Antimicrobial Efficacy of Metal-Barbiturate Conjugates Against Pathogenic Strains of *Escherichia coli* and *Staphylococcus aureus*

Ashfaq Mahmood Qureshi*[,1], Mehmood Qadir[2], Abdul Rauf[4], Muhammad Idrees[2], Siara Mumtaz[1], Muhammad Najam-ul-Haq[1], Aziz-ur-rehman[4], Muhammad Ismail[1], Muhammad Athar[1], Rashid Khushal[3], Sara Riaz[1] and Habib Bokhari*[,2]

[1]*Department of Chemistry, Baha-uddin Zakriya University, Multan, Pakistan*

[2]*Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan*

[3]*Astra Zeneca, Leicester, UK*

[4]*Department of Chemistry, The Islamia University, Bahawalpur, Pakistan*

Received April 11, 2011: Revised July 27, 2011: Accepted August 12, 2011

Abstract: Resistance to commercially available antimicrobials is increasing at alarming rate therefore there is an urgent need of new antimicrobial compounds and materials with novel modes of action to tackle the problem of resistance in pathogenic gram positive and gram negative bacteria. The current study involves the design and synthesis of new molecular structures which should not resemble the basic molecular structures of the existing classes of antimicrobial agents in order to avoid the risks of cross resistance development. A total of 14 metal-barbiturate complexes (newly synthesized compounds) were evaluated for their antimicrobial properties against multiple drug resistance clinical isolates of S. aureus and E. coli. Broth dilution method was used to determine minimum inhibitory and bactericidal activity of the newly synthesized compounds. The results showed that the metal-barbiturate complexes with cobalt ions were more effective against E. coli and S. aureus as compared to other compounds. Moreover the activity of these compounds was higher against S. aureus as compared to E. coli which indicates species specific variable antimicrobial effects of these compounds.

Keywords: Antimicrobial, Barbiturate Complexes, Drug resistance, *E. coli*, *S. aureus*.

INTRODUCTION

Antimicrobial resistance is one of the major threats to the life quality of human beings as well as animals. Since the discovery of first antibiotic, the new antibiotics have been discovered but the incidence of antimicrobial resistance in the bacteria has also been reported continuously [1-2]. Hence, continuous burden of infectious diseases and emergence of drug resistance is a global issue [3-4]. The resistance to commercially available antimicrobials is increasing day by day by various bacteria [5]. Therefore, there is an urgent need of new compounds with new modes of action and combination therapies to tackle the problem of resistance in gram positive and gram negative bacteria [6].

Staphylococcus aureus is a gram-positive bacterium which colonizes the skin and is present in nares of 25-30% of healthy population [7] and is one of the most common causative agents of nosocomial infections worldwide. Staphylococcal infections are of particular concern because of the causative bacteria offering resistance to a wide range of commonly used antibiotics. On the other hand *E. coli* have certain well known resistance determinants therefore they are used as indicator bacteria internationally for antimicrobial resistance [8-10].

*Address correspondence to these authors at the Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan; Tel: 0092-3005127684; Fax: 0092-514442805; E-mail: habib@comsats.edu.pk

Department of Chemistry, Baha-uddin Zakriya University, Multan, Pakistan; Tel: 0092-3064782968; Fax: 0092619210138; E-mail: shamashfaq@yahoo.com

Barbiturates are a class of drugs that are used as anesthetics and sleep inducing agents [11]. However, some previous studies have already shown that barbiturate such as methohexitone (1%) and thiopentone (2.5%) are bactericidal against coagulase-negative staphylococci [12-13]. Moreover, thiopentone (2.5%) has already shown bactericidal activity against *E. coli* and *P. aeruginosa* [14].

Pathogenic bacterial organisms on the other hand under constant exposure to antibacterials are evolving rapidly and emerging as more resistant entities to existing antimicrobial agents [5, 15]. Therefore it is envisaged that new compounds with new modes of action and combination therapies are required to tackle the problem of resistance in gram positive and gram negative bacteria [6].

Structurally similar antimicrobials are likely to face the similar fate i.e. resistance to one may result in resistance to others. In order to combat the development of cross resistance, we intended to explore the basic ring structures different from existing classes of antimicrobial compounds. So we used barbiturate as the basic ring structure with metal-barbiturate complex formation at various positions of the basic ring in order to explore the best suitable metal ligand or position on the ring to show maximum antimicrobial activity. In this way we can counteract the phenomenon of cross resistance by exploring the chemical formulas and ring structures dissimilar to the existing antimicrobial compound structures. For instance, PNU-286607, a barbiturate derivative displays little cross-resistance with marketed antibacterial agents and is active against methicillin-resistant

1570-1808/11 $58.00+.00

staphylococcus aureus (MRSA) and fluoroquinoline-resistant bacterial strains [16]. Some other examples of related compounds are thioxanthene flupenthixo Prochlorperazine, and fluphenazine [17-18]. These are different antipsychotic compounds which have already been found to be effective as antimicrobial agents by their *in vitro* studies.

The objective of the current study was to evaluate the efficacy of newly synthesized metal-barbiturate complexes against resistant bacterial isolates. Antimicrobial effects of some of the newly synthesized barbiturate compounds (metal-barbiturate conjugates) against common pathogens including gram negative bacteria (multiple drug resistant *E. coli* causing Urinary Tract Infections) and gram positive bacteria (multiple drug resistant *S. aureus* causing skin infections) were identified.

MATERIAL AND METHODS

Synthesis of Compounds (1-3)

All chemicals used in this study are purchased from Sigma-Aldrich (Czech Republic). Synthesis of all ligands was done according to Scheme **1**. In a round bottom flask, fitted with reflux condenser, a mixture of equimolar barbituric acid and aromatic aldehyde was refluxed in absolute ethanol for about 4 hrs to overnight. After the formation of precipitates, the reaction mixture was cooled and filtered off. The precipitates were washed several time with hot ethanol, dried in an oven and subjected for further characterization i.e., m.p (John Fisher melting point apparatus), I.R, ^{1}HNMR, ^{13}C NMR (Jan19 NMR spectrometer, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C) and mass spectrometry (on JEOL JMS600 instrument).

5-(5-hydroxy-2-nitrobenzylidene)pyrimidine-2,4,6(1H,3H, 5H)-trione *(1)*

Light grey powder, 61%; m.p 260 ^{o}C; $_{max}$(ν KBr)/cm^{-1} 1220 (C-N str), 1425 (C=C str), 1500 (-NO_2 antisymmetric str), 1610, 1710 (C=O str), 3050 (=C-H str), 3069 (-N-H str), 3300 (-OH str); δ_H(300 MHz; DMSO) 6.78 (1H, d, *J* 2.1, Ar*H*), 6.95 (1H,dd, *J* 9.1, 2.5, Ar*H*), 8.15 (1H, d, *J* 9.1, Ar*H*), 8.52 (1H, s, C*H*=C), 11.12 (1H, s, N*H*), 11.19 (1H, s, N*H*), 11.45 (1H, s, O*H*); ^{13}CNMR(d_6-DMSO, δ, ppm): 116.1 (C), 116.9 (CH), 119.7 (CH), 125.4 (CH), 130.8 (C), 137.3 (C), 146.0 (CH), 149.8 (C), 152.0 (C=O), 161.3 (C=O), 164.2 (C=O); *m/z* (EI) 277.9 (1), 230 (100), 217.9 (17), 187.8 (100), 174.9 (27), 160 (24), 148.9 (75), 135 (29), 127.8 (68), 119 (33), 105 (39), 92 (34), 77 (20), 63 (50), 51 (13).

5-(4-methoxybenzylidene)pyrimidine-2,4,6(1H,3H,5H)-trione (2)

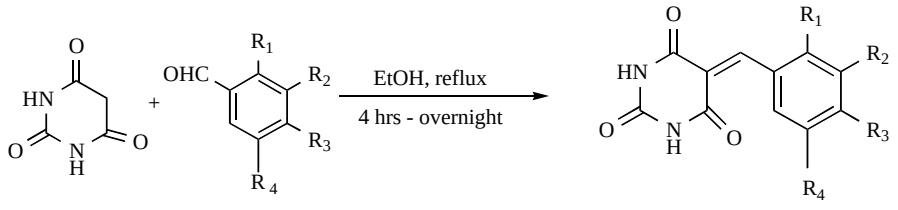
Orange yellow powder, 78%; m.p 255 ^{o}C; $_{max}$(ν KBr)/cm^{-1}1212 (C-N str), 1507 (C=C aromatic str), 1600, 1674, 1730 (C=O str), 2840 (C-H aliphatic str), 3071 (=C-H str), 3208 (N-H str); δ_H(300 MHz; DMSO) 3.86 (3H, s, OCH_3), 7.05 (2H, d, *J* 9, Ar*H*), 8.24 (1H, s, C=C*H*), 8.36 (2H, d, *J* 9, Ar*H*). 11.16 (1H, s, N*H*), 11.28 (1H, s, N*H*); 66.2 (CH_3), 118.0 (C), 118.6 (CH), 118.8 (CH), 123.5 (CH), 123.8 (CH), 125.0 (C), 148.7 (CH), 151.7 (C), 153.1 (C=O), 164.0 (C=O), 164.1 (C=O); *m/z* (EI) 246 (100), 245 (87), 231 (10), 215 (20), 202 (65), 172 (13), 159 (19), 132 (17), 117 (18), 89 (25), 61.9 (11).

5-(3-bromobenzylidene)pyrimidine - 2, 4, 6 (1H, 3H, 5H)-trione (3)

Bright yellow powder, 20%; m.p 247 ^{o}C; $_{max}$(ν KBr)/cm^{-1} 516 (C-Br str), 1274 (C-N str), 1577 (C=C str), 1678, 1704, 1752 (C=O str), 3098 (=C-H str), 3372 (N-H str); δ_H (300 MHz; DMSO) 7.41 (1H, t, *J* 7.9, Ar*H*), 7.69 (1H, d, *J* 8.8, Ar*H*), 7.88 (1H,d, *J* 7.9, Ar*H*), 8.21 (1H, s, Ar*H*), 8.28 (1H, s, C=C*H*), 11.26 (1H, s, N*H*), 11.41 (1H, s, N*H*); 116.4 (C), 117.6 (CH), 125.3 (C), 128.0 (CH), 128.2 (CH), 128.5 (CH), 146.4 (CH), 154.8 (C), 155.0 (C=O), 162.0 (C=O), 162.4 (C=O); *m/z* (EI) 294.9 (83), 251.9 (40), 224.9 (7.4), 215 (100), 172 (75), 155 (4), 128 (18.5), 116 (16), 101 (43), 89 (21), 78 (64), 75 (32), 61.9 (85).

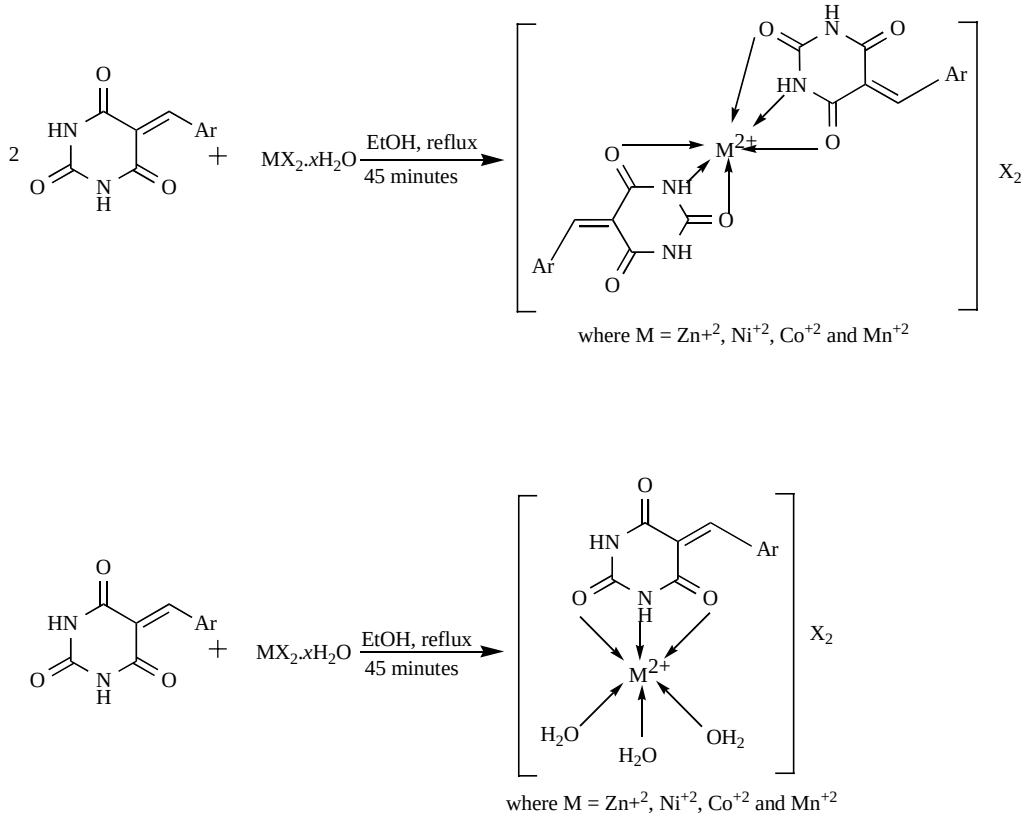
Synthesis of Compounds (4-14)

Barbituric acid can act as an alterdentate ligand as it has more than one equivalent coordination sites available to a metal ion. In this regard rearrangement is possible in which metal is transferred from one site to another. Three barbiturates used in this study for preparation of metal-barbiturate complexes are compounds **1-3**. All the complexes of barbiturate were prepared in two different molar ratios of metal: ligand i.e., 1:1 and 1:2 by dissolving ligand and metal salt separately in minimum amount of ethanol. Then the two solutions were mixed followed by addition of small amount of ammonia in a round bottom flask and refluxed for about 45 minutes. The precipitates formed were filtered, washed with hot ethanol, dried in an oven at 50 ^{o}C and characterized by IR and UV and then subjected to biological activity.



Where (**1**) = R_1 =NO_2, R_2, R_3 =H and R_4 =OH
(**2**) = R_1 = R_2 =R_4 = H and R_3 =OCH_3
(**3**) = R_1 = R_3 = R_4 = H and R_2 =Br

Scheme 1. Preparation of Barbiturate Ligands used for complexation.



Scheme 2. Preparation of metal barbiturate complexes.

Antimicrobial Studies

Bacterial Strains

All clinical isolates used in the present study were isolated from patients in the hospital. All chemicals were purchased from Sigma unless otherwise stated.

E. coli

The strains were isolated from Urine samples collected from Urinary Tract infection patients. Urine samples were immediately cultured on CLED (cysteine lactose electrolyte–deficient) agar. The bacterial growth was subcultured on MacConkey agar. Lactose- and indole-positive colonies were presumptively identified as *E. coli.* The antimicrobial resistance profiles for *E. coli* isolates were determined by standard disk diffusion techniques (Neo-Sensitabs; Oxoid Ltd), according to the recommendations of CLSI (Clinical Laboratory Standards Institute) whereas virulence factors profile was also determined as described in our previous study [19]. The clinical strain used in this study was resistant to Nalidixic acid, Ceftazedime, Ciprofloxacin, Sulphamethoxazole Trimethoprim and Ampicillin.

S. aureus

The clinical samples from patients suffering from multiple skin diseases were obtained from the skin lesions for the isolation of *S. aureus* with the help of sterile swabs which were then placed back in the sterile Mannitol Salt Broth and transported to the laboratory. Samples were then inoculated on to Mannitol Salt Agar for the selective growth of *S. aureus*. *S. aureus* isolates were then subjected to disk diffusion method for susceptibility pattern. The strain was resistant to Nalidixic acid, Sulphamethoxazole-Trimethoprim, Ampicillin, Trimethoprim. The multidrug resistant strain of *S. aureus* was selected for antimicrobial assay.

Antimicrobial Assay

Broth dilution method was used to determine minimal concentration of newly synthesized compounds to inhibit or kill the microorganism. LB broth was prepared by adding 2g of media in 100ml distilled water and sterilized using autoclave at 121ºC for 15 min. Bacterial isolates and quality control organisms' suspensions (10^7cfu/ml) in PBS (phosphate buffer solution) were prepared by making dilution using plate count method.10 µl inoculums of these suspensions (10^7cfu/ml) were used in the assay.

Stock solutions of barbituric acid and 14 newly synthesized compounds were prepared by dissolving 5 mg of each compound in 1ml mixture of DMSO (50% v/v) and PBS (50% v/v). Two fold serial dilutions for each newly synthesized compound from its stock solution were prepared in DMSO/ PBS mixture (50 % / 50 % v/v). For each compound, test tubes were labeled with different dilution concentrations. Assay was performed by adding broth media and compound dilutions in each test tube and the volume of broth/compound mixture was adjusted to 1 ml i.e. containing variable amount of broth and newly synthesized compound dilutions.

10 µl of prepared bacterial suspension was added into each test tube and inoculated tubes were then incubated for

18 – 24 hr at 37ºC. Positive control was performed by adding 10 µl of *S. aureus* (ATCC 6538 – Oxoid Basingstoke UK) and *E. coli* (ATCC 8739 – Oxoid Basingstoke UK) to separate test tubes containing broth media only whereas uninoculated tube containing broth and synthetic compound serve as negative control for turbidity measurement.

After incubation effects of synthetic compounds on bacterial count in each test tube were recorded by finding out the turbidity as compared to the control tubes. Minimum inhibitory concentration was determined by visual inspection of the tube as MIC is the lowest concentration of compound that inhibits visible growth. Tubes without growth were vortexed vigorously for 15 seconds to resuspend any bacteria that might have adhered to the wall of the tube and were reincubated for additional 4 hours. After 4 hours of additional incubation, visually clear tubes were again vortexed and 0.1 ml from each test tube was spread across the surface of LB agar plates with the help of sterile glass rod. The plates were incubated at 37ºC for 18-24 hours and after incubation the concentration in which the petriplate showed either no growth or reduction of 99.9% from inoculums was considered as bactericidal concentration of the specific compound.

RESULTS AND DISCUSSION

The spectral data of the synthesized compounds is found to be supportive. Table **1** shows HRMS and IR data of synthesized compounds.

Table 1. Spectral Data of Barbiturate/ Barbiturate Metal Complexes

Compound	Ligand:Metal	HRMS	Selected I.R data (ν cm^{-1})
1	L-1	$C_{11}H_7N_3O_6$ 277.18978(Cal) 277.18981(Found	1220 (C-N), 1425 (C=C), 1500 (-NO_2), 1610, 1710 (C=O), 3050 (=C-H), 3069 (-N-H), 3300 (-OH)
2	L-2	$C_{12}H_{10}N_2O_4$ 246.2188(Cal) 246.2195(Found)	1212 (C-N), 1507 (C=C), 1600, 1674, 1730 (C=O), 2840 (C-H), 3071 (=C-H), 3208 (N-H)
3	L-3	$C_{11}H_7BrN_2O_3$ 295.08888(Cal) 295.08919(Found)	516 (C-Br), 1274 (C-N), 1577 (C=C), 1678, 1704, 1752 (C=O), 3098 (=C-H), 3372 (N-H)
4	L-1: Zn (1:1)	$C_{11}H_{13}Cl_2ZnN_3O_9$ 467.55062(Cal) 467.55078(Found)	1696 (C=O), 1594 (C=C), 3137 (=CH), 3444 (-NH), 851 (C-N), 1347 (NO_2), 549 (M-N)
5	L-1: Mn (1:1)	$C_{11}H_{13}Cl_2MnN_3O_9$ 457.079665(Cal) 457.079698(Found)	1674 (C=O), 1622 (C=C), 3050 (=CH), 3420 (-NH), 850 (C-N), 1348 (NO_2), 525 (M-N)
6	L-1: Ni (1:1)	$C_{11}H_{13}Cl_2NiN_3O_9$ 460.83502(Cal) 460.83499(Found)	1691 (C=O), 1594 (C=C), 3010 (=CH), 3384(-NH), 851 (C-N), 1346 (NO_2), 520 (M-N)
7	L-1: Co (1:1)	$C_{11}H_{13}Cl_2CoN_3O_9$ 461.074815(Cal) 461.074845(Found)	1692 (C=O), 1586 (C=C), 3015 (=CH), 3418 (-NH), 859 (C-N), 1350 (NO_2), 519 (M-N)
8	L-1: Co (1:2)	$C_{22}H_{14}Cl_2CoN_6O_{12}$ 684.218755(Cal) 684.218777(Found)	1694 (C=O), 1585 (C=C), 3195 (=CH), 3384 (-NH), 851 (C-N), 1346 (NO_2), 541(M-N)
9	L-2: Mn (1:1)	$C_{12}H_{16}Cl_2MnN_2O_7$ 426.108685(Cal) 426.108697(Found)	1692 (C=O), 1594 (C=C), 3040 (=CH), 3376 (-NH), 851 (C-N), 532(M-N)
10	L-2: Co (1:1)	$C_{12}H_{16}Cl_2CoN_2O_7$ 430.103835(Cal) 430.103877(Found)	1682 (C=O), 3195 (-NH), 3398 (-OH), 518 (M-N)
11	L-2: Co (1:2)	$C_{24}H_{20}Cl_2CoN_4O_8$ 622.276795(Cal) 622.276799(Found)	1703 (C=O), 3202 (-NH), 3413 (-OH), 517 (M-N)
12	L-3: Mn (1:1)	$C_{11}H_{13}BrCl_2MnN_2O_6$ 474.978765(Cal) 474.978789(Found)	1694 (C=O), 1589 (C=C), 3010 (=CH), 3405 (-NH), 850, 1348 (NO_2), 540(M-N)
13	L-3: Ni (1:1)	$C_{11}H_{13}BrCl_2NiN_2O_6$ 478.73412(Cal) 478.73455(Found l)	1694 (C=O), 1583 (C=C), 3055 (=CH), 3400 (-NH), 851 (C-N), 1346 (NO_2), 517 (M-N)
14	L-3: Co (1:2)	$C_{22}H_{14}Br_2Cl_2CoN_4O_6$ 720.016955(Cal) 720.016972(Found)	1682 (C=O), 3144 (-NH), 3400 (-OH), 518 (M-N)

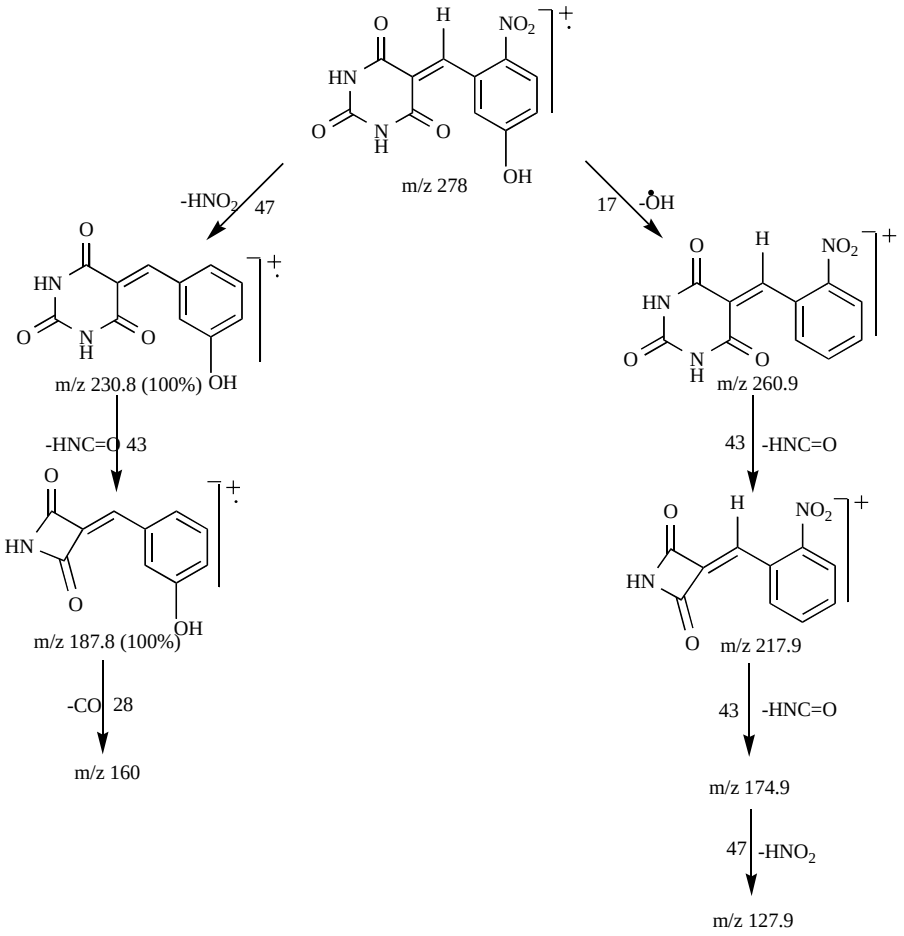


Fig. (1). Mass fragmentation pattern of Compound **1**.

The ^{13}CNMR spectrum showed resonance of three carbonyl groups at each instance ranging from 152-164.2 ppm. The carbon of alkene moiety resonated at 117.6-119.7 ppm and its adjacent carbon resonated highfield in the range 149.8-154.8 ppm due to two carbonyl groups in its vicinity.

The mas spectra of all the compounds showed molecular ions of different intensity, which confirmed their molecular weights. The mass fragmentation pattern of compound (**1)** is speculated in Fig. (**1**). The base peak at m/z 230.8 showed the most stable fragment formed in this case by the loss of HNO_2.

Antimicrobial Assay

The study has shown that some of the metal-barbiturate complexes have at least some bacteriostatic activity against uropathogenic *E. coli*. The bactericidal effects of some of the barbiturates were observed against the tested *S. aureus*. The variations in the effects of these compounds indicates that there are some of the metals which when attached to the barbiturate, the compound become antimicrobial or its activity is enhanced, while the other metal-barbiturate complexes are less effective.

The solvent system for dissolving compounds was DMSO, so antimicrobial assay was performed to evaluate the antimicrobial effect of DMSO on the studied strains. MIC of DMSO for *E. coli* was 17 % (v/v) (IC50 = 12 %), and MBC at 29 % (v/v) whereas the MIC for *S. aureus* was 8 % (v/v) (IC50 = 6 %) and MBC was 20 % (v/v). Barbituric acid was the parent compound for the syntheses of derived metal-barbiturate complexes. Antimicrobial properties of Barbituric acid were studied to see the antimicrobial effects of the parent compound. Barbituric acid did not show any bacteriostatic or bactericidal effects against *E. coli* and *S. aureus* at concentrations upto 2000 µg/ml. The antimicrobial effects of compounds (**1-3)** were also studied. (**1)** did not show any bacteriostatic or bactericidal effects against *E. coli* and *S. aureus* till 2000 µg/ml. IC 50 values of (**2**) (1350 µg/ml for *S. aureus*; 1300 µg/ml for *E. coli*) and (**3**) (1150 µg/ml of *S. aureus*; 1100 µg/ml for *E. coli*) were higher as compared to metal-barbiturate complexes (**10)** and (**11)** which indicate that metal-barbiturate complexes are more effective as compared to parent compounds.

We used a total of 14 newly synthesized Barbiturate derivative compounds to evaluate their antimicrobial properties against clinical multiple drug resistance uropathogenic *E. coli* and *S. aureus*. Out of the 14 barbiturate derivatives, 9 compounds (**1**, **4-10** and **14**) did not show any bacteriostatic or bactericidal effects against *E. coli* and *S. aureus* till 2000 µg/ml except 4 which started inhibition of bacterial growth at 1500 µg/ml for *S. aureus* (IC 50 = 1825 µg/ml) with no activity for *E. coli* till 2000

Table 2. Melting Temperatures, Solubilities and Antimicrobial Activities (IC 50) of Compounds Against *E. coli* and *S. aureus*

Entry	Ligand:Metal	Melting Point °C / Decomposition Point	IC 50 *(E. coli)*	IC 50 (*S. aureus*)	Solvent
1	L-1	260°C (Sharp)	----	----	DMSO
2	L-2	255 °C (Sharp)	1300 µg/ml	1350 µg/ml	DMSO
3	L-3	247 °C (Sharp)	1100 µg/ml	1150 µg/ml	DMSO
4	L-1: Zn (1:1)	255 °C (decomp)	----	1825 µg/ml	DMSO
5	L-1: Mn (1:1)	140 °C (decomp)	----	----	DMSO
6	L-1: Ni (1:1)	300 °C (decomp)	----	----	DMSO
7	L-1: Co (1:1)	195 °C (decomp)	----	----	DMSO
8	L-1: Co (1:2)	220 °C (decomp)	----	----	DMSO
9	L-2: Mn (1:1)	255 °C (decomp)	----	----	DMSO
10	L-2: Co (1:1)	110 °C (decomp)	450 µg/ml	300 µg/ml	DMSO
11	L-2: Co (1:2)	155 °C (decomp)	425 µg/ml	325 µg/ml	DMSO
12	L-3: Mn (1:1)	215 °C (decomp)	----	1400 µg/ml	DMSO
13	L-3: Ni (1:1)	200 °C (decomp)	----	----	DMSO
14	L-3: Co (1:2)	170 °C (decomp)	----	----	DMSO
15	DMSO	----------	12 % (v/v)	6% (v/v)	-------
16	Barbituric acid	----------	----	----	-------

µg/ml. This means that either the sites of metal attachment in these compounds have no role in antimicrobial activity [18, 20] or the type of metal ions ligated at these sites are inert against bacteria. Many of the studies have reported the rising level of resistance against other antimicrobial agents as well in *S. aureus* [21-22] and uropathogenic *E. coli* [23-24].

Metal-barbiturate complex (**12**) has shown no bacteriostatic activity against *E. coli* upto 2000 µg/ml however it was bacteriostatic against *S. aureus (*IC 50 = 1400 µg/ml*)*. On the other hand four compounds (**2-3**, **10-11**) had bacteriostatic effects against uropathogenic *E. coli* out of which (**10**) (IC 50 = 450 µg/ml) and (**11**) (IC 50 = 425 µg/ml) were bacteriostatic at lower concentrations (Fig. **2**, Table **2**). Of these (**4**) compounds, (**2**) (IC 50 = 1350 µg/ml) and (**3**) (IC 50 = 1150 µg/ml) were bacteriostatic while the other two i.e., (**10**) (IC 50 = 300 µg/ml) and (**11**) (IC 50 = 325 µg/ml) have bactericidal activity (Fig. **3**, Table **2**) against *S. aureus*. Similar antimicrobial effects have already been reported by different cyclophenamides against *S. aureus* and *E. coli* [17]. The results indicate that (**10**) and (**11**) were more effective against *S. aureus* as compared to *E. coli*. IC 50 value of (**10**) for *S. aureus* was 300 µg/ml and 450 µg/ml for *E. coli*. On the other hand IC 50 values of (**11**) for *S. aureus* was 325 µg/ml while 425 µg/ml for *E. coli*.

Our observed differences between antimicrobial activities (IC 50 values) of (**2)** (1350 µg/ml for *S. aureus*; 1300 µg/ml for *E. coli*), (**3)** (1150 µg/ml of *S. aureus*; 1100 µg/ml for *E. coli*), **(11)** (325 µg/ml for *S. aureus*; 425 µg/ml for *E. coli*) and (**10)** (300 µg/ml for *S. aureus*; 450 µg/ml for *E. coli*) against *E. coli* and *S. aureus* may be due to difference of susceptibility levels between different species due to the wall structure i.e. Single layer of cell wall of gram positive (*S. aureus*) while the other has double layer (*E. coli*). The wall structure may result in different outcomes when similar drugs used on two different bacteria e.g. Penicillin is more effective against Gram positive bacteria as compared to the Gram negative bacteria [25].

It was also noted that the antimicrobial effects of different compounds (**2)** (IC 50 = 1350 µg/ml), (**3)** (IC 50 = 1150 µg/ml), (**10)** (IC 50 = 300 µg/ml) and (**11)** (IC 50 = 325 µg/ml)) against the same species, i.e., *S. aureus*, were not of the same level; some were bacteriostatic (**2-3**) and the others were bactericidal (**10-11**).

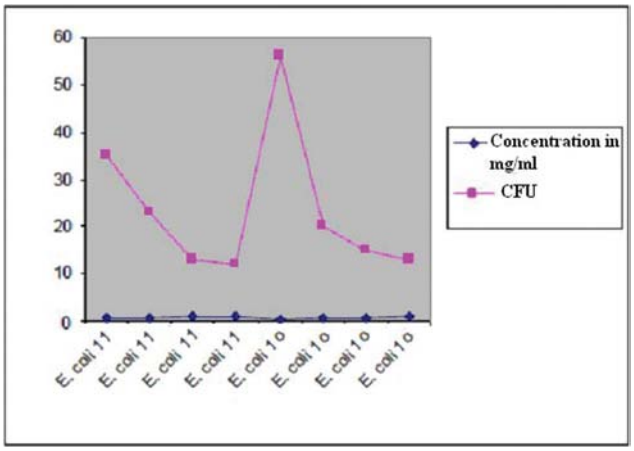


Fig. (2). Antibacterial effects of (**10)** and (**11)** on Multidrug Resistant Uropathogenic *E. coli.*

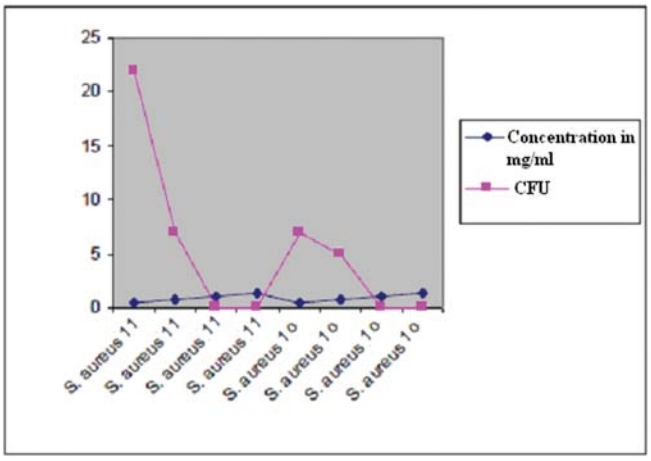


Fig. (3). Antibacterial effects of (**10**) and (**11)** on Multidrug Resistant *S. aureus* isolated from infected skin.

We can further explore the choice of metal ions and positions on molecule which would show even better antimicrobial effects at lower concentrations of the compounds [26]. For example a trifluoromethyl substituent at position 2 in antipsychotic thioxanthene flupenthixol provides a distinct antimicrobial property to this antipsychotic drug [18].

CONCLUSION

The compounds which we evaluated for their antimicrobial activity, had different metal ions ($Zn^{+2,}$ Ni^{+2}, Co^{+2} and Mn^{+2}) ligated to coordination sites of barbiturate with 1:1 and 1:2 metal: ligand ratio. The biologically active complexes were found to be of Co^{+2} (**10** and **11**). These are cobalt complex of 5-(4- methoxybenzylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**2**) in 1:1 and 1:2 metal: ligand ratio respectively. These (**10-11**) were found to have the highest antimicrobial activity amongst the compounds we studied. These results suggests that antimicrobial activity of the compounds may have been regulated by the position at which metal ion is ligated.

The Barbituric acid derivatives have a possible risk of side effects due to sedative, hypnotic and anesthetic effects. The pharmacological effects of barbiturates scale from mild sedation to anesthesia. These effects can be minimized by applying the antimicrobial properties of the derived compounds to treat skin infections with susceptible pathogens (for example *S. aureus*) and other conditions where no oral or systemic administration of the compounds would be required.

Our study shows that barbiturate compounds when complexed with cobalt (Co^{+2}) metal at specific position show antibacterial activity against multiple drug resistant *E. coli* and *S. aureus*.

ACKNOWLEDGEMENT

H. Bokhari wants to thank Higher Education Commission of Pakistan (HEC) for providing research support to carry out this work. Furthermore, A. M. Qureshi is extremely grateful to Bahauddin Zakriya University to provide generous funding and support for carrying out the work related with chemistry.

REFERENCES

[1] Nikaido, H. Prevention of drug access to bacterial targets: permeability barriers and Active efflux. *Science*, **1994** *264*, 382–388.

[2] McManus, M.C. Mechanisms of bacterial resistance to antimicrobial agents. *Am. J. Health Syst. Pharm*., **1997,** *54*, 1420-1433.

[3] Walker, G.C. The SOS response of *Escherichia coli* in *Escherichia coli* and *Salmonella*: cellular and molecular biology 2nd edn, ASM Press, Washington, D.C. **1996,** 1400-1416.

[4] Thanacoody, H.K.; Thioridazine: resurrection as an antimicrobial agent. *Br. J. Clin. Pharmacol.*, **2007,** *64*, 566-574.

[5] Bush, K.; Macielag, M. New approaches in the treatment of bacterial infections. *Curr. Opin. Chem. Biol.*, **2000,** *4*, 433-439.

[6] Stavri, M.; Piddock, L.J.V.; Gibbons, S. Bacterial efflux pumps inhibitors from natural sources. *J. Antimicr. Chemother*, **2007**, *59*, 1247-1260.

[7] Casewell, M.W.; Hill, R.L. The carrier state: methicillin-resistant *Staphylococcus aureus*. *J. Antimicrob. Chemother*., **1986**, *18*, 1-12.

[8] Witte W Selective pressure by antibiotic use in livestock. *Int. J. Antimicrob. Agents*, **2000,** *16*, S19-S24.

[9] Heritage, J.; Ransome, N.; Chambers, P.A.; Wilcox, M.H. A comparison of culture and PCR to determine the prevalence of ampicillin-resistant bacteria in the faecal flora of general practice patients. *J. Antimicrob. Chemother*., **2001**, *48*, 287-289.

[10] Catry, B.; Laevens, H.; Devrese, L.A.; Opsomer, G.; de Kruif, A. Antimicrobial resistance in livestock. *J. Vet. Pharmacol. Ther*., **2003**, *26*, 81-93

[11] Refat, M.S.; El-Korashy, S.A.; Ahmed, A.S. A convenient method for the preparation of barbituric and thiobarbituric acid transition metal complexes. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.*, **2008,** *71*, 1084-1094.

[12] Berry, C.B.; Gillespie, T.; Hood, J.; Scott, N.B. Growth of microorganisms in solutions of intravenous anaesthetic agents. *Anaesthesia*, **1993**, *48*, 30-32.

[13] Sosis, M.B.; Braverman, B. Growth of *Staphylococcus aureus* in four intravenous anesthetics. *Anesth. Analg.* **(1993),** *77*, 766–768.

[14] Crowther, J.; Hrazdil, J.; Jolly, D.T.; Galbraith, J.C.; Greacen, M.; Grace, M. Growth of microorganisms in propofol, thiopental, and 1:1 mixture of propofol and thiopental. *Anesth. Analg.*, **1996**, *82*, 475-478.

[15] Craven, S.H.; Neidle, E.L. Double trouble: medical implications of genetic duplication and amplification in bacteria. *Future Microbiol*., **2007**, *2*, 309-321.

[16] Ruble, J.C.; Hurd, A.R.; Johnson, T.A.; Sherry, D.A.; Barbachyn, M.R.; Toogood, P.L.; Bundy, G.L.; Graber, D.R.; Kamilar, G.M. Synthesis of (-)-PNU-286607 by asymmetric cyclization of alkylidene barbiturates. *J. Am. Chem. Soc.*, **2008**, *131*, 3991-3997.

[17] Rani, B.L.; Mazumbar, K.; Dutta, N.K.; Karak, P.; Dastidar, S.G. Antibacterial property of the antipsychotic agent prochlorperazine, and its synergism with methdilazine. *Microbiol. Res.*, **2005**, *160*, 95-100.

[18] Jeyaseeli, L.; Gupta, A.D.; Asok, K.K.; Mazumdar, K.; Dutta, N.K.; Dastidar, S.G. Antimicrobial potentiality of the thioxanthene flupenthixol through extensive *in vitro* and *in vivo* experiments. *Int. J. Antimicrob. Agents*, **2006,** *1*, 58-62.

[19] Idrees, M.; Mussarat, U.; Badshah, Y.; Qamar, R.; Bokhari, H. Virulence factors profile of drug- resistant *Escherichia coli* isolated from urinary tract infections in Punjab, Pakistan. *Eur. J. Clin. Microbiol. Infect. Dis.*, **2010** (In press)

[20] Loza-Mejia, M.A.; Olvera-Vazquez, S.; Maldonado-Hernandez, K.; Guadarrama-Salgado, T.; Gonzalez-Sanchez, I.; Rodriguez-Hernandez, F.; Solano, J.D.; Rodriguez-Sotres, R.; Lira-Rocha, A. Synthesis, cytotoxic activity, DNA topoisomerase-II inhibition, molecular modeling and structure-activity relationship of 9-anilinothiazolo 5, 4- quinoline derivatives. *Bioorg. Med. Chem.*, **2009**, *17*, 3266-3277.

[21] Idrees, F.; Jabeen, K.; Khan, M.S.; Zafar, A. Antimicrobial resistance profile of methicillin resistant *Staphylococcal aureus* from skin and soft tissue isolates. *J. Pak. Med. Assoc*., **2009,** *59*, 266-269.

[22] Lin, J.; Yeh, K.S.; Liu, H.T.; Lin, J.H. *Staphylococcus aureus* isolated from pork and chicken carcasses in Taiwan: prevalence and antimicrobial susceptibility. *J. Food Prot.,* **2009,** *72*, 608-611.

[23] Kwan, C.W.; Onyett, H. Community-acquired urinary tract pathogens and their resistance patterns in hospitalized children in southeastern Ontario between 2002 and 2006. Paeds & Child Health, **2008,** *13*, 759-762.

[24] Ho, P.L.; Yip, K.S.; Chow, K.H.; Lo, J.Y.; Que, T.L.; Yuen, K.Y. Antimicrobial resistance among uropathogens that cause acute uncomplicated cystitis in women in Hong Kong: a prospective multicenter study in 2006 to 2008. *Diagn. Microbiol. Infect. Dis.*, **2009,** *66*, 87-93.

[25] Yurchenco, J.A.; Hopper, M.W.; Vince, T.D., Warren, G.H. Cyclacillin, a Semisynthetic Aminoalicyclic Penicillin I. Antibacterial Activity *in vitro* and *in vivo* compared with Ampicillin and Cephalexin. *Chemotherapy*, **1970,** *15*, 209-221.

[26] Wang, K.R.; Zhang, B.Z.; Zhang, W.; Yan, J.X.; Li, J.; Wang, R. Antitumor effects, cell selectivity and structure-activity relationship of a novel antimicrobial peptide polybia-MPI. *Peptides*, **2008**, *29*, 963-968.