Antibiofilm Activities of some Synthesized Hydrazones on Solid Surfaces

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***Abstract*- Hydrazones are interesting group of compounds possessing diverse biological and pharmaceutical properties such as antimicrobial, anti-inflammatory, antimalarial, antimycobacterial, antiviral, anticancer, antibiofilm activities, etc. The present study highlights the synthetic aspects of4-aminobenzoylhydrazones of3,5-dimethyl-2,6-diphenyl piperidin-4-one and 3-ethyl 2,6-diphenyl piperidin-4-one.The synthesized compounds are characterized by IR, NMR and Mass spectral studies. Formation of hydrazonecompounds were ascertained by IR,1HNMR and13C NMR spectralstudies and also molecular ion peak in mass spectra confirm the formation of hydrazones. Antibacterial,antifungal and antibiofilmpotential of the synthesized hydrazones are explored by agar well diffusion method and the measurement of zone of inhibition shows that they exhibit significant activity against tested bacteria and fungus.**

Key words: Antibiofilm, Biological activities, Piperidone derivatives

I. INTRODUCTION

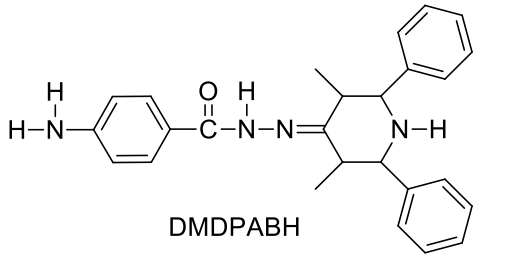
Interest in the study of microbial biofilms has increased greatly in recentyears due in large part to the profound impact biofilms have in clinical,industrial, and natural settings. Traditionally, the study of biofilms hasbeen approached from an ecological or engineering perspective; using acombination of classical microbiology and advanced microscopy.[1]What is a biofilm? This definition, by necessity, may be quite broad becauseit is clear that many organisms can attach to a variety of surfaces underdiverse environmental conditions. Therefore, in the context of this articlewe will operationally define a biofilm as bacteria that are attached to asurface in sufficient numbers to be detected macroscopically.

Hydrazones constitute important group of biologically active organic compounds which haveinfluenced the attention of chemist due to their vast applications in pharmacology.These compounds are synthesized as drug molecules to fight diseases with minimal toxicity and maximum efficiency. A number of hydrazones derivatives of aldehydes and ketones have been synthesizedand their biological activities like antimicrobial, antibiofilm,antitubercular,antitumoral, antimalarial activities are well reported in literature [(2-9)]. Heterocyclic compounds containingpiperidine skeleton are wellestablished group of compounds possessing interestingpharmacological activities and their wide occurrence in nature.Piperidines with aryl substituent at carbons 2 and 6 of piperidine ring have been reported as potent antimicrobial agent[(10-12)].

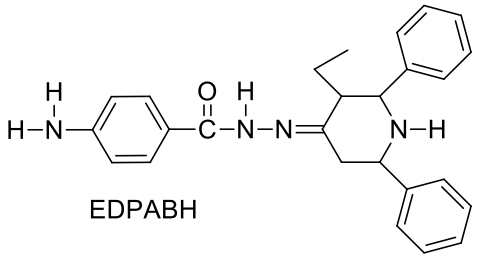
In the present study two new 4-aminobenzoylhydrazones derived from 3,5-dimethyl-2,6-diphenyl piperdin-4-one (DMDPABH)and3-ethyl-2,6-diphenyl piperdin-4-one(EDPABH) were synthesized.The formation of hydrazones is confirmed by means of IR,[1]H NMR,[13]C NMR and Mass spectral data.Antimicrobial potential testing is important for novel drug molecule discovery,epidemiology and antibiofilmoutcome. To assess antibacterial, antifungal and antibiofilmsusceptibility,the synthesized hydrazones are tested for activity against gram negative bacteria (*Escherichiacoli* and *Pseudomonas aeruginosa*), gram positive bacteria (*Bacillus subtilis and Staphylococcus aureus*), fungus*Candida albicans*and gram negative bacteria (*Pseudomonas aeruginosa*) respectively.

II. EXPERIMENTAL

All chemicals were reagent grade from Merck andwere used as such. IR spectra of the compound were recorded on BrukerTensor FT IR spectrometer using KBr pellets.[1]H and [13] C NMR were recorded onBruker Advance III 500 MHZspectrometer using $CDCl_3$ as solvent. Mass spectra were recorded on Jeol GC MATE II instrument. The heterocyclic ketones 3,5-dimethyl-2,6-diphenyl piperidin-4-one and 3-ethyl-2,6-diphenyl piperidin-4-one were prepared as per the procedure reported by Baliah[(13).] The hydrazones were prepared by refluxing 4-Aminobenzoic acid hydrazide(10 mmol) and appropriate ketone(10 mmol) in ethanol for 4 hrs and the resulting solution was kept overnight. The resultant solid compound was filtered, washed,dried and recrystallised from ethanol.The hydrazones are insoluble in water and soluble in ethanol,methanol,DMF, DMSO and Chloroform.The Yield was about 60%.The melting point was recorded in an open capillary and it was uncorrected.



DMDPABH



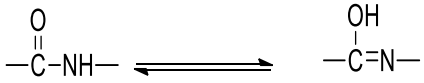
EDPABH

The synthesized compounds were tested for antibacterial,antifungal and antibiofilm activitiesby Well diffusion method as reported by *Perez et al* [14] using Muller Hinton Agar medium for bacteria and Sabourad Dextrose Agar for fungus.The medium was prepared and autoclaved at 15lbs.The medium was cooled to 50-55ºC and poured into sterile Petridish to a uniform depth of 4 mm which is equivalent to approximately 25- 30mL on 90 mm plate.Once the medium was solidified,standardized bacterial or fungal suspension was swabbed on the medium within 15 min of adjusting the density of the inoculums.The plated were undisturbed for 3 to 5 min to absorb the excess moisture.Sterilized 9 mm cork borer was used to make agar well and DMSO as a control. Tetracycline(30µg/ml) for bacterial and Amphoterecein-B (30µg/ml) for fungi suspended in sterile glass distilled water used as positive control.It was incubated for 24hrs at 37ºC.After incubation period, zone of inhibition were measured by 1 mm accuracy.The diameter of zone of inhibition denotes qualitatively about the antimicrobial potential of the compounds.

III. RESULTS AND DISCUSSION

The HydrazonesDMDPABH and EDPABH were prepared by refluxing the appropriateheterocyclic ketones and 4-aminobenzoic acidhydrazide.The physical data of the hydrazones are summarized in Table-I. The main absorptionfrequencies inthe IR spectra and NMR chemical shifts data are listed inTable-II and Table-IIIrespectively.In the IR spectra, bands appearing at 1703and 1608 cm^{-1} in DMDPABH and1705 cm^{-1} and 1606 cm^{-1} in EDPABH are attributed to amide I vibrations.Bands occurring at 1538 $cm^{-1}$and 1497 $cm^{-1}$are due to υ C=N.Bands at 1499 and 1457 cm^{-1} are due to Amide II and δNH.Amide III vibrations are located at 1276 cm^{-1} and 1289 cm^{-1}[15].

^{1}H NMR spectra of hydrazones ofDMDPABH and EDPABH show signal at 7.45and 7.67 ppm attributed to amide proton (-CONH-). The protons in $–NH_2$ resonate at 6.7 ppm.The peaks observed as multiplets at 7.2 -7.6 ppm can be assigned to aromatic protons.In^{13}C NMR spectra,the aromatic carbons are identified by their characteristic absorption around128 ppm.The *ipso* carbons should absorb at downfield (around 140 ppm) compared to other carbons.The absence of signal around 200 ppm in EDPABH reveals it exists as equilibrium mixture of amido and imidol forms in solution.



The signals at 150 ppm are due to C4 in the heterocyclic ring and correspond to (>C=N).The alkyl carbonsappearat 10.5ppm and 11.7ppm in DMDPPABH and EPABH [13] respectively.Mass spectral analysis of hydrazones shows m/z at 412 and is of M^{+} and they correspond to molecular weight of the synthesized compounds.

The antibacterial and antifungal activities of the synthesized hydrazoneswere assayed against two gram negative stain (*Escherichiacoli* and *Pseudomonas aeruginosa*),two gram positive bacteria (*Bacillus subtilis and Staphylococcus aureus*) and fungi (*Candida albicans*)by agar well diffusion method at different concentrations (1000, 1500 and 2000µg/ml in DMSO. The standard used was Tetracycline (30µg/ml) for antibacterial and Amphotericin-B(30µg/ml) for antifungal studies. The antibiofilmactivity of the synthesized hydrazones was assayed against one gram negative stain (*Pseudomonas aeruginosa)* by agar well diffusion method at different concentrations (500, 250 and 125µg/ml) in DMSO.Solvent control was also performed to know the activity of the solvent.

The activity was measured by measuring the diameter of the inhibited zone in mm and it is furnished in Table-IVand Table-V.The activity increases with increase in concentration of the test solution.Both the hydrazonesshow more activity towards *Pseudomonas aeruginosa* at higher concentration and exhibit significant and similar activity towards all tested bacteria.DMDPABHandEDPAH show good activity towards *Candidaalbicans*.Zone of inhibition increases with the concentration and DMDPABH ismore active when compared with EDAPBH. Both the synthesised compounds show very good biofilm activity with increase in concentration of the compounds.

IV. SUMMARY:

The two new amino benzoyl hydrazones of3,5-dimethyl-2,6-diphenyl piperidin-4-one and 3-ethyl-2,6-diphenyl piperidin-4-one synthesized show significant antibacterial, very good antifungal and excellent antibiofilm activities.

TABLE - I
Physical and m/z(Mass spectral) data of Hydrazones

Compound	Molecular formula	Colour	Yield %	Melting point ºC	m/z value
DMDPABH	$C_{26}H_{28}N_4O$	Pale brown	60	120-121	412 (M+)
EDPABH	$C_{26}H_{28}N_4O$	Pale yellow	60	209-211	412(M+)

TABLE - II
Infrared spectral data of Hydrazones (cm^{-1})

Compounds		Assignment
DMDPABH	EDPABH	
3427,3356,3230	3306,3030	υ_{N-H} of ring and amide NH and NH_2
1703,1608	1705,1606	Amide I band
1538	1497	=C=N-(azomethine)
1499	1457	Amide II and δ N-H
1450	1457	Aromatic C-C skeletal vibration
1276	1289	Amide III band.

TABLE-III
NMR Spectral data of Hydrazones

	^{1}H NMR chemical shifts in ppm				^{13}C NMR chemical shifts in ppm						
Compound	Aromatic ring protons	NH_2	Piperidin ring protons	Methyl Protons	Amide Carbon	C2	C3	C4 (C=N)	C5	C6	Methyl
DMDPABH	7.4-7.2	-	3.6-2.7	1.2	211.2	68.9	-	142.0	29.7	-	10.5
EDPABH	7.6-7.2	6.6	4.1-2.5	1.2	-	63.3	40.4	150.0	29.6	61.1	11.7

TABLE IV
Antimicrobial activity ofsynthesized compounds

Microbes		Conc. µg/ml	Zone of Inhibition (mm)		
			DMDPABH	EDPABH	Standard#(30 µg/ml)
Gram negative bacteria	*Escherichiacoli*	1000	-	10±0.70	Resistant against the drug
		1500	10±0.70	12±0.84	
		2000	13±0.91	14±0.98	
	Pseudomonas aeruginosa	1000	11±0.77	14±0.98	27.00±1.00
		1500	13±0.91	15±1.05	
		2000	18±1.26	19±1.33	
Gram positive bacteria	*Bacillus subtilis*	1000	-	-	26.33±0.57
		1500	-	-	
		2000	15±1.05	13±0.91	
	Staphylococcus aureus	1000	10±0.70	-	26.00±1.00
		1500	14±0.98	10±0.70	
		2000	17±1.19	14±0.98	
Fungus	*Candida albicans*	1000	15±1.05	14±0.98	18±1.26
		1500	20±1.4	15±1.05	
		2000	22±1.54	20±1.4	

Tetracycline for bacterial andAmphotericin-B for fungi.

TABLE-V
Antibiofilm activity of synthesized compounds

Microbe		Conc. µg/ml	Inhibition of Bio-film formation(mm)	
			DMDPABH	EDPABH
Gram negative bacteria	*Pseudomonas aeruginosa*	125 µg/mL	63.21±4.42	56.43±3.95
		250 µg/mL	75.28±5.27	75.01±5.25
		500 µg/mL	99.32±6.95	90.94±6.37

REFERENCES

[1] J. W. Costerton, Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott,*Annu. Rev. Microbiol*. 49, 711 (1995).
[2] O'Toole, G. A., Pratt, L. A., Watnick, P. I., Newman, D. K., Weaver, V. B. and Kolter,
[3] R. 1999. *Genetic approaches to study of biofilms*. Methods Enzymol.,310: 91-109.
[4] Sevim Rollas and Guniz Kuculguzel.S, *Molecules*, 12(2007) 1910
[5] Singh. M and Raghav. V , *International Journal of Pharmacy and Pharmaceutical sciences*3 (4), (2011) 975
[6] Lakshmi Narayana.S, Young Kyo Seo, Sung Ok Baek ,Varada Reddy A, *E. Journal of Chemistry* ,9(3) (2012)1288
[7] MdRahmat Ali and Akranth Marella, Md. Tauquir Alam, Ruskar Naz, Mymoona Akhter, Md. Shaquiquzzamam, Rikta .S, Omprakash. T, Md. Mumtaz Alam, Jyoti Hooda, *Indonesian J.Pharm*. 23,No 4(2012)126
[8] Padmini K, Jayapreethi P ,Divya M, Rohini P, Lohita. M, Swetha. K, Kaladar. P *International Journal of Pharma Research and Review*.2(8) (2013)43
[9] Riyaz Ali Osmani, Rahul L Jadhavetal,American Journal of Advanced Drug Delivery (1)5 (2013)724.
[10] Praveen Kumar, Amit Rai, Mahendrasingh, Durgesh Kumar, Anil Kumar.S and Vinit Raj *Pharmaceutical Science*2.3(2016)278
[11] Natesh Rameshkumar, Anantharaman Veena, RajuI lavarasn, Mandaleeswaran Adiraj, Shanmugapandian P, Seshaih Krishnan Sridhar.*Bio.PharmBull*.26(2) (2003)188
[12] Murugaesan Srinivasan, Subbu Perumal, Sangavanaicker Selvaraj, *Chem. Pharm. Bull* 54 (6)(2006)795
[13] Greeshma.K. P and Muthulingam S,*International Journal of Pharmaceutical sciences and Research*, Volume 8(2017)1967
[14] NollerC.R.,BaliahV,*J.Am.Chem.Soc* .,70(1948)3853
[15] Perez C.,Pauli.M and Bazarque P., *Acta.Biol Med. Exp.*,15 (1990)113
[16] Manimekalai A, SenthilSivakumar B and Maruthavanan .T,*Indian Journal Of Chemistry*, 43B(2004)1753