DOCUMENTATION OF SIGNIFICANT HETEROCYCLIC LIGAND WITH SPECIFIC MICROBIAL ACTIVITY

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ABSTRACT

Thirty-one differently substituted pyrimidine bases were tested for their inhibitory effect on the glucuronidation of 4-nitrophenol and phenolphthalein by rat liver microsomes. 4,6-dihydroxy-5-nitropyrimidine were the most potent and selective inhibitors of 4-nitrophenol glucuronidation, without any effect on the phenolphthalein conjugating activity of UDP-glucuronyltransferase (UGT). It becomes a challenge for the society to find new chemical entities which can treat microbial infections. The present review aims to focus on account of important chemical moiety, that is, pyrimidine and its various derivatives as antimicrobial agents. In the current studies we represent pyrimidines as antimicrobial agents with different mono-, di-, tri-, and tetrasubstituted classes along with in vitro antimicrobial activities of pyrimidines derivatives which can facilitate the development of more potent and effective antimicrobial agents.

KEY WORDS: 7-amino-2,4,10-trihydroxy-9,9-dimethyl-5-nitrosopyrimidine(ATDNP),

Oxidative polycondensation, thermal, conductivity sand microbiological sampling.

INTRODUCTION

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Of the 2 million people who acquire bacterial infection in US hospitals each year, 70% of cases now involve strains that are resistant to at least one drug [1]. In communities and hospitals around the world, the number of patients with antibiotic-resistant infections continues to climb [2]. A major cause for concern in the UK is methicillin-resistant Staphylococcus aureus (MRSA), which was at low levels a decade ago but now accounts for ca. 50% of all S. aureus isolates [3]. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted. The causes of antimicrobial resistance are multifactorial. In case of an antibiotic, it has been well documented that resistance is mainly caused by continued overreliance on and imprudent use of these antibacterial agents [4] and increasing evidence is being obtained suggesting that the same may be true for the emergence of biocide resistance [5-6]. Of particular concern is the possible cross-resistance of antibiotics and biocide due to common resistance mechanism [7-8]. Metal resistance is being observed as the result of polluted environments [9-10]. The consequence of continued exposure to antibacterial environment is an enrichment of bacteria that are intrinsically resistant to antimicrobials or have acquired resistance mechanism to these substances [11-12].
Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics [13-14]; hence, they have attracted considerable attention in the design of biologically active molecules [15-16] and advanced organic chemistry [17-18]. Also in the family of heterocyclic compounds nitrogen containing heterocycles are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes [19].

However, the current review intends to focus on the significance of pyrimidine class of antimicrobial agents along with clinical and in vitro applications of pyrimidine derivatives to facilitate the development of more potent as well as effective antimicrobial agents.

EXPERIMENTAL

Materials and Methods: All materials were of analytical grade and were used as such. Sodium hypochloride (NaOCl) (30%) aqueous solution was used.

Analytical procedures : Elemental analysis was carried out with standard procedures[20]. The ultraviolet visible spectra were measured by a Perkin Elmer Lambda UV Chemito Spectroscan UV 2600.The infrared spectra were measured by a Perkin Elmer FT-IR spectrum one (4000-550cm-1). ^{1}HNMR and ^{13}C-NMR spectra (Bruker Avance DPX-400 and 100.6MH_3 respectively) were recorded at 25°C by using deuterated DMSO as a solvent.

Synthesis of ATDNP: ATDNP was prepared by the condensation of 2,2 – dimethyl-3-hydroxypropanldehyde (1.38g, 0.01 mole) with 4-amino-2,6-dihydroxy-5-nitrosopyridine (0.98g, 0.01mole) in ethanol (25ml) and aqueous potassium hydroxide, achieved by heating the mixture in microwave conditions. The Precipitate was collected, recrystallised from methanol and dried (yield – 70%). ^{1}HNMR (DMSO); 13.50 (S, 1H – OH) ; 9.35 (S, 1H – CH = N) ; 6.64 (d, 1H – H_a) ; 8.61 (d, 1H – H_b) ; 7.90 (q, 1H– H_c). **C^{13} NMR (DMSO):** 70-100(N=C-H);142(C=C); 160-185(C=C-OH); 58(CH_2OH).

Synthesis of ATDNP- metal complexes : Solutions of M$(OAc)_2$.x H_2O (M=Rh,Ru,Ir,Li) ; (x= 4,1,3,2,): (1m mol) in CH_3CH_2OH (10 ml) were added to a solution of ATDNP (2m mol/unit) in THF (20 ml). The mixture was stirred and heated at 70°C for 8min. The precipitated complex was filtered, washed with cold methanol / THF (1:1) and dried in oven at 110°C (Table 1).

Table 1: Elemental analyses data and yield of metal complex compounds.

Compound	Calculated(found)				Yield	Colour
	C	H	N	Metal		
ATDNP	45(46)	5(5.67)	23.33(25)	-	70%	Brown
ATDNP –Rh	31.48(33)	3.49(4)	16.32(17.4)	30.02(32)	62%	Pale Brown
ATDNP –Ru	31.67(33.07)	3.51(4.02)	16.42(17.8)	29.61(30.05)	67%	Yellowish brown
ATDNP –Ir	25(26.03)	2.77(3)	12.96(14)	44.44(45)	59%	Dark yellow
ATDNP –Li	43.72(44)	4.85(5)	22.67(23)	2.83(2.98)	52%	White

Results and Discussion:

(a)Solubility: (i) ATDNP was brown colored crystalline, soluble in methanol , ethanol, acetone, DMF and DMSO.

(ii) Metal complexes were insoluble in any of the selected solvents.

(b) Spectral: (i) UV-vis (methanol and DMSO) of ATDNP (Fig.1).

(ii) In the FT-IR spectra of ATDNP bands of – OH and – CH=N groups were observed at 2795 and 1654cm^{-1} respectively (Table 2).

(iii)In the ^{1}H-NMR, the signals of phenyl – OH and –CH=N groups were obtained at 13.50 and 9.30 ppm and 11.55, 9.94 ppm respectively.

(iv)^{13}C-NMR: 218(C-C=O); 150(C_5H_4N); 87(N=C-H);125(C=C); 177(C=C-OH).

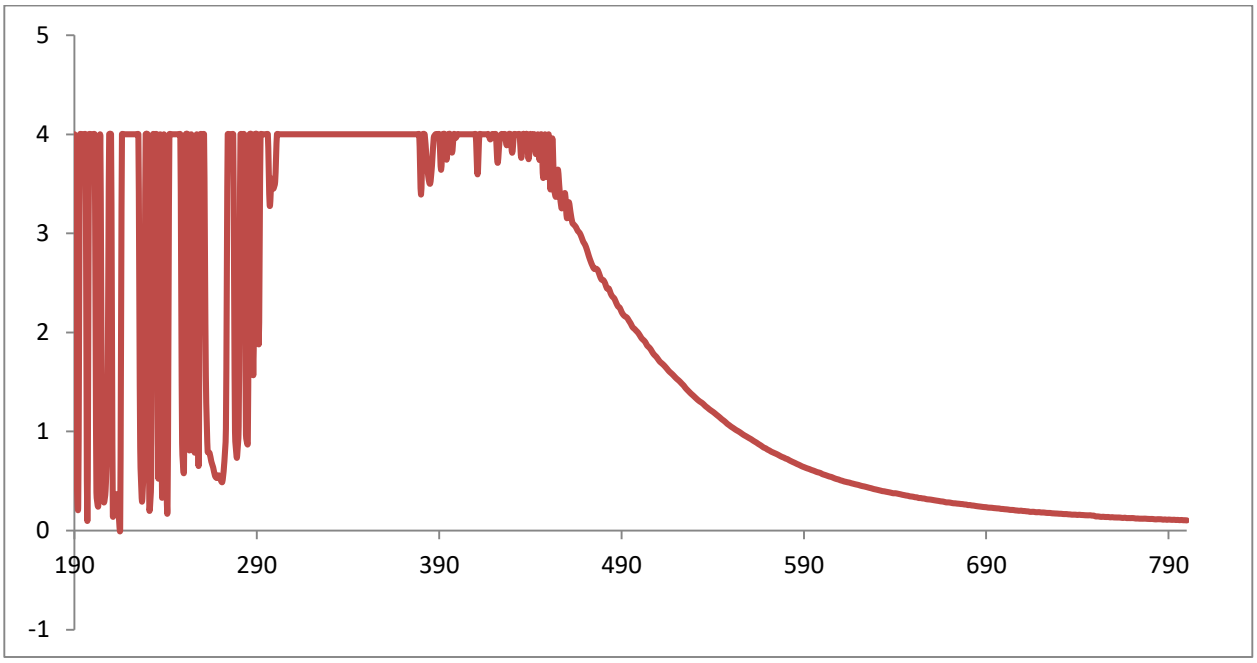


Fig.1: UV-vis (methanol and DMSO) of ATDNP

Table 2: FT-IR data of ATDNP and its metal complexes.

Compound	Wave numbers (cm^{-1})				
	-OH	Ar-C-H	-CH=N	-C=C	M-N
ATDNP	2795	2899	1654	1587	700
ATDNP –Rh	2790	3056	1632	1570	679
ATDNP –Ru	3262	3059	1646	1580	687
ATDNP –Ir	3274	3062	1648	1568	685
ATDNP –Li	3270	3025	1627	1560	683

Medicinal Properties of Ligand

The presence of pyrimidine base in ligand (ATDNP) is one possible reason for their widespread therapeutic applications. The pyrimidines represent one of the most active classes of compounds possessing wide spectrum of biological activities like significant in vitro activity against unrelated DNA and RNA, viruses including polioherpes viruses, diuretic, antitumour, anti-HIV, and cardiovascular [21]. The result indicated that a wide range of pharmacological activities are exhibited by the compounds. In addition to this, various analogs of pyrimidines have been found to posses antibacterial [22–23], antifungal [24], antileishmanial [25], anti-inflammatory [26], analgesic [27], antihypertensive [28], antipyretic [29], antiviral [30], antidiabetic [31], antiallerggic [32], anticonvulsant [33], antioxidant [34], antihistaminic [35], herbicidal [36] and anticancer activities [37].

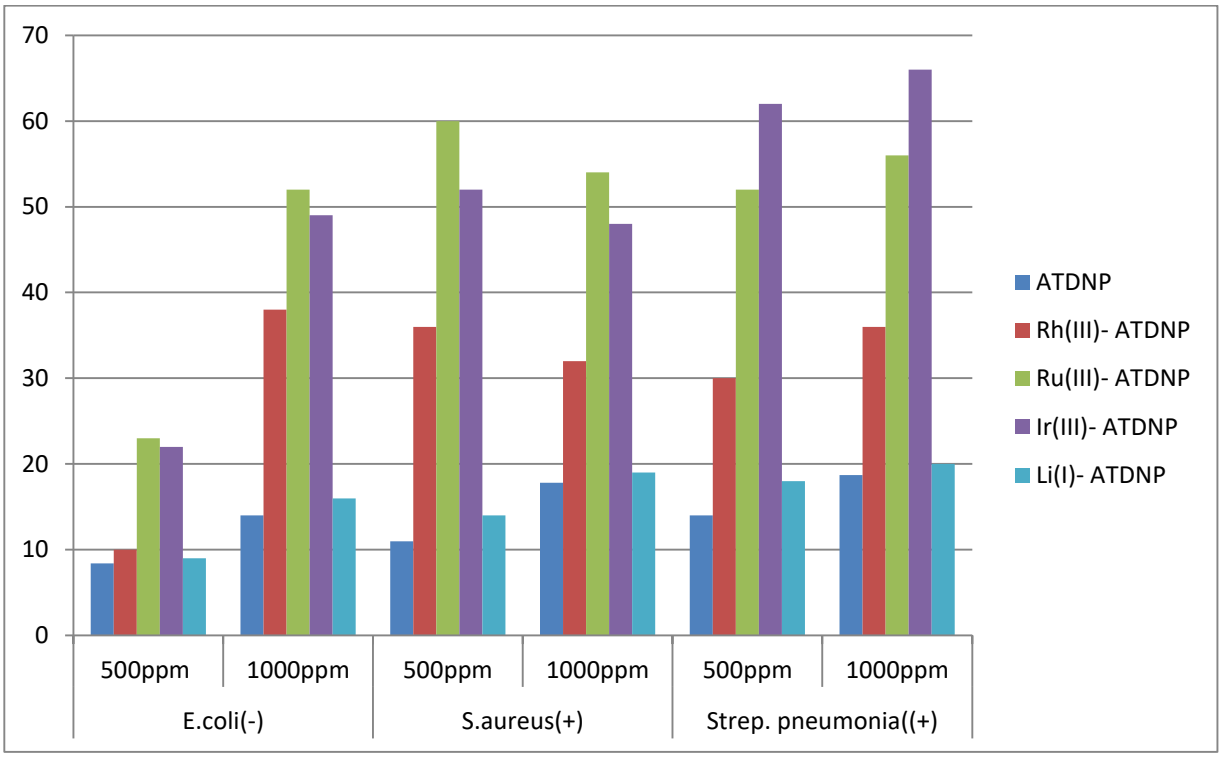
Microbail Screening :

Antibacterial and Antifungal Activity: To carry out the determination of MIC, a microbiologic suspension was prepared by diluting cultures incubated for24 hours (bacteria) and 48 hours (fungi) with sterilized physiological solution. For the standardization of the microbiological suspension we used a spectrophotometer (λ= 580nm), where the above mentioned suspension was diluted with sterilized physiological solution (0.9% NaCl) until we obtained a 25% transmittance reading.

Table 3- Antibacterial analysis of the ligand and its complexes.

compound	E.coli(-)		S.aureus(+)		Strep. pneumonia (+)	
	500ppm	1000ppm	500ppm	1000ppm	500ppm	1000ppm
ATDNP	8.4	14	11	17.8	14	18.7
Rh(III)-ATDNP	10	38	36	32	30	36
Ru(III)-ATDNP	23	52	60	54	52	56
Ir(III)-ATDNP	22	49	52	48	62	66
Li(I)- ATDNP	9	16	14	19	18	20

Fig.2: Antibacterial analysis of the ligand and its complexes.



CONCLUSION

In nut shell, a convenient and efficient route for the synthesis of ATDNP with no side reactions and satisfactory isolation possessed. Significant antibacterial and antifungal properties decide its use as a therapeutic agent.

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