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Synthesis of some novel piperazine salts and their antimicrobial property against *Escherichia coli* and *Bacillus subtilis*

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ABSTRACT

A series of substituted piperazine salts have been synthesized and tested for antimicrobial activity. The antimicrobial activity was tested against Escherichia coli and Bacillus subtilis. The study indicates that some of these derivatives showed significant activity against tested microbes. Based on the reported results, it may be concluded that an electronegative group is very vital in eliciting the desired biological effect.

Keywords: Piperazine salts, Antimicrobial activity, *Escherichia coli*, *Bacillus subtilis*.

INTRODUCTION

The chemistry of piperazine derivatives continues to draw attention of synthetic organic chemists due to varied biological activities [1, 2]. The pharmaceutical importance of these compounds lies in the fact that they are important pharmacophores that can be found in many marketed drugs such as Crixivan [3], and drugs under development [4]. Piperazinyl-linked ciprofloxacin dimmers reported as potent antibacterial against resistant strains [5], a novel class of mixed D_2/ D_4 receptor antagonists [6], dual calcium antagonist [7], antimalarial agents [8] and potential antipsychotic agents [9]. Piperazine derivatives have also been reported as antifungal agents [10]. Indoles and imidazoles have been found to possess marked clinical uses [11, 12], like for treatment of several diseases like epilepsy, diabetes, anti-fertility, antimicrobial, anticancer etc [13-17].

In view of these facts, we report here the synthesis of some derivatives of the title structure type containing the above mentioned moieties for evaluation of their antimicrobial activity.

Table 1: Antimicrobial activities of compounds 3a-e

Compound No.	R	R'	Minimum inhibitory concentration (MIC) in µg/ml	
			E. coli	*B. subtilis*
3a	Phenyl	Cyclohexylidene	>100	>100
3b	Phenyl	Indolidene	25	3.175
3c	Phenyl	Indolidene	25	>100
3d	Phenyl	p-Chlorobezylidene	3.175	25
3e	Phenyl	p-Hydroxybenzylidine	>100	>100
Ampicilin	-	-	6.25	-
Erythromycin	-	-	-	2.87

MATERIALS AND METHODS

All melting points are uncorrected. Purity of the compound was checked by TLC. IR spectra (ν_{max} in cm^{-1}) were recorded in KBr on a FTR 8201vc spectrometer ^1H NMR spectra on a DRX (200 MHz) and DRX (300 MHz) NMR spectrometer using TMS as internal standard (chemical shift in δ ppm). The physical data has been tabulated in **Table 2** below.

2-Aryl/alkyl-4-arylidene/alkylidene-1,3-oxazol-5-one 1a-e. A mixture of carbonyl compound and benzoyl/acetyl glycine (0.03mole), acetic anhydride (20 ml) and anhydrous sodium acetate (0.03 mole) was stirred mechanically and refluxed on a water bath for 2 hours. Subsequently 100 ml ethanol was added to it and allowed to stand overnight. A yellow solid which separated out, was filtered off and washed successively with cold water. It was recrystallized from benzene.

1a - IR (KBr): 1436 (C=C skeletal), 1820 (C=O), 1678 (C=N), 2948 (ArC-H str.), 1120 (C-C); ^1H NMR ($CDCl_3$): δ 7.22-7.51 (m, 5H, Ar-H), 1.19-1.27 (m, 10H, CH_2 in cyclohexylidene ring)
1a: Mass (FAB): 676 (M^+), 649, 444, 588, 578, 550, 416, 332, 341, 354, 315, 239, 163, 135, 121 (Base peak);

2-Aryl/alkyl-4-arylidene/alkylidene-imidazol-1-benzene-4-carboxylic acid 2a-e. A mixture of 2-Aryl/alkayl (0.1 mole) and p-aminobenzoic acid (0.15 mole) in anhydrous pyridine (50 ml) was heated under reflux for 6 hours under anhydrous reaction conditions. The resultant solution was cooled and treated with dilute HCl (100 ml). The solid which separated out was filtered and washed with water (200 ml). It was dried at 100°C and recrystallised from ethanol as light brownish solid mass.

2a: IR (KBr): 1625 (C=N), 1652 (ter. amido C=O), 1442 (C=C, skeletal), 2931 (ArC-H str.), 1372 (COOH str.), 2855 (C-H str.);^1H NMR ($CDCl_3$): δ 1.22-2.82 (m, 10H, CH_2 in cyclohexylidene ring), 6.74-7.91 (m, 9H, Ar-H), 11.2 (brs, 1H, s, exchangeable COOH);

2b: IR (KBr): 1634 (ter. Amido C=O), 1628 (C=N), 1382 (COOH str.), 2936 (N-H), 3014 (Ar-H), 2128 (N=C-N-); $^1HNMR(CDCl_3)$:δ 6.12-7.72 (m,13H,Ar-H),7.61 (broad,s,1H,N-H),10.16 (brs,1H,s, exchangeable COOH);

2c: IR (KBr): 1680 (sec, amido C=O), 1645 (tert. amido C=O), 1620 (C=N), 1110 (C-C), 1635 (C=C), 3480 (N-H), 2945 (C-H);^{1}H NMR ($CDCl_3$): δ 7.1-7.8 (m, 8H, Ar-H), 2.8 (m, 3H, CH_3), 10.8 (brs, s, exchangeable COOH), 7.79 (broad s, 1H, -NH-);

2d:IR(KBr):702(Ar-Cl),2145(-N=C-N),3020(Ar-),1390(COOHstr.), 1633 (C=N), 1649 (ter.Amido); ^{1}HNMR($CDCl_3$):δ 6.35-7.67 (m,13H,Ar-H), 11.18 (brs,1H,s, exchangeable COOH);

2e: IR (KBr):1639(C=N), 1643 (ter.amido), 1410 (COOH, str.), 3365 (Ar-OH), 2990 ((Ar-H), 2152(-N=C-N); ^{1}HNMR ($CDCl_3$):δ 6.23-7.98 (m.13H, Ar-H), 11.21 (brs, 1H, s, exchangeable COOH), 4.86 (s, 1H, Ar-H) ;

Piperazine1-4-bis-[-2-aryl/alkyl-4-arylidene/alkylidene-5-oxo-imidazolyl]-1-phenyl-4-carboxylates 3a-e. A mixture of 2-aryl/alkyl-4-arylidene/alkylidene-imidazol-1-benzene-4-carboxylic acid (0.02 mole) and piperazine hexahydrates (0.01 mole) in ethanol (50 ml) was heated and refluxed for 2 hours. The solvent was distilled off and the crude piperazine salts thus obtained was recrystallized from acetone.

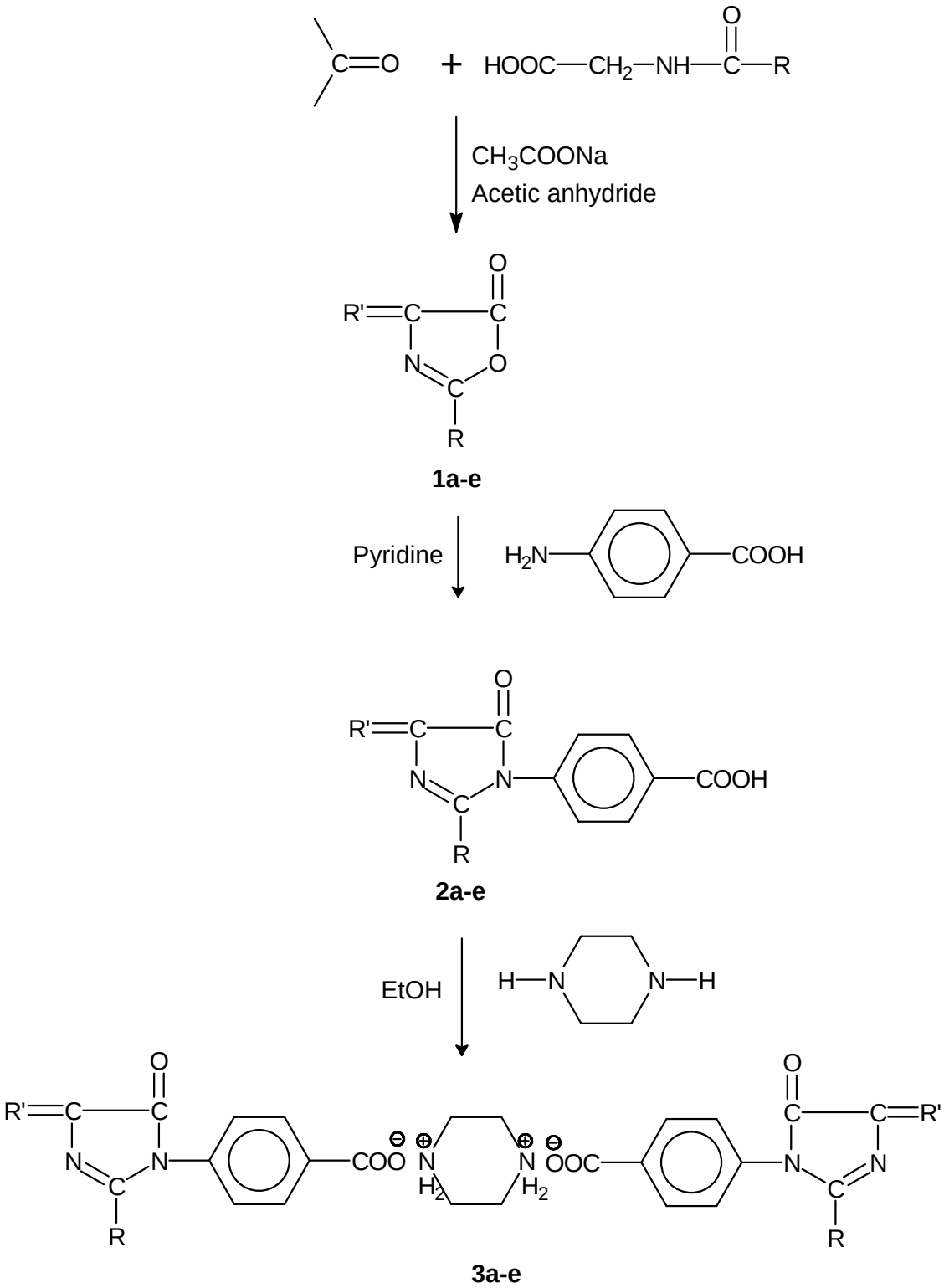
3a: IR(KBr): 3110 (amine salt), 3014 (Ar), 1695 (C=O), 1635 (C=N), 1375 (COOH str.), 2675 (sec.NH_2 ,1640 (C=C), 1445 (C=C skeletal); ^{1}H NMR ($CDCl_3$): δ 7.25-7.77 (m,18H,ArH), 2.02 (t, 20H, CH_2 in cyclohexylidene), 8.25 (brs, 4H, CONH piperazine), 1.92-1.5 (m, 8H, CH_2).

3b:IR(KBr): 3122 (Amine salt), 1655 (sec.amide,C=O), 2662 (sec.NH_2), 1636 (ter.amidoC=O), 2925 (N-H), 3035 (Ar-H);^{1}HNMR($CDCl_3$):δ .5-7.80 (m,26H,Ar-H) 8.12 (brs,4H,CONH piperazine), 7.69 (broad,s,1H,NH-indole), 1.82-2.21 (m,8H,CH_2)

Table 2: Physical data of compounds 1, 2 and 3a-e.

Compd.	R	R'	1a-e			2a-e			3a-e		
			m.p.	Yield (%)	N (%) Found (Calc)	m.p. °C	Yield (%)	N (%) Found (Calc)	m.p.	Yield (%)	N (%) Found (Calc)
a	Phenyl	Cyclohexylidene	160	80	5.78 (5.80)	123	73	7.82 (7.77)	102	80	10.51 (10.56
b	Phenyl	2-oxo Indolidene	291	75	9.61 (9.65)	242	45	10.26 (10.22)	203	65	12.37 (12.41)
c	Methyl	2-oxo Indolidene	215	65	9.25 (12.28)	202	55	12.05 (12.01)	214	60	14.40 (14.43)
d	Phenyl	p-chlorobenzylidene	226	70	4.67 (4.70)	160	65	6.90 (6.89)	97	65	9.45 (9.48)
e	Phenyl	p-hydroxy benzylidene	181	67	5.34 (5.28)	142	68	1.36 (7.38)	185	55	9.7 (10.00)

3c: IR (KBr): 1672 (sec.amideC=O),1642 (tert.amidoC=O),1628 (C=N), 1116(C-C),3390 (N-H), 2980 (C-H); ^{1}HNMR($CDCl_3$):δ 6.9-7.7 (m,16H,Ar-H), 2.6 (m,6H,CH_3), 7.82 (broad,s,1H,-NH-indole), 8.28 (brs,2H,CONH piperazine),1.90-2.12 (m,8H,CH_2).



1a-e

2a-e

3a-e

3d: IR(KBr) :707 (Ar-Cl), 2616 (sec.NH_2), 1642 (sec.amido), 1639 (ter.amido), 3028 (Ar-H), 3142 (aminesalt), 1620 (C=N);^{1}HNMR($CDCl_3$):δ 6.6-7.85 (m,26H,Ar-H), 8.09 (brs,2H,CONH piperazine), 1.86-2.17 (m,8H,CH_2);

3e: IR (KBr): 3542 (Ar-OH), 3032 (Ar-H), 1631 (sec.amido), 3129 (amine salt), 6151 (ter.amido), 1618(C=N); ^{1}HNMR ($CDCl_3$): δ 6.2-7.79 (m, 26H, Ar-H), 8.15 (brs, 2H, CONH piperazine), 1.79-2.15 (m,8H,CH_2), 4.95(s, 1H, Ar-OH);

RESULT AND DISCUSSION

Compound **3a-e** were screened for their antibacterial activity against *Escherichia coli* and *Bacillus subtilis in vitro* involving the two fold serial dilution technique. The initial concentration of the test compound is taken 1 mg/ml in dimethylsulphoxide (DMSO) for carrying out antibacterial activity. The culture was prepared by mixing 20 ml of plane broth and 1 ml of bacterial growth-containing broth. From this 1.8 ml of the culture was taken in one tube and 1 ml of the culture was put down in six other sterilized tubes. At last one control tube containing no antibiotic sample was kept. All the tubes were incubated at 37°C. after 24 hours. The bacterial growth was examined by appearance of turbidity. The tube in which there was no growth of bacteria, the concentration of the sample was calculated which provided the value minimum inhibitory concentration (MIC µg/ml) of the compound under investigation. Out of five, three compounds of this category were found to show definite activity against *E. coli* and two such compounds could not exhibit measurable degree of activity. **3b** and **3c** were found moderately active while **3d** (R=phenyl, R' = para chlorophenyl) showed highly satisfactory antimicrobial activity against *E. coli* with a MIC value of 3.175. Interestingly, the compound **3e** (R=phenyl, R' = para hydroxyphenyl) was found completely inactive with a MIC value >100. Considering *B. subtilis* activity one compound **3b** (R = phenyl, R'= indolidenes) showed remarkably high order of activity like the standard drug moxiflaxoin. It is interesting to observe here that the replacement of phenyl group by a methyl group (compound **3c**) causes the complete loss of antimicrobial activity against *B. subtilis*, this drastic change in activity seems mainly due to a change from aromatic to aliphatic system. These results clearly demonstrate that the substituents have greater role to play in such molecular architecture. It also implies that an electronegative group is very vital in eliciting the desired biological effect and a change in R is also responsible for the decrease or increase in bioactivity of such compounds.

CONCLUSION

We have synthesized a series of piperazine-1,4-bis-[2-aryl/ alkyl-4-arylidene/ alkylidene-5-oxo-imidazolyl]-1-phenyl-4-carboxylate derivatives as potent antibacterial compounds. **3b** and **3d** are acceptably potent compounds inhibiting *E. coli* and *B. subtilis* respectively to the greatest extent. In comparison with antibiotics commonly used in therapy these compounds show similar or slightly less antibacterial activity (Table 1). This class of compounds could be new potent antibacterial agents. Resistance to antimicrobial drugs is increasing all over the world. Both Gram positive and Gram negative strains are involved in this process. New types of compounds like **3b** and **3d** with antimicrobial activity could diminish this negative tendency.

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