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Synthesis, Characterization and Docking Study of Some Novel Bis-phthalimide for their Antimicrobial Potential

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ABSTRACT:

A series of Bis-Phthalimides derivatives was synthesized and characterized by physicochemical and spectral means. The synthesized compounds were evaluated for *in-vitro* antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* by tube dilution method. The most promising antimicrobial activity of derivatives (K1H and K5H) was further docked to study their binding efficacy to the active site of Code 1KZN. All the compounds possessed significant antimicrobial activity with MIC in the range of 40µg/ml to 80µg/ml. The molecular docking studies of potent antimicrobial compounds (PMO, K1H and K5H) showed their putative binding mode and significant interactions with *DNA gyrase subunit b* as prospective agents.

Keywords: Bis-Phthalimide derivatives, docking, antimicrobial activity, Mannich reaction.

INTRODUCTION:

Heterocycles are widely used in the development of several pharmaceutically important compounds. The nitrogen and sulphur heterocyclic systems are very interesting because of their physicochemical properties with relevance to the design of new drugs. The chemical core of phthalimide (-CO-N(R)-CO-) shows they are hydrophobic and this increases their potential to cross biological membranes in-vivo [1]. The most important biological activity properties that have been reported for bis-phthalimide derivatives are anti-cancer [2], anti-microbial [3,4], anti-oxidant [5], anti-inflammatory [6,7], Anti-bacterial [8], anti-fungal, [9], anti-convulsant [10], CNS depressant [11], anti-tumor [12,13].

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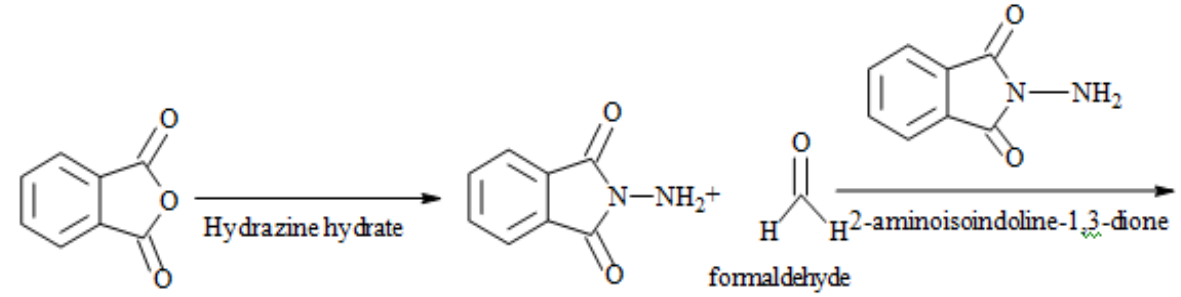
EXPERIMENTAL WORK:

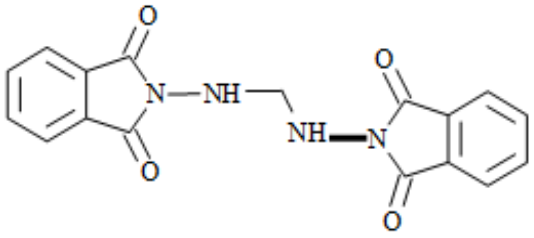
Reagents and chemicals of analytical grade were purchased from commercial sources and used as such with further purification. Melting point was determined in laboratory by open capillary method. The Ultraviolet absorption spectra were determined in methanol on Shimadzu, UV1800, and Visible double beam spectrophotometer. The IR spectra of the synthesized compounds were recorded on Shimadzu, 1S Furior, Affinity spectrometer. The ^{1}H NMR spectra are recorded in DMSO using NMR Bruker Avance Neo 500 MHz spectrometer. Mass spectra of synthesized compounds were recorded on LCMS-ion trap Mass spectrometer.

Chemical Synthesis:



Isobenzofuran-1, 3-Dione

2-aminoisoindoline-1, 3-dione



2, 2'-(methylenebis(azanediyl)bis(isoindoline-1,3-dione)

Synthesis of Phthalimide:

In a round bottom flask, 10g (0.0675mol) of phthalic anhydride and 10.5 ml of concentrated ammonia solution was placed. It was heated on a sand bath, gradually at first until the mixture was in a state of quiet fusion and formed a homogenous melt. The flask was shaken occasionally during the heating and the material which sublimes was pushed down. The contents of the flask were poured into the porcelain basin. It was allowed to cool and grind to a fine powder in a mortar. It was recrystallized from ethanol.

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Synthesis of N-amino phthalimide:

In a 500 ml beaker, 1 mole (7.35g) of phthalimide and hydrazine 1 mole (2.5 ml) in 90- 100 ml of absolute ethanol was placed and stirred under water-ice bath at 0-5℃ for 25 minutes. 2ml of hydrazine was added to beaker drop by drop. It was continuously stirred for 2 hrs. It was then filtered with vacuum pump. The precipitate obtained was washed with 5 ml of water and dried well.

Synthesis of Bis-phthalimide derivatives:

To the mixture of formaldehyde (0.1 mol, 1.7 ml), N-amino phthalimide (0.1 g) in 20 ml of ethanol was placed in round bottom flask. It was refluxed for 5 h. The reaction mixture was cooled and poured into ice-cold water. The precipitate was collected by filtration. The precipitate was dried and recrystallized from ethanol.

Docking study:

VLife MDS software 4.6 and free online software was used to perform all docking simulations. The molecular docking study of newly synthesized bis-phthalimides was done with DNA gyrase subunit b (PDB- 1KZN) which is key enzyme present in bacteria required for protein synthesis. [14,15].

Antimicrobial Screening:

The antibacterial activity of synthesized compounds was evaluated against bacteria by using the MIC (double strain nutrient broth) [16,17] and well diffusion method [18]. Amikacin was employed as standard drug to compare the results. The bacterial cultures were developed by selective nutrient broth. Nutrient broth was used for the preparation of inoculums of the bacteria and nutrient agar was used for the screening method

RESULT AND DISCUSSION:

All synthesized molecules were characterized by physico-chemical and analytical methods. All molecules were obtained in good yields after their purification by using a mixture of ethyl acetate and hexane. Their structure was elucidated by 1H NMR, FTIR and Mass spectroscopy.

The purity and homogeneity of the compounds were checked and monitored by TLC as the compounds were found to give single spot. The UV-Visible spectra of compounds have shown absorption maxima in between 209- 272nm.

The spectral data of synthesized compounds are listed in Table. The compounds (K1H, K2H, K5H, K7H) display characteristic absorption bands in IR spectrum in between 2975.25-3420.81 cm^{-1}due to C-H stretching, All compounds display characteristic absorption band in

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between 1601.20-1683.25 cm^{-1} due to C=O stretching. The compound (K1H, K5H, K7H, K8H) display characteristic absorption bands in IR spectrum in between 3109.30-3446.21 cm^{-1} due to secondary amine stretching.

The 1- H NMR spectra of synthesized compounds (K1H, K5H, K7H and K8H) have shown in ppm about 6.667-8.420 aromatic CH, ppm about1.038-1.850 for aliphatic CH and ppm about 4.54-6.20 for secondary amine.

Mass spectra of synthesized compounds (K1H, K5H) shows molecular ion (M+) peak at 419 and 389 respectively, also other frequencies after their fragmentation was shown in results.

Derivatives of Bis-Phthalimides:

2, 2'-methylene bis (azanidyl) bis (isoindoline-1,3-dione) [PMO]

Yield 62%, m.p. 216-218°C, R*F* 0.63, IR (KBr) in (cm^{-1}) 3413.10 (N-H), 2975.25 (C-H) 2896.17 (C-H), 1663.63 (C=O), 1571.05 and 1458.21 (C=C), 1341.51 (C-N).

2, 2'-[Methylene-bis-(azanidyl)]-bis-(4-nitro isoindoline-1, 3-dione) [K1H]

Yield 68%, m.p. 228-230°C, R*F* 0.59, , IR(KBr) in (cm^{-1}) 3109.30 (N-H), 3000 (C-H), 2784.29 (C-H), 1685.81 (C=O), 1560.44 and 1521.86 (C=C), 1195.89 (C-N), 1353.09 and1560.44 ($-NO_2$), 1H NMR (DMSO) (ppm), 7.949-8.434 (m Ar-H), 1.857 (s- CH_2), 4.563-4.542 (N-H), MS, (M+) ion m/e 419.4 g/mol

2, 2'-[Methylene-bis- (azanidyl)]-bis-(4- hydroxyl-isoindoline-1,3dione) [K2H]

Yield 72%, m.p. 238-240°C, R*F* 0.62, IR (KBr) in (cm^{-1}) 3166.21 (N-H), 3032.15 (C-H), 2900.09 (C-H), 1663.63 (C=O), 1555.62 and 1493.89 (C=C), 1349.23 (C-N), 3600(OH).

2, 2'-[methylene-bis- (azanidyl)]-bis-(3,4-dinitro isoindoline-1,3-dione) [K4H]

Yield 62%, m.p. 234-236°C, R*F* 0.60, IR (KBr) in (cm^{-1}) 3095.80. (N-H), 2962.71(C-H), 2781.40(C-H), 1669.42 (C=O), 1528.61 and 1472 (C=C), 1310.65 (C-N), 1528.61 and

1348.27 ($-NO_2$).

2, 2'-[Methylene-bis- (azanidyl)]-bis-(4-ethyl isoindoline-1,3-dione) [K5H]

Yield 69%, m.p. 207-209°C, R*F* 0.63, IR (KBr) in (cm^{-1}) 3420.81 (N-H), 976.21(CH), 2494.97 (C-H), 1659.77 (C=O), 1560.44 and 1490.04 (C=C), 1390.70 (C-N), ^{1}H NMR (DMSO) (ppm), 6.667-8.434 (mAr–H), 1.038(s-CH_2), 2.679-2.505 (t-CH_3CH_2-), 4.46 (–NH), MS (M+) ion m/e 393.6 g/mol

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2,2'-[Methylene-bis- (azanidyl)]-bis-(4-amino isoindoline-1,3-dione) [K7H]

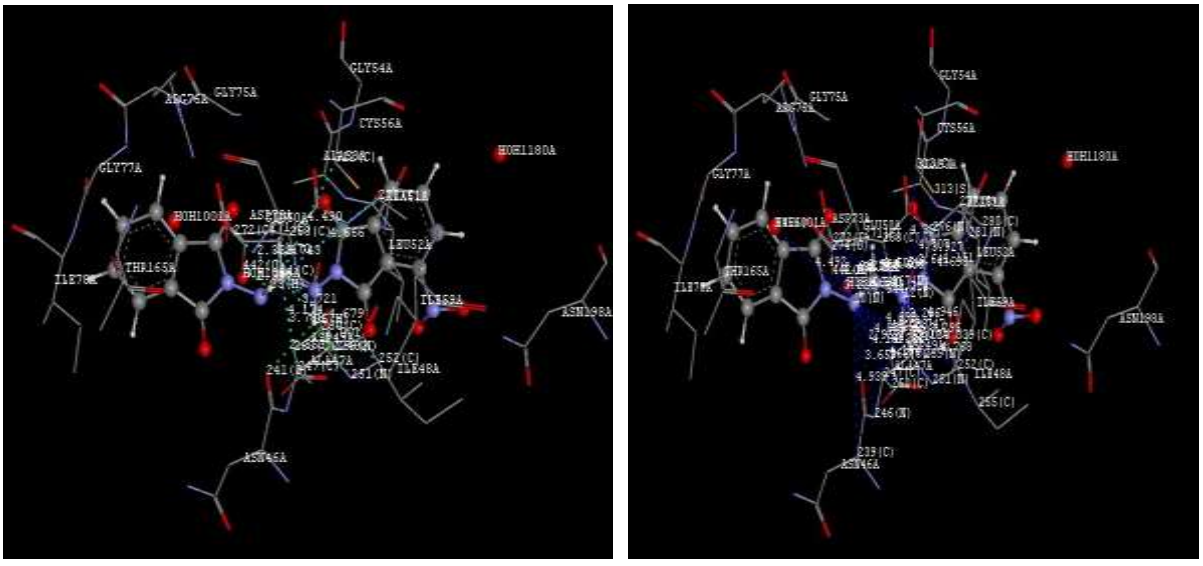
Yield 71% , m.p. 214-216°C, R*F* 0.59, IR (KBr) in (cm^{-1}) 3392.85 (N-H), 2975.25(C-H), 2800 (C-H), 1661.70 (C=O), 1539.22 and 1606 (C=C), 1382.02 (C-N), 3392.85(-NH_2), ^{1}H NMR (DMSO) (ppm), 7.80 -7.56 (m Ar – H), 1.77 (s- CH_2), 6.62 (-NH).

2, 2'-[Methylene-bis- (azanidyl)]-bis-(4- methyl isoindoline-1,3-dione) [K8H]

Yield 67%, m.p. 232-234°C, R*F* 0.68, IR (KBr) in (cm^{-1}), 3446.85 (N-H), 3000 (C-H), 2800 (C-H), 1654.95 (C=O), 1600 and 1400 (C=C), 1011.68 and 1036.76 (C-N), ^{1}H NMR (DMSO) (ppm), 8.379-7.146 (m Ar–H), 1.723 (s-CH_2), 2.205-2.243 (Ar-CH_3), 4.56(- NH).

Molecular docking

VLife MDS software 4.6 and free online software was used to perform all docking simulations which has shown the acceptable results. A set of new Bis-Phthalimides derivatives were subjected to docking with DNA gyrase subunit b (PDB ID: 1KZN), from the Protein Data Bank (http://www.rcsb.org/pdb). The 2D structures of the synthesized ligands (PMO, K1H, K2H, K5H & K7H) were drawn and converted to energy minimized 3D structures in the PDB file format. By removing the hetero atoms, water molecule and cofactors, the target protein file was prepared by leaving the associated residue with protein by using VLife MDS software 4.6., and docking results tabulated in table 1 and 2.



(a) (b)

Hydrophobic[a] and Charge Interactions [b] of K1H

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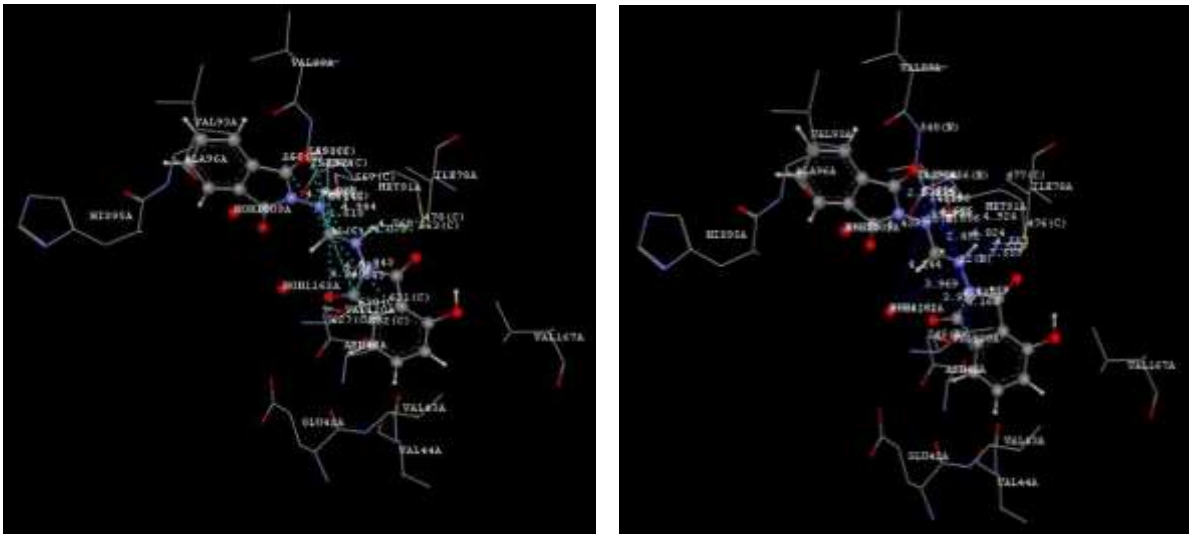
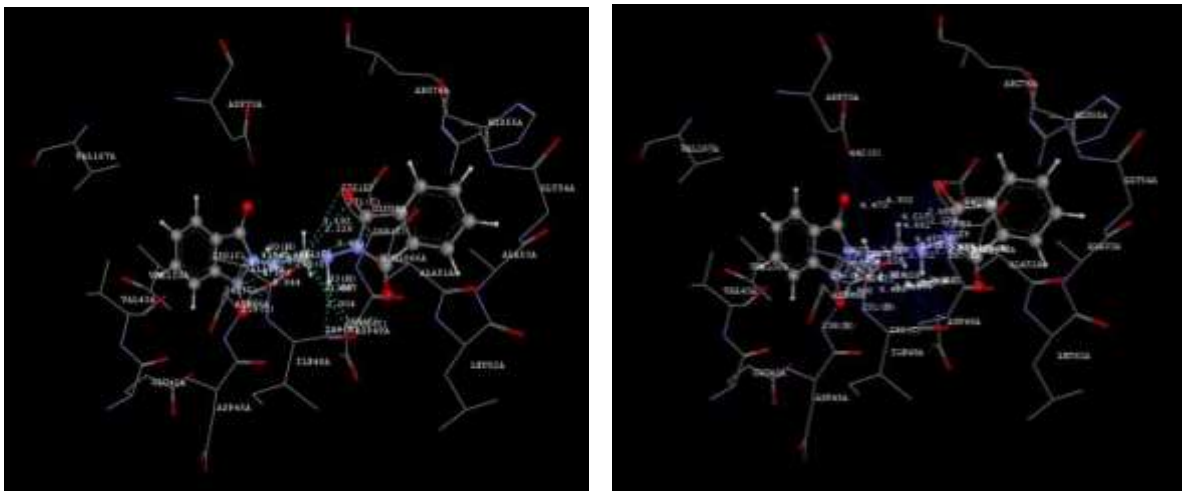


(a) (b)

Hydrophobic [a]and charge interactions[b] of K2H



(a) (b)

Hydrophobic [a] and charge interactions [b] of PMO

Table 1: Dock score with optimized energy of synthesized compounds

Sr. No.	Dock score (kj/mol)	Optimized energy
1.	-180.60	135.43
2.	-153.64	128.09
3.	-164.83	125.60
4.	-186.93	133.08
5.	-190.57	122.81

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Antibacterial activity

The antibacterial activity of synthesized compounds was evaluated against bacteria by using the MIC (double strain nutrient broth) and Amikacin was employed as standard drug. The results were tabulated in Table 3 and Table 4.

Table 2: MIC data of synthesized compounds

Sr. No.	Code	MIC at Conc. (µg/ml)
1.	K1H	40
2.	K2H	80
3.	K4H	60
4.	K5H	40
5.	K7H	40
6.	K8H	60
7.	PMO	60

Table 3: Zone of Inhibition of synthesized compounds

Sr. No.	Code	Concentration	*S. aureus*	*E. Coli*
1	**K1H**	40	6	7
		60	8	9
		80	11	12
		100	14	13
2	**K2H**	40	6	5
		60	7	6
		80	9	7
		100	9	8
3	**K4H**	40	6	5
		60	8	7
		80	9	8
		100	10	9
4	**K5H**	40	7	8
		60	9	11
		80	12	13

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Sr. No.	**Code**	**Concentration**	***S. aureus***	***E. Coli***
		100	17	16
5	**K7H**	40	6	9
		60	9	12
		80	11	13
		100	16	16
6	**K8H**	40	7	5
		60	8	7
		80	8	7
		100	9	8
7	**Standard**	40	10	11
		60	11	13
		80	13	14
		100	15	16

The antibacterial activity of synthesized derivatives was tested against *S. aureus* and *E. coli*. The compounds PMO, K1H and K5H were found to possess good antimicrobial activity against these microorganisms at concentration of 40 ug/ml. The compounds PMO, K1H and K5H were found to possess good zone of inhibition against *S. aureus* and *E. coli.*

The compounds (PMO, K1H, and K5H) shows good results in the form of dock score, hydrophobic bonding and Vander wall bonding against *DNA gyrase subunit*.

CONCLUSION:

The research study reported the efficient synthesis of novel bis-Phthalimide derivatives. All compounds were characterized by standard spectroscopic techniques and evaluation of the antibacterial activity of all new compounds was carried out and proved significant to moderate activity. These compounds also subjected to docking study and have shown good binding energies with the target protein which will be helpful for elaborating the mode of action.

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