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Original article

Synthesis, Characterization and Antibacterial and Antioxidant Activity of Bis Phthalimide Derivatives

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ABSTRACT:
A new series of bis-Phthalimide derivatives 3a-3c had been synthesized by reaction of diamines and substituted phthalic anhydride in presence of glacial acetic acid. The synthesized compounds were tested for antibacterial activity and antioxidant activity. The structures of new sulfanilamide derivatives were characterized by elemental analysis, IR spectroscopy, and mass spectrometry (MALDI-TOF). The synthesized compounds were tested for their in vitro antibacterial activity using the broth diffusion method against *E. coli* and S. mutants. MBC for the compound 3a was 120 and 150µg/ml, compound 3b was 150 and 140 µg/ml and for compound 3c, MBC was 100and 150 µg/ml against *E. coli* and *S. mutants*, indicating that all the tested compounds exhibited better activity as compared to standard drug ampicillin. Lower the IC50 value of the compound, stronger the ability of the antioxidant compound to scavenge the DPPH free radical. Our results showed that compounds 3a, 3b, and 3c exhibited the lowest IC50value 22.39±0.21, 11.62±0.28 and 0.27±0.02 µg/ml respectively. The IC50 values of hydrogen peroxide scavenging activity for the compounds 3a, 3b and3c were calculated as 1.13±0.003, 1.03±0.05 and 1.54±0.01 mg/ml, respectively. The antioxidant assay results established that compound 3ashowed a greater rate of H2O2 scavenging activity than other compounds.
Keywords: phthalic anhydride, DPPH scavenging, Hydrogen peroxide scavenging, Antibacterial.

1. INTRODUCTION

Mounting infectious diseases and the developing number of multidrug-resistant microbial pathogens still make the treatment of particular diseases an important and serious global problem. Therefore, extensive research for the discovery and synthesis of new classes of antimicrobial agents is needed.

Phthalimide possesses a structural feature$C_8H_5NO_2$ and an imide ring which help them to be biologically active and pharmaceutically useful [1]. Among bicyclic non-aromatic nitrogen heterocycles, phthalimides are an interesting class of compounds. Phthalimides have served as starting materials and intermediates for the synthesis of many types of alkaloids and Pharmacophores [2]. Phthalimide is an imido derivative of phthalic acid. In organic chemistry, imide is a functional group consisting of two carbonyl groups bound to nitrogen. They are hydrophobic and neutral, and can therefore cross biological membranes *in vivo*. These compounds are structurally related to acid anhydrides [1].

Phthalimide and some of its derivatives proved to have received awareness due to their antibacterial, antifungal, analgesic, antitumor, anxiolytic and anti-HIV-1 activities. When phthalimide is subjected to Mannich condensation, it may yield Mannich bases which may display more potent biological activities. The present research focuses on novel synthesized phthalimides having significant biological activities [2] Amongst heterocyclic scaffolds, phthalimides are of particular biological interest and have been reported as herbicides, insecticides and anti-inflammatory agents. Phthalimides are an important class of drugs exhibiting anxiolytic, antimicrobial, antituberculosis, hypolipidemic, analgesic, anticancer, acetylcholinesterase inhibitors and inhibitor of human neuronal nitric oxide synthase [3]. Isoindole-1,3(2H)-dione is an aromatic imide, containsisoindole moiety, which is motif in nature. In the combinedform with maleimides and succinimides, isoindole-1,3 (2H)-diones used as plastic modifiers to improve heat resistance, antioxidant and anti-foulant properties [4].

Herein, we report the synthesis and chemistry of phthalimide derivatives by reacting phthalic anhydride with diamine and its antibacterial and antioxidant studies.

2. EXPERIMENTAL

2.1 Materials

All chemicals and reagents were used are of analytical grade. Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with iodine vapor. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. binding of potent Schiff base derivatives to Ct-DNA was investigated by fluorescence quenching, absorption spectroscopy, circular dichroism, and viscosity measurements.

2.2 Synthesis

2.2.1 General Procedure for the Synthesis of Bis-phthalimide Derivatives (3a- 3c)

An appropriate amount of corresponding diamines (10 mmol) in 2:1 molar ratio in 50ml glacial acetic acid was slowly added to a solution of 20 mmol phthalic anhydride, tetrachlorophthalic anhydride and tetrabromophthalic anhydride in 100 ml glacialacetic acid. The coloured solutions were refluxed with constant stirring for 8 h at 100^{o}C and poured into cold water. The coloured precipitates were filtered off and finally dried in vacuum dessicator on fused $CaCl_2$ and recrystallized in DMSO. The progress of the reaction was monitored by thin layer chromatography (TLC) in methanol: dichloromethane (1:4).

2-(5-(1,3-Dioxoisoindolin-2-yl)-2H-1, 2, 4-triazol-3-yl) isoindoline-1, 3-dione (compound 3a)

^{1}H NMR spectrum (400 MHz DMSO-d6) δ, ppm: 7.60 (d, 4H, Ar-H), 7.69 (d, 4H, Ar-H), 12.16 (s, 1H, -NH).

^{13}C NMR (100 MHz, DMSO-d6) δ inppm: 173.66, 173.60, 149.51, 129.11, 128.25, 125.42, 123.96. MS (*m/z*): 360.01(M+1). Found, %: C 60.12; H 2.47; N 19.54. C18H9N5O4. Calculated, %: C 60.17; H2.52; N 19.49.

4,5,6,7-Tetrachloro-2-(5-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)-2H-1,2,4-triazol-3-yl)isoindoline-1,3-dione (compound 3b)

^{1}H NMR spectrum (400 MHz DMSO-d6) δ, ppm: 9.65 (s,1H, -NH).

^{13}C NMR (100 MHz, DMSO-d6) δ in ppm: 163.28, 159.12, 138.15,133.69, 131.33, 130.63, 127.20. MS (*m/z*): 635.90 (M+1). Found, %: C 34.03; H0.20; N 10.98. C18HN5O4Cl8. Calculated, %: C 34.05; H 0.15; N 11.03.

4,5,6,7-Tetrabromo-2-[3-(4,5,6,7-tetrabromo-1,3-dioxo-2,3-dihydro-1H-isoindol-2-yl) -1H-1,2,4-triazol-5-yl]-2,3-dihydro-1H-isoindole-1,3-dione (compound 3c)

^{1}H NMR spectrum (400 MHz DMSO-d6) δ, ppm: 9.46 (s, 1H, NH).

^{13}C NMR(100 MHz, DMSO-d6) δ in ppm: 166.63, 145.02, 138.41, 133.29, 131.51, 129.19,128.38, 126.22, 118.93, 116.30. MS (*m/z*): 991.58 (M+1). Found, %: C 21.87; H0.11; N, 7.05. C18HN5O4Br8. Calculated, %: C 21.82; H 0.11; N 7.05.

3.2. Biological evaluation

3.2.1. Antimicrobial study

Agar well-diffusion method [5] was applied to study the antibacterial activity of the synthesized **Bis-phthalimide** derivatives (3a-3c) against bacterial *E. coli* and *S. mutans*, (Table 2). The concentration for each compound was 2 mg/ml, using DMSO as a solvent and control. Ampicillin was used as standard drug for anti-bacterial screening. For all synthesized compound the minimum inhibitory concentration and zone of inhibition were measured against all tested microorganism.

3.2.2 *Antioxidant Assay*

3.2.2.1 DPPH Radical Scavenging Activity

2,2-Diphenyl-1-picryl-hydrazyl is a stable free radical which was used for the estimation of antioxidant activity. The free radical scavenging ability of the test compounds was studied using the DPPH assay [6]. The test compounds in ethanol were prepared separately and added to the ethanol solution containing DPPH (0.01 mmol) within the range of 0.5–2.5 mg/ml and adjusted to a final volume of 3 mL using ethanol as solvent. The scavenging ability of the test compounds was monitored spectrophotometricly by measuring the absorbance at 517 nm after 20 min. The % inhibition was obtained using equation. The 50 % decline in absorbance of the DPPH solution was derived from the graph with the concentration (μM) plotted against the absorbance (Table 3). These concentration values were used to determine the IC_{50} values in μM. (Table 5)

$$\%\ of\ inhibition = \frac{ABScontrol - ABSsample}{ABScontrol} \times 100$$

3.2.2.2. Hydrogen Peroxide Scavenging Activity

The ability of the synthesized compounds to scavenge hydrogen peroxide was measured by using standard H2O2 scavenging assay method [7]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (10x, pH 7.4). A series of various concentrated solution of each of the synthesized coumarin compounds (0.5 – 2.5 mg/ml) were prepared in ethanol (95%) and added (1 ml) to the hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution. Ascorbic acid was used as standard. All the experiments were carried out in triplicates in dark condition. The percentage of scavenged hydrogen peroxide (Table 4) was calculated by using the following equation,

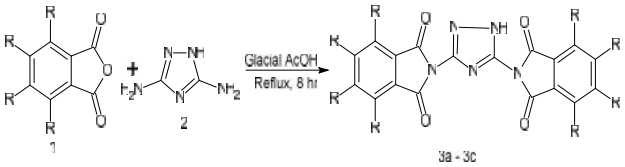
Percentage of scavenged H2O2 = [(Ai – At) / Ai] × 100

Where Ai is the absorbance of control and At is the absorbance of test.

H2O2 scavenging activity was expressed in terms of IC50 value. (Table 5)

4. RESULT AND DISCUSSION

4.1 Synthesis



3a; R = H

3b; R= Cl

3c; R=Br

Table 1: Physiochemical character of synthesized compounds

Sl. No.	Compound	Molecular formula	Molecular weight	Melting point (°C)	% of yield	Nature
1	**3a**	C18H9N5O4	360.01	> 300	66	White solid
2	**3b**	C18HN5O4Cl8	635.90	> 300	73	Yellow solid
3	**3c**	C18HN5O4Br8	991.58	> 300	60	White solid

4.2 *Antibacterial Activity*

Investigation of *in vitro* antimicrobial activity was carried out to for the estimation of potency of bis-phthalimides (**3a-3c)** the results **(Table 5.2)** revealed that all three compounds are significantly active against the selected bacterial strains *E.coli* and *S. mutants*. Minimum bactericidal concentration (MBC) was determined by dilution method. It is noteworthy that the MBC for the compound **3a** was 120 and 150µg/ml, compound **3b** was 150 and 140 µg/ml and for compound **3c**, MBC was 100and 150 µg/ml against *E. coli* and *S. mutants*, indicating that all the tested compounds exhibited better activity as compared to standard drug ampicillin. Lipophilicity of the compounds promotes their permeability into the cells and increases the inhibition efficiency and further inhibits growth of the bacteria [1-10].

Table 2: Antibacterial activity of bis-phthalimide derivatives

Compounds	MBC (µg/ml)		Zone of inhibition (mm)	
	E. coli	*S. mutans*	*E. coli*	*S. mutans*
3a	120	150	11	8
3b	150	140	10	8
3c	100	150	12	9
Ampicillin	30	30	17	21

4.3 Antioxidant Activity

4.3.1 DPPH Radical Scavenging Activity

2,2-Diphenyl-1-picryl-hydrazyl is used for the evaluation of antioxidant property of the target compounds with ascorbic acid as a positive control. The IC50 value can be calculated from the graph (**Figure 2**) and given in **Table 5** and % antioxidant activity **Table 3**. Lower the IC50 value of the compound, stronger the ability of antioxidant compound to scavenge the DPPH free radical. Our results showed that compounds **3a, 3b** and **3c** exhibited lowest IC50value 22.39±0.21, 11.62±0.28 and 0.27±0.02 µg/ml respectively, which reveals that bis-phthalimides **3a**, **3b** and **3c** are best antioxidant for scavenging activity [11, 12].

Table 3: DPPH Radical Scavenging Activity of compound 3a – compound 3c

Concentration (mg/ml)	% Antioxidant activity		
	Compound 3a	Compound 3b	Compound 3c
0	0	0	0
0.5	50	58	60
1	65	65	54
1.5	70	65	57
2	72	80	59
2.5	90	92	65

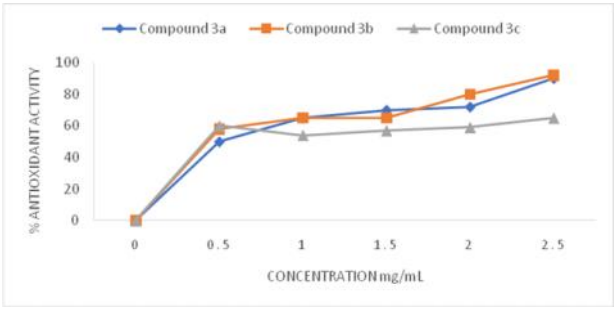


Fig 2: DPPH free radical assay of the bis-phthalimide derivatives (3a, 3b and 3c).

4.3.2. Hydrogen Peroxide Scavenging Activity

Antioxidant potential of test compounds was also estimated by hydrogen peroxide scavenging assay [6]. The IC50 values of scavenging activity for the compounds **3a, 3b** and**3c** were calculated as 1.13±0.003, 1.03±0.05 and 1.54±0.01 mg/ml, respectively. The antioxidant assay results established that compound **3a** showed the greater rate of H_2O_2 scavenging activity than other compounds. (Table 4, figure 3). The IC_{50}data were shown in table 5 and figure 4.

Table 4: Hydrogen Peroxide Scavenging Activity of compound 3a – compound 3c

Concentration (mg/ml)	% Antioxidant activity		
	Compound 3a	Compound 3b	Compound 3c
0	0	0	0
0.5	25	44	36
1	42	50	40
1.5	65	56	48
2	80	58	55
2.5	86	65	62

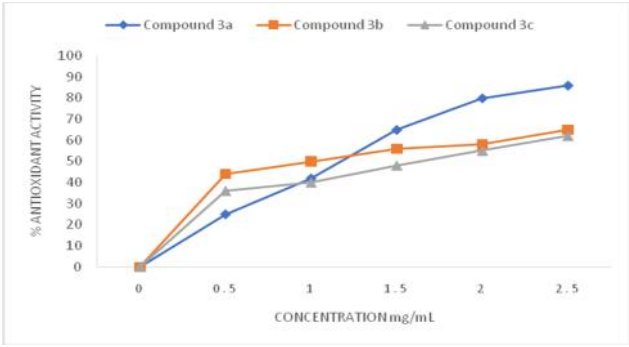


Fig 3: Hydrogen peroxide scavenging activity of the bis-phthalimide derivatives (3a, 3b and 3c).

Table 5: Antioxidant activity IC_{50} of the bis-phthalimide derivatives

Compounds	Hydrogen Peroxide Assay IC50 (µg/ml)	DPPH Free Radical Assay IC50 (µg/ml)
3a	1.13±0.003	0.022±0.21
3b	1.03±0.05	0.011±0.28
3c	1.54±0.01	0.74±0.07
Ascorbic acid	1.11±0.057	0.012±0.56

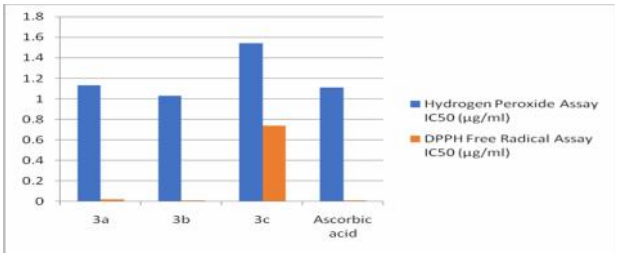


Fig 4: IC_{50} of the bis-phthalimide derivatives

5. CONCLUSION

The derivatives developed during the study proved to be effective in antibacterial as well as antioxidant activity. Hydrogen peroxide radical scavenging activity found to be superior form 3(a), 3(b) and 3(c) compound.

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