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Synthesis and biological evaluation of some novel pyrido[2,3-d]pyrimidine derivatives as antimicrobial and antioxidant agents

[1] Vaijinath A. Verma* and [2] Rajkumar S. Meti.

[1] Department of chemistry, Shri Prabhu Arts, Science & J. M. Bohra commerce Degree College, Shorapur, Karnataka, India.

[2]Department of Biochemistry, Mangalore University, P.G. Centre Chikka Aluvara Kodagu, Karnataka, India.

* **Corresponding Author:** E-Mail**:** drvermachem@gmail.com

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ABSTRACT

As a part of systemic investigation a new series of indole analogues containing 2-Amino-4-(5'-substituted 2'-phenyl-1*H*-indol-3'-yl)-6-phenylpyridine-3-carboxamides (3a-c), 5-(5'-Substituted 2'-phenyl-1*H*-indol-3'-yl)-2,7-diphenylpyrido[2,3-d]pyrimidin-4(3*H*)-ones (4a-c) and 5-(5'-Substituted 2'-phenyl-1*H*-indol-3'-yl)-7-phenylpyrido[2,3-d]pyrimidin-4(3*H*)-ones (4d-f) were synthesized in good yield. The structure of synthesized compounds were established by spectroscopic (FT-IR, ^{1}H and ^{13}C NMR, Mass) and elemental analyses. The newly synthesized compounds were evaluated for their antimicrobial and antioxidant activities. Some of these compounds are found to exhibit strong antimicrobial and antioxidant activities.

Keywords: Indole; Pyrido[2,3-d]pyrimidine; Antimicrobial; Antioxidant.

1. INTRODUCTION

The increasing incidence of infection caused by the rapid development of microbial resistance to most of the known antibiotics is a serious health problem. Antimicrobial resistance refers to micro-organism developing the ability to inactivate, exclude or block the inhibitory or lethal mechanism of the antimicrobial agent [1]. As multi-drug resistant microbial strains proliferate, the necessity for effective therapy has stimulated research on the synthesis of novel antimicrobial molecules [2]. Electronic-rich nitrogen heterocyclic compounds are the most reinforcing and extensively developing discipline of heterocyclic chemistry [3]. These heterocyclics possess broad-spectrum of biological activities such as anticancer[4], antidiabetic [5], anti-inflammatory [6], anti-HIV [7], antimalarial [8], antimicrobial [9] and antioxidant [10,11]. Furthermore, the structural diversity and biological importance of indole have been explored for synthesis and various biological applications such as, antimicrobial [12-14], anticancer [15], antidiabetic [16], anti-inflammatory[17], antitumor[18], gastric ulcer [19] activities. Also the properties of indole attracted chemists attention because of the correlation of serious diseases with oxidant damage of the membrane, DNA and RNA-induced radicals [20]. Careful literature survey reveled that targets containing pyridine and pyrimidine moieties are well known for their manifold uses because of their potential pharmacological activities as antimicrobial [21], antioxidant [22], anticancer [23] and antitumor [24] activities. These observations of fused indole and pyrido[2,3-d]pyrimidine prompted their synthesis and screening for antimicrobial and antioxidant activities.

2. MATERIAL AND METHODS

2.1. Chemistry

All the reagents were obtained commercially. Melting points were determined by open capillary method and were uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated aluminium plates (Merck). The IR spectra were recorded on Thermo Fischer (id S-5) FT-IR spectrometer. The ^{1}HNMR were recorded on a Bruker NMR (500MHz) and chemical shifts were expressed in ppm (δ scale) downfield from TMS. ^{13}C NMR (100 or 125 MHz, DMSO) spectra recorded on Bruker NMR. Mass spectral data were recorded by electron impact method on JEOL GCMATEII GC-MS mass spectrometer at 70ev.

2.2. The general procedures illustrated in scheme 1 and details are listed 3-(5'-Substituted -2'-phenyl-1H-indol-3'-yl)-1-phenylprop-2-en-1-ones (1a–c) and 2-Amino-

4-(5'-substituted-2'-phenyl-1H-indol-3'-yl)-6-phenylpyridine-3-carbonitriles (2a–c)

These compounds were prepared by reported method [25-28].

2.3. 2-Amino-4-(5'-substituted-2'-phenyl-1*H*-indol-3'-yl)-6-phenylpyridine-3- carboxamides (3a-c)

To 100 mL of alcoholic solution of KOH (5%), compounds (2a-c) (0.01 mol) were added and the reaction mixture was refluxed for 30 min. After cooling, the reaction mixture was diluted with water and the solid formed was filtered off, washed with water and recrystallized from dimethyl formamide to give (3a-c).

2.4. 5-(5'-Substituted-2'-phenyl-1*H*-indol-3'-yl)-2,7-diphenylpyrido[2,3-d]pyrimidin-4(3*H*)-ones (4a-c)

A solution of 3a-c (0.01mol) in benzoyl chloride (8 mL) was heated under reflux for 6 h. The excess of benzoyl chloride was removed under reduced pressure. After cooling, the product separated was filtered, washed with cold ethanol, dried and recrystallized from dimethyl formamide.

2.5. 5-(5'-Substituted-2'-phenyl-1*H*-indol-3'-yl)-7-phenylpyrido[2,3-d]pyrimidin-4(3*H*)-ones (4d-f)

A mixture of 3a-c (0.01 mol) and triethyl orthoformate (0.01mol) in ethanol (5 mL) was heated under reflux for 3 h. After cooling the product separated was filtered, washed with ethanol and recrystallized from ethanol.

2.6. 2-Amino-4-(5'-chloro-2-phenyl-1*H*-indol-3'-yl)-6-phenylpyridine-3-carboxamide (3a)

Yellow crystal, yield: 82 %, mp: 250-51 ^{o}C; FTIR (KBr) cm^{-1}: 3416 (Indole NH), 2917, 2849 (NH), 1715, 1627(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm); 12.20 (s, 1H, indole NH), 7.00-8.20 (m, 13H, Ar-H and pyridine-NH_2), 5.80(s, 2H, NH_2); ^{13}C-NMR (DMSO-d_6,125MHz,d); 166.6, 161.5, 158.1, 134.6, 133.5, 133.2, 132.9, 131.1, 130.9, 130.0, 129.3, 129.1, 128.8, 128.7, 127.8, 125.6, 123.3, 119.1, 117.6, 40.16; MS (EI) *m/z* 438 (M^+); 440 (M^++2); Anal. % $C_{23}H_{19}N_5OCl$: C, 71.15; H, 4.36; N, 12.77. Found: C, 71.16; H, 4.37; N, 12.79.

2.7. 2-Amino-4-(5'-methyl 2'-phenyl-1H-indol-3'-yl)-6-phenylpyridine-3-carboxamide (3b)

Orange needles, yield: 71 %, mp: 240-41 ºC; FTIR (KBr) cm^{-1}: 3435 (Indole NH), 3010,2998 (NH), 1705, 1607(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm); 12.30 (s, 1H, indole NH), 7.11-8.15 (m, 17H, Ar-H and pyridine-NH_2), 5.50(s, 2H, NH_2), 2.25(s, 3H, CH_3); Anal. % $C_{27}H_{22}N_4O$: C, 77.49; H, 5.30; N, 13.39. Found: C, 77.46; H, 5.34; N, 13.35.

2.8. 2-Amino-6-phenyl-4-(2'-phenyl-1*H*-indol-3'-yl)pyridine-3-carboxamide (3c)

Colourless solid, yield: 78 %, mp: 229-30 ºC; FTIR (KBr) cm^{-1}: 3423 (Indole NH); 3100, 3045 (NH), 1700, 1594(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm); 12.30 (s, 1H, indole NH), 7.20-7.95 (m, 14H, Ar-H and pyridine-NH_2), 5.65(s, 2H, NH_2); Anal. % $C_{26}H_{20}N_4O$: C, 77.21; H, 4.98; N, 13.85. Found: C, 77.23; H, 5.00; N, 13.86.

2.9. 5-(5'-Chloro-2'-phenyl-1*H*-indol-3'-yl)-2,7-diphenylpyrido[2,3-d]pyrimidin-4(3H)- one (4a)

Pale yellow crystal, yield: 73 %, mp: 270-71 ^{o}C; FTIR (KBr) cm^{-1}: 3405 (indole NH), 3150 (NH), 1693 (C=O), 1594(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.53(s, 1H, indole NH), 10.15 (s, 1H, pyrimidine-NH), 7.20-8.20 (m, 20H, Ar-H);^{13}C-NMR (DMSO-d_6, 125 MHz, d), 163.3, 157.5, 156.6, 156.4, 138.1, 134.1, 133.5, 132.4, 132.0, 130.8, 130.4, 130.2, 129.5, 129.1, 128.9, 128.8, 125.8, 125.6, 124.1, 122.6, 118.4, 40.0: MS (EI) *m/z* 554 (M^+); 556 (M^++2); Anal. % $C_{33}H_{21}N_4OCl$: C, 75.50; H, 4.03; N, 10.67. Found: C, 75.52; H, 4.03; N, 10.69.

2.10. 5-(5'-Methyl-2'-phenyl-1*H*-indol-3'-yl)-2,7-diphenylpyrido[2,3-d]pyrimidin-4(3*H*)-one (4b)

Orange solid, yield: 65 %, mp: 292-93 ^{o}C; FTIR (KBr) cm^{-1}: 3444 (indole NH), 3214 (NH), 1699 (C=O), 1612, 1592(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.12(s, 1H, indole NH), 10.01 (s, 1H, pyrimidine-NH), 7.00-8.20 (m, 20H, Ar-H), 2.31 (s, 3H, CH_3); Anal. % $C_{34}H_{24}N_4O$: C, 80.93; H, 4.79; N, 11.10. Found: C, 80.96; H,4.80; N, 11.13.

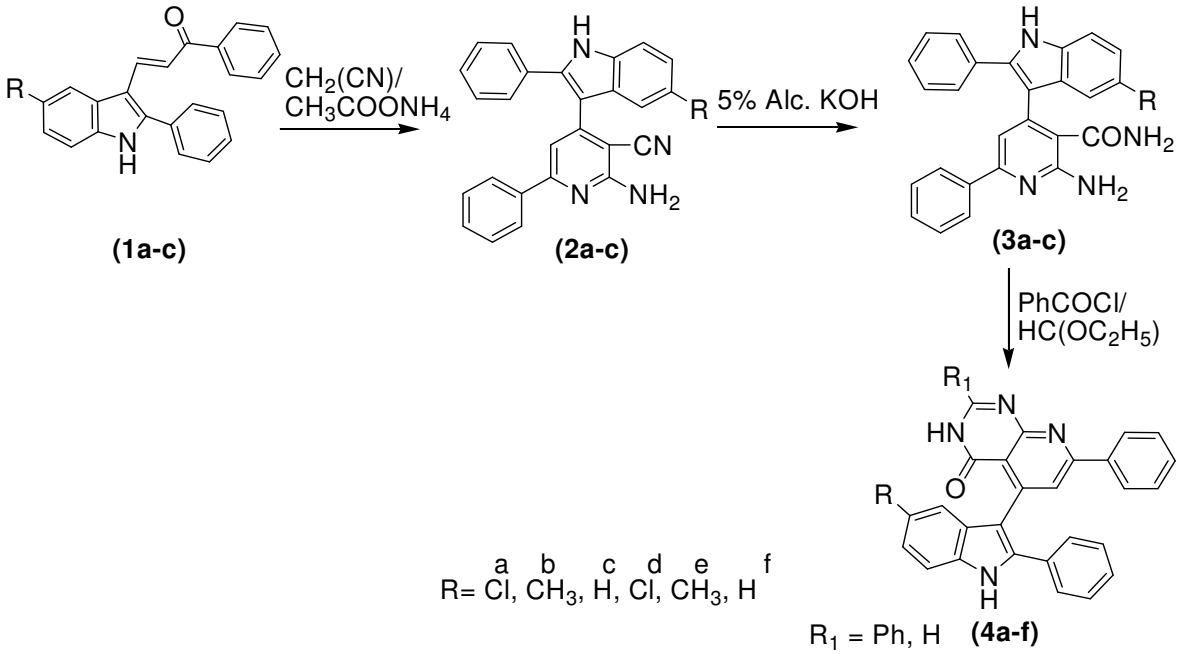
2.11. 2,7-Diphenyl-5-(2'-phenyl-1*H*-indol-3'-yl)pyrido[2,3-d]pyrimidin-4(3*H*)-one (4c)

Brown powder, yield: 67 %, mp: 285-86; FTIR (KBr) cm^{-1}: 3444 (indole NH), 3214 (NH), 1699 (C=O), 1612, 1592(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.10(s, 1H, indole NH); 10.10 (s, 1H, pyrimidine-NH), 7.35-7.00 (m, 20H, Ar-H); Anal. % $C_{33}H_{22}N_4O$: C, 80.80; H, 4.52; N, 11.42. Found: C, 80.83; H, 4.58; N, 11.46.

2.12. 5-(5'-Chloro-2'-phenyl-1*H*-indol-3'-yl)-7-phenylpyrido[2,3-d]pyrimidin-4(3*H*)-one (4d)

Yellow solid, yield 75 %, mp: 282-83 ^{o}C; FTIR (KBr) cm^{-1}: 3312 (indole NH), 3148 (NH), 1718 (C=O), 1620, 1598(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.50(s, 1H, indole NH), 10.13 (s, 1H, pyrimidine-NH), 7.00-8.15 (m, 15H, Ar-H); Anal. % $C_{27}H_{17}N_4OCl$: C, 72.24; H, 3.82; N, 12.48. Found: C, 72.20; H, 3.85; N, 12.50.

2.13. 5-(5'-Methyl-2'-phenyl-1*H*-indol-3'-yl)-7-phenylpyrido[2,3-d]pyrimidin-4(3*H*)-one (4e)



Scheme -1

Orange powder, yield: 76 %, mp: 280-81 ^{o}C; FTIR (KBr) cm^{-1}: 3389 (indole NH), 3200 (NH), 1700 (C=O), 1621, 1600(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.24(s, 1H, indole NH), 10.00 (s, 1H, pyrimidine-NH), 7.08-8.08 (m, 15H, Ar-H), 2.21(s,3H,CH_3); Anal. % $C_{28}H_{20}N_4O$: C, 78.49; H, 4.70; N, 13.08. Found: C, 78.50; H, 4.66; N, 13.03.

2.14. 7-Phenyl-5-(2'-phenyl-1*H*-indol-3'-yl)pyrido[2,3-d]pyrimidin-4(3*H*)-one (4f)

Brown needles, yield: 78 %, mp: 265-66 ^{o}C; FTIR (KBr) cm^{-1}: 3441 (indole NH), 3125(NH), 1685 (C=O), 1621, 1600(C=N); ^{1}H NMR (DMSO-d_6, δ, ppm): 12.11(s, 1H, indole NH), 10.00 (s, 1H, pyrimidine-NH), 7.10-8.20 (m, 17H, Ar-H); Anal. % $C_{27}H_{18}N_4O$: C, 78.24; H, 4.38; N, 13.52. Found: C, 78.22; H, 4.38; N, 13.54.

3. RESULTS AND DISCUSSION

3.1. Antimicrobial activity

The *in vitro* biological screening of the synthesized compounds was carried out against bacterial species, *E. coli*, *B. subtilis* and *K. pneumonia* and fungal species, *A. niger*, *A. flavus* and *A. fumigatus* by cup plate method [29] using nutrient agar and PDA medium for antibacterial and antifungal activity, respectively. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (5mg/5mL in DMF), standard solution (5mg/5mL in DMF), which were further diluted in distilled water to get concentrations of 25, 50, 75 and 100 µg/Ml. DMF was used as control. The plates were incubated at 37°C for 24 hrs and 72 hrs for the evaluation of antibacterial and antifungal activities, respectively. The diameter of the inhibition zones for all the test compounds were measured in mm and the results were compared with the results obtained by using streptomycin and fluconazole as positive reference drugs for antibacterial and antifungal activities, respectively. The susceptibility was assessed on the basis of diameter of zone of inhibition against bacteria and fungi. Inhibition zones were measured and compared with standards. The results are tabulated in Table 1. Further, for precise value that hindered the growth of pathogens to nil, MIC (Minimal Inhibition Concentration) and IC_{50} values was appraised by broth dilution method. The MIC values of compounds were determined as the lowest concentration (highest dilution) of the compounds at which there was no visible growth of the microorganism as detected by unaided eye. The minimal inhibitory concentrations (MIC/IC_{50}) of antibacterial and antifungal activities values carried out with synthesized compounds which display good zones of inhibition at concentration 100 µg/mL by the broth micro dilution method [30-32]. IC_{50} values were dictated to be the concentration of each sample required to scavenge 50% of microbial growth. Compounds which exhibited best activities at concentration of 100µg/mL were dealt with for MIC and IC_{50} values. The MIC/IC_{50} values of superscripted reference in table 2.

The outcome of antimicrobial studies of newly synthesized compounds revealed that these compounds have significant antibacterial and antifungal activities. The results of antibacterial screening showed that compounds 3a and 4e demonstrated good zone of inhibition against *E. coli*. Compounds 3b, 3c and 4f exhibited maximum

growth inhibition against *K. pneumonia* and *S. aureus*. In the case of chloro inserted substituent in C-5 position in indole ring compounds 4a and 4d increment activity were observed enormously and showed remarkable broad spectrum of potency against all bacteria and found to be almost equally potent as the reference drugs. On the other hand antifungal activities of test compounds reveled that, compound 3a exhibited good zone of inhibition against *A. niger*. Compound 3c showed maximum zone of inhibit against *A. flavus*. Compounds 3b, 4b, 4c and 4f showed activity against *A. fumigatus.* The compounds 4a and 4d exhibited strong antifungal activity against all fungi.

Interpretation of the obtained results and considering the structure activity relationship (SAR) of the tested compounds clearly suggested that with respective MIC/IC_{50} values, compounds 4a and 4d were found to exhibit the most potent activity against all bacteria and fungi. SAR studies to be found that presence of an electron withdrawing group chloro substituent on C-5 position in the indole amplified the activity and antimicrobial activity, decrease in the presence of electron donating groups.

3.2. Antioxidant activity

Conversely to have an overview of free radicals and the detrimental effects caused by the same, the emphasis on antioxidants becomes imperative. Antioxidants are substances that neutralize free radicals or their actions; on the contrary free radical refers to any chemical species capable of independent existence that contains unpaired electron. These are derived from oxygen (reactive oxygen species, ROS) and nitrogen (reactive nitrogen species, RNS) and are entwined in the etiology of a variety of diseases. They can adversely alter various biological molecules as a consequence of which they lose their function which in turn leads to diseased conditions. Here is where antioxidants emerge as defense system against the damage induced by free radicals acting at various levels. Evidently the implication of antioxidants on free radical scan impediment the damage caused by free radicals. In the present antioxidant study,

3.2.1. The DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging by Hatano's method[33].

The RSA of the synthesized compounds were carried out by DPPH, a stable free radical that can accept hydrogen radical or an electron and gets converted to a stable diamagnetic molecule. In the course of determination of RSA, compounds of different concentrations (25, 50, 75 and 100µg/mL) were prepared in methanol; 1,2,3 and 4 mL of the above mentioned concentrations were taken respectively in different test tubes along with 1 mL DPPH to each test tube followed by the addition of methanol to adjust the volume to 5 mL. The test tubes were shaken vigorously after the addition and incubated in dark for 20 min at room temperature. A DPPH blank was prepared without the compound. Changes in the absorbance at 517 nm were measured using a UV-Visible spectrophotometer. Scavenging of DPPH free radicals was calculated from the following equation

DPPH radical scavenging (%) = [(Ac-As/Ac)X 100]

Where, Ac is the absorbance of the control reaction and As is the absorbance of the sample or standards.

The Radical scavenging activity (RSA) results indicate that, compound 4a was found to enhance the RSA by 82.13 and 89.18% at concentrations 75 and 100 µg/mL, respectively. Compound 3a exhibited 70.02% RSA at concentration 100 µg/mL concentrations. The compound 4d was found to enhance RSA to 75.82 and 80.46% at 75 and 100 µg/mL concentrations. The higher activity of 4a may be due to presence of enolizable aminde group in pyrimidine ring and introduction of electron withdrawing chloro substitution at 5-position of indole which lead to the aromatic system. The plausible mechanism for hydrogen donation to (DPPH) free radical and stabilization of free radical formed is shown in Scheme 2 and 3 for compound of 4a. While other compounds were found to augment RSA to lesser extent figure 1.

3.2.2. Ferric ion (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power of the synthesized compounds were determined according to the method of Oyaizu [34]. Different concentrations (25, 50, 75 and 100µg/mL) of the sample in DMSO were mixed with phosphate buffer (2.5 mL, 0.2M, pH=6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min after which 2.5 mL of trichloroacetic acid (10%) was added to the mixture and centrifuged for 10 min at 1000rpm. The upper layer solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.01%) and absorbance at 700 nm was measured in UV-Visible spectrophotometer.

The ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) results are shown in Fig. 2 revealed that compound 4e showed higher activity by introduction of methyl substitution at C-5 position of indole. Compound 4b exhibited promising activity at 50, 75 and 100 µg/mL concentrations. Whereas other compounds

exhibited either moderate or poor FRAP activity. In other words the compound showed the ability of electron donor to scavenge free radicals. The reducing ability of the synthesized compounds indicated that increase in the concentration of samples increases the reductive ability. Thus higher the absorbance of compounds, greater is the reducing power.

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

The chelating activity of the ferrous ions (Fe^{2+}) towards the test compounds and standards was determined by the Denis method [35]. In this method, the test samples of concentration 25, 50, 75 and 100µg/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 (0.02 mL, 5 mM) and the total volume was adjusted to 4 mL with ethanol. The mixture was shaken vigorously and kept at room temperature for 10 min and then the absorbance at 517 nm were measured using a UV-Visible spectrophotometer. The percentage of inhibition of the ferrozine complex formation was calculated using the following equation

Ferrous ion chelating effect (%) = [(Ac-As/Ac)] X 100

Where, Ac is the absorbance of control and As is the absorbance of test sample or standards

The ferrous ion (Fe^{2+}) chelating activity results of test compounds are shown in figure 3. The results of the title compounds indicate that compound 4f exhibited higher metal chelating activity. Compounds 3c, 4c and 4d exhibited good metal chelating activity at all concentrations, where as other compounds exhibited either moderate or less chelating activity.

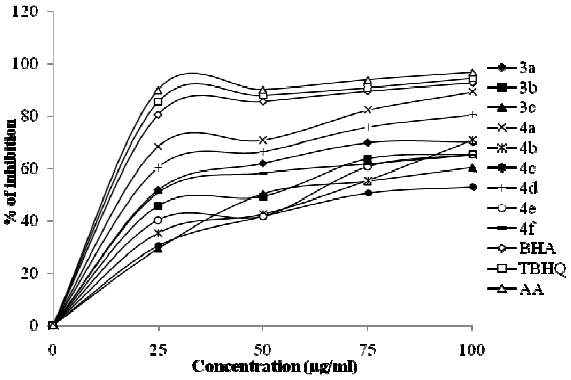


Figure - 1: RSA of compounds 3a-c and 4a-f at different concentrations (25, 50,75,100 µg/ml).

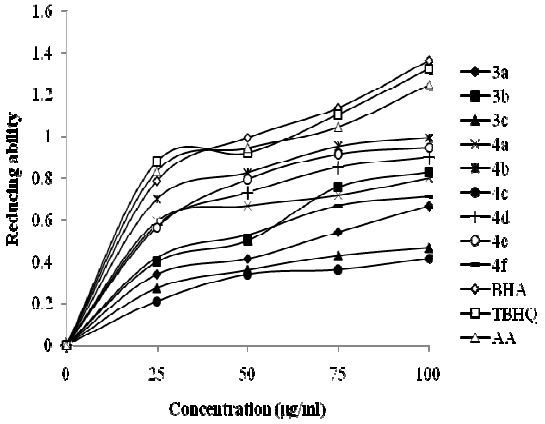


Figure - 2: FRAP of compounds 3a-c and 4a-f at different concentrations (25, 50,75,100 µg/ml).

Table -1: Antibacterial evaluation of compounds 3 and 4.

Compounds			Concentration of compounds (µg/mL) Zone of inhibition (mm)											
			E. coli				*K. pneumonia*				*S. aureus*			
	R	R_1	25	50	75	100	25	50	75	100	25	50	75	100
3a	Cl	-	15	14	15	12	10	10	12	13	14	14	16	18
3b	CH_3	-	10	14	15	16	07	08	11	17	03	05	11	17
3c	H	-	12	13	16	16	01	10	12	15	-	04	12	16
4a	Cl	Ph	19	19	21	22	17	17	20	21	18	19	21	23
4b	CH_3	Ph	12	12	14	15	09	10	12	15	04	10	12	16
4c	H	Ph	10	13	14	15	10	12	12	17	11	12	14	15
4d	Cl	H	17	18	19	21	16	17	19	20	17	18	21	22
4e	CH_3	H	10	16	18	20	09	14	19	20	01	04	18	21
4f	H	H	10	13	13	15	08	08	12	16	10	10	13	16
S_1	-	-	20	20	22	24	18	19	21	23	19	20	23	25
Control DMF				-	-	-	-	-	-	-	-	-	-	-

S_1 = Streptomycin, - = No activity

Table - 2: Antifungal evaluation of compounds 3 and 4.

Compounds			Concentration of compounds (µg/mL) Zone of inhibition (mm)											
			A. niger				*A. flavus*				*A. fumigatus*			
	R	R_1	25	50	75	100	25	50	75	100	25	50	75	100
3a	Cl	-	13	12	16	17	14	14	15	17	10	11	12	16
3b	CH_3	-	11	10	12	15	12	06	09	10	08	08	12	13
3c	H	-	10	10	13	13	11	04	11	12	06	11	13	15
4a	Cl	Ph	18	19	21	22	17	19	23	25	18	17	19	22
4b	CH_3	Ph	12	12	14	15	11	10	18	17	13	12	14	15
4c	H	Ph	09	10	11	14	10	10	14	15	10	12	12	14
4d	Cl	H	17	17	19	20	16	18	21	23	17	16	19	20
4e	CH_3	H	10	11	14	16	12	10	13	15	10	11	13	15
4f	H	H	9	13	13	15	10	10	11	13	08	08	12	14
S_2	-	-	19	20	22	24	18	20	23	26	19	19	20	23
DMF			-	-	-	-	-	-	-	-	-	-	-	-

S_2 = Fluconazole, - = No activity

Table - 3: The Minimum inhibitory Concentration (MIC, 100 µg/mL) and IC_{50} of compounds 3 and 4.

Compd	Minimum inhibitory Concentration (µg/mL)											
	E. coli		*K. pneumoneae*		*S. aureus*		*A. niger*		*A. flavus*		*A. fumigatus*	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
3a	5.6	2.3	4.9	2.6	10.2	5.0	3.3	1.2	4.2	2.1	1.2	0.5
3b	4.2	2.1	-	-	11.3	5.3	3.4	1.4	3.4	1.7	-	-
3c	3.6	2.4	5.4	2.3	9.2	3.7	2.3	1.2	2.2	1.1	0.7	1.4
4a	6.7	3.1	6.2	3.2	13.3	6.3	6.6	3.2	6.2	3.2	3.3	1.7
4b	4.4	2.2	3.4	1.4	9.2	3.3	4.6	1.4	4.7	2.6	1.3	0.9
4c	4.6	2.1	5.4	2.3	7.2	3.7	2.3	1.1	3.2	1.2	0.9	1.4
4d	6.2	2.9	6.1	3.1	13.1	6.2	6.4	3.0	6.3	3.1	3.1	1.5
4e	5.1	3.8	3.5	1.5	8.2	3.2	3.1	1.1	4.1	2.1	1.0	0.6
4f	4.6	2.4	5.4	2.3	6.2	2.7	6.3	3.2	6.2	3.1	3.1	1.4
S_1	6.25	3.1	6.25	3.3	13.25	6.3	-	-	-	-	-	-
S_2	-	-	-	-	-	-	6.7	3.3	6.5	3.3	3.5	1.9

S_1 and S_2 =Standards, - = No activity

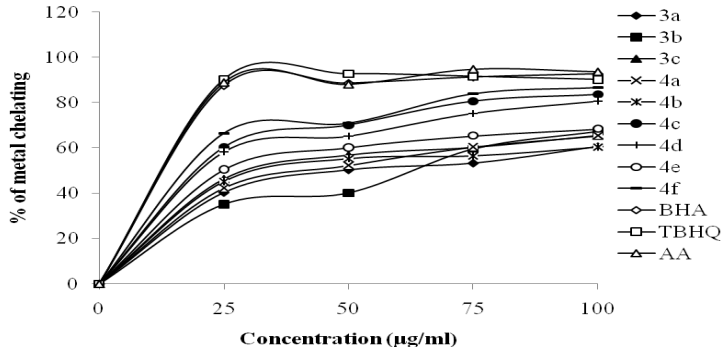


Figure - 3: Metal chelating activity of compounds 3a-c and 4a-f at different concentrations (25,50,75,100 µg/ml).

4. CONCLUSION

The study revealed that fused pyrido[2,3-d]pyrimidine and electronic withdrawing chloro substituent at C-5 position on indole demonstrated excellent antimicrobial activity. The hydrophobic nature of the halogen atoms changes the physiochemical properties and these changes can be reflected in altered biological activity. This property is useful for compounds to diffuse through biological membranes and reach its site of action. For, reason of hydropholicity was found to be directly related to antimicrobial activity[36]. It seems obvious that the antioxidant properties of the newly synthesized compounds are attributed to indole fused pyrido[2,3-d] pyrimidine derivatives in which the pyrimidine ring is bearing amide group and C-5 position on indole substituents.

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