial and antioxidant activities.

4. Experimental Protocols

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method and were uncorrected. Purity of the compounds was checked by TLC using silica-gel-G-coated aluminium plates (Merck), and spots were visualized by exposing the dry plates to iodine vapors. The IR (KBr) spectra were recorded with a Perkin-Elmer spectrum one FT-IR spectrometer. The ^1H NMR ($\text{DMSO}-d_6$) spectra were recorded on Bruker NMR (500 MHz), and the chemical shifts were expressed in ppm (δ scale) downfield from TMS. Mass spectra were recorded with a JEOL GC/MATE II GC-MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer.

5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**2**) was prepared by following the reported method [27].

4.1. General Procedure for the Synthesis of *N*-[(5'-Substituted 2'-phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amines (3a-c**).** A solution of compound **2**

FIGURE 6: Metal-chelating activity of compounds (5).

(0.01 mol) and 5-substituted 2-phenylindole-3-carboxaldehyde (0.01 mol) in 1,4-dioxane (40 mL) containing glacial acetic acid (2 mL) was refluxed for 8 hrs. The solvent was distilled off at reduced pressure. The reaction mixture was cooled and poured into ice-cold water. The separated product was filtered, washed thoroughly with cold water, dried, and recrystallized from ethanol to furnish **3a-c**.

N-[(5'-Chloro-2'-phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**3a**), yellow crystals, yield 75%, mp 288-89°C, Rf, 0.61 ethylacetate: ethanol; IR (KBr): ν/cm^{-1} 3465 (NH), 1573 (C=N); ^1H NMR ($\text{DMSO}-d_6$): δ 12.40 (s, 1H, indole NH), 9.00 (s, 1H, N=CH), 8.10 (s, 1H, indole-H₄), 8.00 (d, 1H, indole-H₆), 7.60 (d, 1H, indole-H₇), 7.00-7.55 (m, 5H, Ar-H and 4H, pyridine-H); Mass m/z : 399 (M^+), 401 (M^++2); Anal. Calcd. for $\text{C}_{22}\text{H}_{14}\text{N}_5\text{OCl}$: C, 66.09; H, 3.53; N, 17.52. Found: C, 66.00; H, 3.60; N, 17.54%.

N-[(5'-Methyl-2'-phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**3b**), colorless needles, yield 63%, mp 285-86°C, Rf, 0.49 ethylacetate: ethanol; IR (KBr): ν/cm^{-1} 3400 (NH), 1590 (C=N); ^1H NMR ($\text{DMSO}-d_6$): δ 12.00 (s, 1H, indole NH), 8.85 (s, 1H, N=CH), 7.60-8.00 (m, 5H, Ar-H and 4H, pyridine-H), 7.55 (s, 1H, indole-H₄), 7.45 (d, 1H, indole-H₆), 6.95 (d, 1H, indole-H₇), 2.30 (s, 3H, CH₃); Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{N}_5\text{O}$: C, 72.81; H, 4.52; N, 18.46. Found: C, 72.78; H, 4.45; N, 18.38%.

N-[(2'-Phenyl-1*H*-indol-3'-yl)methylene]-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**3c**), white solid, yield 67%, mp 240-41°C, Rf, 0.62 ethylacetate: ethanol; IR (KBr): ν/cm^{-1} 3375 (NH), 1595 (C=N); ^1H NMR ($\text{DMSO}-d_6$): δ 12.10 (s, 1H, indole NH), 9.00 (s, 1H, N=CH), 7.00-8.40 (m, 5H, Ar-H, 4H, indole-H and 4H, pyridine-H); Anal. Calcd. for $\text{C}_{22}\text{H}_{15}\text{N}_5\text{O}$: C, 72.32; H, 4.14; N, 19.17. Found: C, 72.35; H, 4.09; N, 19.15%.

4.2. General Procedure for the Synthesis of 2-(5'-Substituted 2'-phenyl-1H-indol-3'-yl)-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-ones (4a-c). A mixture of compounds **3a-c** (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 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 from ethanol to get pure **4a-c**.

2-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-one (4a), yellow solid, yield 78%, mp 190-91°C, Rf, 0.65 ethylacetate:benzene (1:2); IR (KBr): ν/cm^{-1} 3444 (NH), 1745 (C=O); ^1H NMR (DMSO- d_6): δ 12.60 (s, 1H, indole NH), 8.50 (s, 1H, indole- H_4), 8.34 (d, 1H, indole- H_6), 7.92 (d, 1H, indole- H_7), 7.00-7.65 (m, 5H, Ar-H and 4H, pyridine-H), 4.80 (s, 1H, CHN), 3.90 (s, 2H, CH_2); Mass m/z : 473 (M^+), 475 ($M^+ + 2$); Anal. Calcd. for $\text{C}_{24}\text{H}_{16}\text{N}_5\text{O}_2\text{S}$: C, 60.82; H, 3.40; N, 14.78. Found: C, 60.79; H, 3.35; N, 14.70%.

2-(5'-Methyl-2'-phenyl-1H-indol-3'-yl)-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-one (4b), white solid, yield 85%, mp 201-02°C, Rf, 0.55 ethylacetate:benzene (1:2); IR (KBr): ν/cm^{-1} 3448 (NH), 1728 (C=O); ^1H NMR (DMSO- d_6): δ 12.00 (s, 1H, indole NH), 7.50-8.00 (m, 5H, Ar-H and 4H, pyridine-H), 8.37 (s, 1H, indole- H_4), 8.24 (d, 1H, indole- H_6), 7.10 (d, 1H, indole- H_7), 5.00 (s, 1H, CHN), 4.00 (s, 2H, CH_2), 2.25 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_5\text{O}_2\text{S}$: C, 66.21; H, 4.22; N, 15.44. Found: C, 66.19; H, 4.20; N, 15.35%.

2-(2'-Phenyl-1H-indol-3'-yl)-3-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]thiazolidin-4-one (4c), yellow solid, yield 82%, mp 180-81°C, Rf, 0.58 ethylacetate:benzene (1:2); IR (KBr): ν/cm^{-1} 3318 (NH), 1730 (C=O); ^1H NMR (DMSO- d_6): δ 12.01 (s, 1H, indole NH), 7.10-8.45 (m, 5H, Ar-H, 4H, indole-H and 4H, pyridine-H), 5.00 (s, 1H, CHN), 3.48 (s, 2H, CH_2); Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$: C, 65.59; H, 3.90; N, 15.94. Found: C, 65.50; H, 3.85; N, 15.99%.

4.3. General procedure for the synthesis of 4-(5'-Substituted 2'-phenyl-1H-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-ones (5a-c). A solution of compounds **4a-c** (0.01 mol) in dioxane (10 mL) was added to a well-stirred mixture of acetyl chloride (0.01 mol) and triethyl amine (0.01 mol) in dioxane (15 mL) during 30 min. The reaction mixture was then stirred for 8 hrs and kept for 24 hrs at room temperature. The product separated was filtered, dried, and recrystallized from ethanol to get pure **5a-c**.

4-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (5a), yellow crystal, yield 65%, mp 202-05°C, Rf, 0.64 benzene: methanol (1:3); IR (KBr): ν/cm^{-1} 3444 (NH), 1766 (C=O); ^1H NMR (DMSO- d_6): δ 12.60 (s, 1H, indole NH), 8.20 (s, 1H, indole- H_4), 8.04 (d, 1H, indole- H_6), 7.92 (d, 1H, indole- H_7), 6.90-7.65 (m, 5H, Ar-H and 4H, pyridine-H), 4.80 (t, 1H, CHN), 4.50 (d, 2H, CH_2); Mass m/z : 441 (M^+), 443 ($M^+ + 2$); Anal. Calcd. for

$\text{C}_{24}\text{H}_{16}\text{N}_5\text{O}_2\text{Cl}$: C, 65.24; H, 3.65; N, 15.85. Found: C, 65.20; H, 3.60; N, 15.85%.

4-(5'-Methyl-2'-phenyl-1H-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (5b), pale yellow crystal, yield 68%, mp 160-61°C, Rf, 0.53 benzene: methanol (1:3); IR (KBr): ν/cm^{-1} 3444 (NH), 1766 (C=O); ^1H NMR (DMSO- d_6): δ 12.60 (s, 1H, indole NH), 8.20 (s, 1H, indole- H_4), 8.04 (d, 1H, indole- H_6), 7.92 (d, 1H, indole- H_7), 6.90-7.65 (m, 5H, Ar-H and 4H, pyridine-H), 4.80 (t, 1H, CHN), 4.50 (d, 2H, CH_2), 2.49 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_5\text{O}_2$: C, 71.25; H, 4.54; N, 16.62. Found: C, 71.20; H, 4.50; N, 16.60%.

4-(2'-Phenyl-1H-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (5c), white solid, yield 71%, mp 164-65°C, Rf, 0.60 benzene: methanol (1:3); IR (KBr): ν/cm^{-1} 3444 (NH), 1766 (C=O); ^1H NMR (DMSO- d_6): δ 12.60 (s, 1H, indole NH), 7.00-8.18 (m, 5H, Ar-H, 4H, indole-H and 4H, pyridine-H), 4.92 (t, 1H, CHN), 4.62 (d, 2H, CH_2); Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{N}_5\text{O}_2$: C, 70.75; H, 4.21; N, 17.19. Found: C, 70.72; H, 4.15; N, 17.13%.

4.4. General Procedure for the Synthesis of 4-(5'-Substituted 2'-phenyl-1H-indol-3'-yl)-3-phenyl-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-ones (5d-f). To a solution of Schiff's base (**3a-c**) (0.02 mol) in dry benzene (30 mL) containing few drops of triethyl amine, phenyl acetyl chloride (0.02 mol) was added dropwise with stirring during 10 mins. After the addition was over, the reaction mixture was refluxed for 1 hr. Triethyl amine hydrochloride formed was filtered off and washed several times with dry benzene. The filtrate and washings were combined and concentrated under reduced pressure. The reaction mixture was cooled to room temperature the product obtained was filtered and washed with petroleum ether (40:60) to remove unreacted Schiff's base and recrystallized from aqueous ethanol to afford (**5d-f**).

4-(5'-Chloro-2'-phenyl-1H-indol-3'-yl)-3-phenyl-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (5d), brown crystal, yield 67%, mp 212-13°C, Rf, 0.63 ethylacetate:benzene (9:1); IR (KBr): ν/cm^{-1} 3447 (NH), 1764 (C=O); ^1H NMR (DMSO- d_6): δ 12.60 (s, 1H, indole NH), 8.20 (s, 1H, indole- H_4), 8.12 (d, 1H, indole- H_6), 7.66 (d, 1H, indole- H_7), 7.00-7.44 (m, 10H, Ar-H and 4H, pyridine-H), 6.30 (d, 1H, CHN), 6.20 (d, 1H, CHCO); Mass m/z : 517 (M^+), 519 ($M^+ + 2$); Anal. Calcd. for $\text{C}_{30}\text{H}_{20}\text{N}_5\text{O}_2\text{Cl}$: C, 69.56; H, 3.89; N, 13.52. Found: C, 65.58; H, 3.92; N, 13.47%.

4-(5'-Methyl-2'-phenyl-1H-indol-3'-yl)-3-phenyl-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (5e), yellow crystal, yield 76%, mp 235-36°C, Rf, 0.69 ethylacetate:benzene (9:1); IR (KBr): ν/cm^{-1} 3400 (NH), 1700 (C=O); ^1H NMR (DMSO- d_6): δ 12.00 (s, 1H, indole NH), 8.00 (s, 1H, indole- H_4), 7.94 (d, 1H, indole- H_6), 7.62 (d, 1H, indole- H_7), 7.00-7.44 (m, 10H, Ar-H and 4H, pyridine-H), 6.00 (d, 1H, CHN), 5.60 (d, 1H, CHCO), 2.32 (s, 3H, CH_3); Anal. Calcd. for $\text{C}_{31}\text{H}_{23}\text{N}_5\text{O}_2$: C, 74.83; H, 4.66; N, 14.08. Found: C, 74.79; H, 4.62; N, 14.00%.

4-(2'-Phenyl-1H-indol-3'-yl)-1-[5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl]azetidin-2-one (**5f**), white solid, yield 75%, mp 261–62°C, Rf, 0.57 ethylacetate : benzene (9 : 1); IR (KBr): ν/cm^{-1} 3415 (NH), 1718 (C=O); ^1H NMR (DMSO- d_6): δ 12.15 (s, 1H, indole NH), 7.15–8.20 (m, 10H, Ar-H, 4H, indole-H and 4H, pyridine-H), 5.92 (d, 1H, CHN), 5.42 (d, 1H, CHCO); Anal. calcd. for $\text{C}_{30}\text{H}_{21}\text{N}_5\text{O}_2$: C, 74.52; H, 4.38; N, 14.48. Found: C, 74.50; H, 4.35; N, 14.42%.

5. Biological Activities

5.1. Antimicrobial Activities. All the synthesized compounds (**3–5**) 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 their antifungal activity against *Aspergillus niger* (MTCC-281), *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus terreus* (MTCC-1782), and *Aspergillus flavus* (MTCC-1973) by the cup-plate method following reported procedure [34]. Holes of 6 mm diameter were punched carefully using a sterile cork borer, and these were filled with test solution (1000, 750, and 500 $\mu\text{g}/\text{mL}$ in DMF), standard solution (1000, 750, and 500 $\mu\text{g}/\text{mL}$ in DMF), and DMF as control. The plates were incubated at 37°C for 24 hrs and 72 hrs in case of antibacterial and antifungal activity, respectively. The diameter of the zone of inhibition (in mm) for all the test compounds was measured, and the results were compared with the standard drugs streptomycin and fluconazole for antibacterial and antifungal activity, respectively. The results are tabulated in Tables 1 and 2.

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 (**3–5**) in methanolic solution at concentrations 25, 50, 75, and 100 $\mu\text{g}/\text{mL}$ containing freshly prepared DPPH solution (0.004% w/v) was carried out, and the results were compared with the standards BHA, TBHQ, and AA by using Hatano's method [35]. All the test analyses were performed on three replicates and averaged. The results in percentage are expressed as the ratio of absorption in the presence of test compounds and absorption of DPPH solution in the absence of test compounds at 517 nm on ELICO SL171 mini spec spectrometer. The percentage scavenging activity of the DPPH free radical was determined using the following equation:

$$\% \text{DPPH radical scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100. \quad (2)$$

The results are shown in Figures 1 and 2.

5.2.2. Ferric Ions (Fe^{3+}) Reducing Antioxidant Power (FRAP). The reducing power of the synthesized compounds (**3–5**) was

determined according to the Oyaizu method [36]. Different concentrations of samples (25, 50, 75, and 100 $\mu\text{g}/\text{mL}$) 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 mins. After which a portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 mins. 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%). Then absorbance at 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are illustrated in Figures 3 and 4.

5.2.3. Ferrous Ions (Fe^{2+}) Metal-Chelating Activity. The chelating activity of ferrous ions by synthesized compounds (**3–5**) and standards was estimated by following the reported method [37]. The test samples (25, 50, 75, and 100 $\mu\text{g}/\text{mL}$) in ethanolic solution (0.4 mL) were added to a solution of ferrous chloride (0.05 mL, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM), and the total volume was adjusted to 4 mL with ethanol. 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 mins. Then the absorbance of the solution was measured spectrophotometrically at 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 formula:

$$\begin{aligned} \text{Ferrous ion chelating effect (\%)} \\ = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100. \end{aligned} \quad (3)$$

The results are exhibited in Figures 5 and 6.

Conflict of Interest

Since, we have procured the IR, NMR and mass spectra of the synthesized compounds from the National Research Centre, 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 include the data provided by them in the research paper, which we have been acknowledged in the acknowledgement 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 section as these are the instrument models, which is mandatory for authors to mention the instrument models which are used to scan the spectra of unknown compounds. Otherwise myself as a corresponding author or co-authors have no-direct financial relation with the commercial identity mentioned in our manuscript in any form.

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References

- [1] R. S. Varma and P. K. Garg, "Synthesis of substituted 5-chloro-3-phenylthio-semicarbazono-2-indolinones as potential antimicrobial agents," *Acta Pharmaceutica Jugoslavica*, vol. 30, no. 4, pp. 199–204, 1980.
- [2] S. P. Singh, V. A. Singh, and K. C. Gupta, "Synthesis of some new 5-bromo-3-(arylthiosemicarbazono)-2-indolinones as antimicrobial agents," *Acta Pharmaceutica Jugoslavica*, vol. 36, no. 1, pp. 19–26, 1986.
- [3] M. G. Bhovi and G. S. Gadaginamath, "1,3-dipolar cycloaddition reaction: synthesis and antimicrobial activity of some new 3-ethoxycarbonyl-5-methoxy-6-bromo-2-triazolylmethylindoles," *Indian Journal of Heterocyclic Chemistry*, vol. 14, no. 1, pp. 15–18, 2004.
- [4] I. M. Labouta, H. M. Salama, N. H. Eshba, and E. El-Chrbini, "Novel indolinones with potential antimicrobial and antineoplastic activities," *Acta Pharmaceutica Jugoslavica*, vol. 38, no. 3, pp. 189–194, 1988.
- [5] A. Heckel, J. Roth Gerald et al., "Preparation of substituted amino methyleneindolinone inhibitors of tyrosine receptor kinase and CDK/cyclin kinase as antitumor agents and inhibitors of cell proliferation," PCT International Applications, WO 01 27, 081 (Cl.C07D209/34), April 2001, DE Appl. 10. 042, 696, 282, August 2000.
- [6] S. P. Singh and R. K. Jha, "Indolinone derivatives as potential antimicrobial agents," *Zentralblatt für Mikrobiologie*, vol. 144, no. 2, pp. 105–109, 1989.
- [7] S. K. Sridhar, S. N. Pandeya, S. K. Bajpai, and H. Manjula, "Synthesis antibacterial and antiviral activities of isatin derivatives," *Indian Drugs*, vol. 36, no. 6, pp. 412–414, 1999.
- [8] D. X. Tan, R. J. Reiter, L. C. Manchester et al., "Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger," *Current Topics in Medicinal Chemistry*, vol. 2, no. 2, pp. 181–197, 2002.
- [9] K. Mogilaiah and K. Vidya, "Synthesis and antibacterial activity of 1,3,4-oxadiazolyl-1,8-naphthyridines," *Indian Journal of Chemistry*, vol. 45B, pp. 1905–1908, 2006.
- [10] K. Mogilaiah and C. S. Reddy, "Chloramine-T mediated synthesis of 1,3,4-oxadiazolyl-1,8-naphthyridines under microwave irradiation," *Indian Journal of Chemistry*, vol. 44B, pp. 768–772, 2005.
- [11] D. Sridhar, M. Arjun, M. Jyothi, T. Raviprasad, and M. Sarangapani, "Synthesis of 5-(benzimidazol-2-yl) benzoxazoles as new antiinflammatory agents," *Indian Journal of Heterocyclic Chemistry*, vol. 16, no. 1, pp. 99–100, 2006.
- [12] K. C. Ravindra, H. M. Vagdevi, V. P. Vaidya, and B. Padmashali, "Synthesis, antimicrobial and anti-inflammatory activities of 1,3,4-oxadiazoles linked to naphtho[2,1-*b*]furan," *Indian Journal of Chemistry*, vol. 45B, pp. 2506–2511, 2006.
- [13] M. S. Y. Khan, G. Chawla, and M. A. Mueed, "Synthesis characterization and biological evaluation of substituted oxadiazole, and triazole derivatives," *Indian Journal of Chemistry*, vol. 43B, pp. 1302–1305, 2004.
- [14] S. L. Vasoya, M. R. Patel, S. V. Dobarra, and H. S. Joshi, "Facile synthesis of some new azetidinones and acetyl oxadiazoles bearing benzo[*b*]thiophene nucleus as a potent biological active agent," *Indian Journal of Chemistry B*, vol. 44, no. 2, pp. 405–409, 2005.
- [15] M. S. Y. Khan, R. M. Khan, and S. Drabu, "Anticonvulsant and antibacterial activity of some new 1,3,4-oxadiazole derivatives," *Indian Journal of Heterocyclic Chemistry*, vol. 11, no. 2, pp. 119–122, 2001.
- [16] R. C. Sharma and D. Kumar, "Synthesis of some new thiazolidin-4-ones as possible antimicrobial agents," *Journal of the Indian Chemical Society*, vol. 77, no. 10, pp. 492–493, 2000.
- [17] H. D. Joshi, P. S. Upadhyay, and A. J. Baxi, "Studies on 4-thiazolidinones—synthesis and pharmacological activity of 1,4-bis[2'-methyl/ethyl/phenyl-2'-substituted styryl-5'-H/methyl/carboxymethyl-4'-thiazolidinon-3'-ylamino] phthalazines," *Indian Journal of Chemistry*, vol. 39, no. 12, pp. 967–970, 2000.
- [18] V. S. Ingle, A. R. Sawale, R. D. Ingle, and R. A. Mane, "Synthesis of new 4-thiazolidinones bearing potentially active heteryl moieties," *Indian Journal of Chemistry B*, vol. 40, no. 2, pp. 124–128, 2001.
- [19] P. Kagthara, T. Upadhyay, R. Doshi, and H. H. Parekh, "Synthesis of some 2-azetidinones as potential antitubercular agents," *Indian Journal of Heterocyclic Chemistry*, vol. 10, no. 1, pp. 9–12, 2000.
- [20] N. Matsui, "Preparation of azetidinones as intermediates from carbapenem antibiotics," *Japanese Kokai Tokkyo Koho*, vol. 7, p. 652, 2000.
- [21] N. Matsui, "Preparation of azetidinones as intermediates from carbapenem antibiotics," *Chemical Abstracts*, vol. 132, Article ID 64109u, 2000.
- [22] S. B. Desai, P. B. Desai, and K. R. Desai, "Synthesis of some thiazolidinone and azetidinone compounds and their antimicrobial activity," *Asian Journal of Chemistry*, vol. 11, no. 4, pp. 1431–1437, 1999.
- [23] K. M. Thaker, V. V. Kachhadia, and H. S. Joshi, "Synthesis of 4-thiazolidinones and 2-azetidinones bearing benzo (b) thiophene nucleus as potential antitubercular and antimicrobial agents," *Indian Journal of Chemistry*, vol. 42B, no. 6, pp. 1544–1547, 2003.
- [24] A. R. Saundane, V. Katkar, M. Yarlakatti, A. V. Vajinath, and W. Prabhaker, "Synthesis and biological activity of some 5-substituted 2-phenyl-3-(6-aryl-3-cyano-2-substituted pyridin-4-yl)indole and their derivatives," *Indian Journal of Heterocyclic Chemistry*, vol. 20, pp. 321–324, 2011.
- [25] A. R. Saundane, V. Katkar, A. V. Vajinath, and W. Prabhaker, "Synthesis, antimicrobial and antioxidant activities of some new indole derivatives containing pyridopyrimidine and pyrazolopyridine moieties," *Medicinal Chemistry Research*, vol. 22, no. 2, pp. 806–817, 2013.
- [26] A. R. Saundane, M. Yarlakatti, and R. Kalpana, "Synthesis, antimicrobial, and antioxidant activities of some new indole analogues containing pyrimidine and fused pyrimidine systems," *Medicinal Chemistry Research*, vol. 21, no. 11, pp. 3809–3817, 2012.

- [27] M. B. Patel, N. R. Modi, J. P. Raval, and S. K. Menon, "Calix[4]arene based 1,3,4-oxadiazole and thiadiazole derivatives: design, synthesis, and biological evaluation," *Organic & Biomolecular Chemistry*, vol. 10, no. 9, pp. 1785–1794, 2012.
- [28] S. P. Hiremath, J. S. Biradar, and M. G. Purohit, "A new route to indolo[3,2-b]isoquinolines," *Indian Journal of Chemistry B*, vol. 21, pp. 249–253, 1982.
- [29] M. Strlic, T. Radovic, J. Kolar, and B. Pihlar, "Anti- and prooxidative properties of gallic acid in fenton-type systems," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 22, pp. 6313–6317, 2002.
- [30] I. Calis, M. Hosny, T. Khalifa, and S. Nishibe, "Secoiridoids from *Fraxinus angustifolia*," *Phytochemistry*, vol. 33, no. 6, pp. 1453–1456, 1993.
- [31] I. Gülçin, M. Elmastaş, and H. Y. Aboul-Enein, "Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies," *Phytotherapy Research*, vol. 21, no. 4, pp. 354–361, 2007.
- [32] I. Gülçin, H. A. Alici, and M. Cesur, "Determination of in vitro antioxidant and radical scavenging activities of propofol," *Chemical and Pharmaceutical Bulletin*, vol. 53, no. 3, pp. 281–285, 2005.
- [33] B. Halliwell, "Reactive oxygen species in living systems: source, biochemistry, and role in human disease," *The American Journal of Medicine*, vol. 91, no. 3, supplement 3, pp. S14–S22, 1991.
- [34] *Indian Pharmacopeia*, New Delhi Appendix IV, Government of India, 3rd edition, 1985.
- [35] T. Hatano, H. Kagawa, T. Yasuhara, and T. Okuda, "Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects," *Chemical and Pharmaceutical Bulletin*, vol. 36, no. 6, pp. 2090–2097, 1988.
- [36] M. Oyaizu, "Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine," *Japanese Journal of Nutrition*, vol. 44, no. 6, pp. 307–315, 1986.
- [37] T. C. P. Dinis, V. M. C. Maderia, and L. M. Almeida, "Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers," *Archives of Biochemistry and Biophysics*, vol. 315, no. 1, pp. 161–169, 1994.