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ANTIBACTERIAL ACTIVITY OF SOME *N,N'*-LINKED BISAZAHETEROCYCLES, BENZIMIDAZOLINE AND QUINOXALINE DERIVATIVES

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ABSTRACT

*Seventeen derivatives of quinazolinonyl-pyrrolidinones/quinazolinediones/ benzodiazepindiones, bisquinazolinones, bisbenzodiazepinones, benzimidazoline and quinoxalines, earlier synthesized by us, are screened for antibacterial activity against Staphylococcus aureus MTCC96 and Escherichia coli MTCC119 by paper disc diffusion method using streptomycin as control. Compound **8** was the most active and equipotent with that of the control at 6.25 μg/disc concentration. Compounds **5**, **8**, **12**, **13a** and **13c** exhibited high antibacterial activity against Escherichia coli MTCC119.*

***KeyWords**: bisazaheterocycles, bisquinazolinones, benzimidazoline, quinoxaline, antibacterial*

INTRODUCTION

N,N'-Linked bisazaheterocycles received considerable attention in recent times because of their pharmacological importance as potential anticonvulsants[1], antidepressants[2], anti-inflammatory[3], antimicrobial[4], antifilarial agents[5], memory enhancers[2] and HMG-CoA reductase inhibitors[6]. Prompted by these observations, we have recently synthesized new *N,N'*-linked bisazaheterocycles. In this paper, the synthesis of 2-phenyl-3-(2-carbomethoxymethyl-2-carbomethoxy-4-oxo-1,2,3,4-tetrahydroquinazolin-3-yl)quinazolin-4(3*H*)-one (**2**), and the results obtained from the screening of a few bisazaheterocycles, previously synthesized[7], for their antibacterial activity are described.

Chemistry:

Reflux of an equimolar mixture of 2-phenyl-3-(2-aminobenzamido)quinazolin-4(3*H*)- one (**1**) and dimethyl acetylenedicarboxylate in methanol yielded a single crystalline product whose EI mass spectrum (70 eV) showed the highest ion peak at m/z 439 (100 %). A sharp singlet peak in the ^{1}H NMR spectrum at δ 2.5 corresponding to CH_2 protons suggested 2-phenyl-3-(2-carbomethoxymethyl-2-carbomethoxy-4-oxo-1,2,3,4- tetrahydro quinazolin-3-yl)quinazolin-4(3*H*)-one (**2**) structure for the product than the expected

compound **3**. The IR spectrum showed four carbonyl absorptions at 1715, 1749 (ester carbonyls), 1673 and 1701 cm^{-1} (quinazolinone carbonyls). Thus, DMAD served as a one-carbon source for bridging the two amino functions of 2-aminobenzamide moiety in **1** (*Scheme-I*).

EXPERIMENTAL

1. Chemistry

Melting point was determined in capillaries using Polman digital melting point apparatus (Model MP-96) and reported in degree centigrade. Infrared spectrum was obtained in KBr pellet on Shimadzu 435 instrument, the position of absorptions are quoted $\pm 2.5 cm^{-1}$. Proton magnetic resonance spectrum was recorded on Varian Gemini (200 MHz) using tetramethylsilane as the reference, dimethyl sulfoxide-d_6 (DMSO-d_6) as the solvent, the chemical shifts are reported in parts per million of tetramethylsilane in δ units. The mass spectrum was recorded on Perkin-Elmer Hitachi RMU-6L instrument using direct inlet probe.

1.1. Synthesis procedure

2-Phenyl-3-(2-carbomethoxymethyl-2-carbomethoxy-4-oxo-1,2,3,4-tetrahydro-quinazolin-3-yl) quinazolin-4(3H)-one **(2)**

A solution of 2-phenyl-3-(2-aminobenzamido)quinazolin-4(3*H*)-one (**1**, 0.35g, 1 mmol) and dimethyl acetylenedicarboxylate (0.2 mL, 1.4 mmol) in methanol (20 mL) was refluxed for 12 h. Product **2** separated out as white solid. It was filtered and recrystallised from ethanol, yield 81%; m.p. 252^{O}C; IR: 3379, 3069, 2951, 1749, 1715, 1701, 1673, 1609, 1569, 1501, 1439, 1399, 1327, 1271, 1218, 1159, 1074, 907; ^{1}H-NMR: δ 2.5 (s, 2H, CH_2), 3.3 (s, 3H, OCH_3), 3.5 (s, 3H, OCH_3), 6.9-8.3 (m, 13H, Ar-H); MS m/z (rel., Int, %): 439 (100), 407 (30), 379 (8), 222 (20), 179 (45), 170 (25), 157 (13), 144 (30), 119 (50), 90 (80), 43 (30).

2. Evaluation of antimicrobial activity

2.1. Organisms

The bacterial strains used for evaluation of antimicrobial activity were *S. aureus* MTCC 96 (Gram positive) and *E. coli* MTCC 119 (Gram negative) and were procured from IMTECH, Chandigarh, India. The activity was determined by employing paper disc diffusion method[8].

2.2. Preparation of sample solution

The samples were dissolved in DMSO (AR grade) and different concentrations of the compounds ranging from 200 to 6.25 μg/disc were prepared by successive dilutions.

2.3. Preparation of medium

The nutrient agar medium was prepared by adding 1.5% agar-agar powder to nutrient broth. The nutrient broth was prepared by dissolving Lablemco/beef extract powder (10 g), peptone powder (10 g) and sodium chloride (5 g) in one liter of distilled water. The pH of the medium was adjusted to 7.2-7.4, and sterilized in the autoclave at 121 ^{o}C (15 lb pressure) for 15 minutes. Sterile medium was cooled to about 50-60 ^{o}C and poured in to sterile petri plates

of 4" diameter with a volume of 20 mL per plate under aseptic conditions and were allowed solidify. The sterile agar plates were used for inoculating the bacterial cultures.

2.4. Bacteriological testing

Actively growing slant culture suspension of *S. aureus* MTCC 96 and *E. coli* MTCC 119 were swab inoculated separately on to the agar plates. Sterile paper discs (6mm diameter) were prepared from standard Whatman No1 filter paper. And these discs impregnated with different concentrations of test compounds (0.01mL/disc). Test compound impregnated discs were gently placed on pre inoculated agar plates. The agar plates were then kept in refrigerator for about thirty minutes to arrest the growth of an organism during the time required for the test compounds to diffuse into the agar medium. Then the plates with test compound discs were incubated at 37 ^{o}C for 24 h.

After 24 h, the petri dishes were checked for growth inhibition zone. The presence or absence of growth inhibition zone around each disc was recorded by comparing with inhibition zone of standard antibiotic disc (Streptomycin, concentration 10 μg/disc). The effectiveness of the test compounds was determined on the basis of presence or absence of growth inhibition zone. Presence of clear zone around the disc indicated that the test compound is effective against the test organism. Absence of clear zone around the disc indicated that the test compound is ineffective against the test organism (*Table-I*).

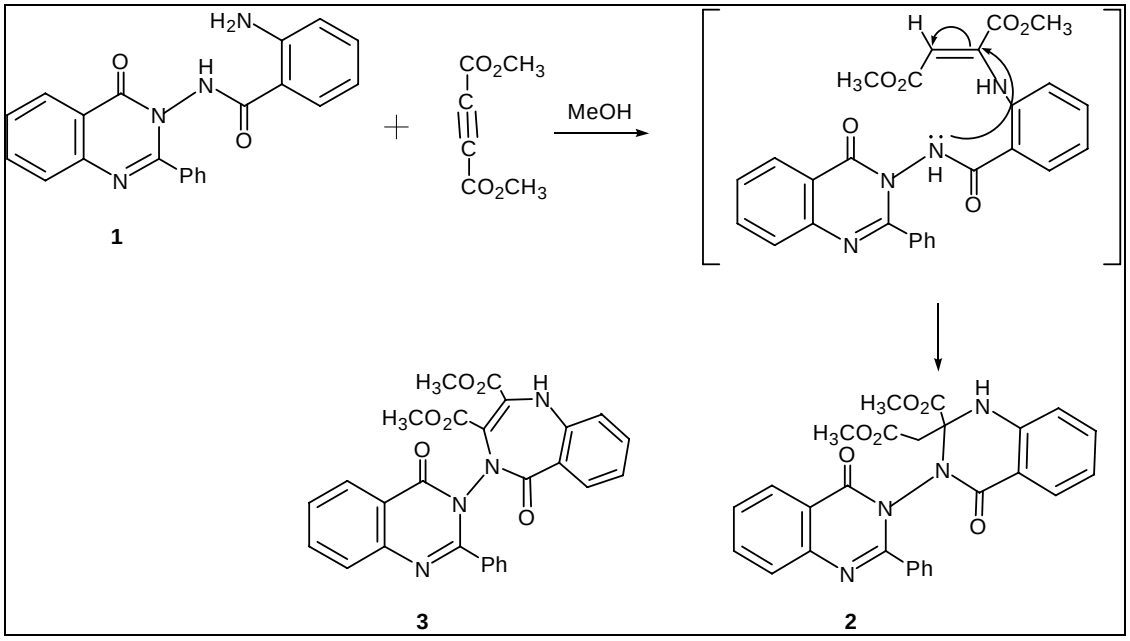
RESULTS AND DISCUSSION

In addition to the bisquinazolinone **2**, the *N,N'*-linked bisazaheterocycles **4**, **5**, **8** and **9**, quinazolinones **6** and **7**, benzamidazoline **13** and quinoxaline **14** (see *Chart-I*), reported by Reddy and Reddy [7] are screened for antibacterial activity. All the seventeen compounds exhibited significant antibacterial activity at different concentrations towards the strains, *Staphylococcus aureus* MTCC96 and *Escherichia coli* MTCC119 The most active of these was 2-phenyl-3-(2,4-dioxo-1,2,3,4-tetrahydroquinazolin-3-yl) quinazolin-4(3H)-one (**8**) which was approximately equipotent in activity with that of streptomycin in concentration as low as 6.25 μg/disc (*Table 1*). Bisazaheterocycles containing tetrahydroquinazolinone unit did not show recognizable activity, suggesting that 2,4-dioxoquinaolin-4(3*H*)-one is the pharmacophore in **8**. The other class of compounds that exhibited significant bactericidal activity against *S. aureus* MTCC96 and *E. coli* MTCC119 are 1,3-dibenzylbenzimidazolines (**13a-e**) up to 12.5 μg/disc concentration. 1-Benzyl-3-methyl-3-*N*-propyl-2-phenyl-1,2,3,4-tetrahydroquinazoline (**14b**) has shown antibacterial activity against *E. Coli* MTCC119 in 200 μg/disc concentrations. 2,2'-Di(2-nitrophenyl)-1,1'-dimethyltetrahydro-3,3'-bisquinazolin-4,4'- dione (**11**) and 1,2-bis(3-nitrobenzylidene)aminobenzoylhydrazine (**10**) have antibacterial activity against *S. aureus* MTCC96 up to 50 μg/disc. Compounds **5**, **8**, **12**, **13a** and **13c** have prominent antibacterial activity against *E. Coli* MTCC119, comparable to that of the control. All these

compounds qualify as potential candidates for antibacterial drugs against both Gram positive and Gram-negative organisms.

CONCLUSIONS

Among the seventeen compounds tested, the bisquinazolinone **8** and the benzimidazoline **13** showed bactereostatatic activity on par with streptomycin against *Staphylococcus aureus* MTCC96 (Gram positive) and *Escherichia coli* MTCC119 (Gram negative) bacteria. In addition, the quinazolinone **5** and the bisbenzodiazepinone **12** inhibited the growth of *E. coli* MTCC119 in very low concentrations. They qualify as drug candidates for further screening and SAR studies.



Scheme-1

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Table-1: Antibacterial activity of compounds

(Solvent: Dimethyl sulfoxide, Concentration 0.01 mL/disc
Control: Streptomycin, Concentration 10 μg/disc)

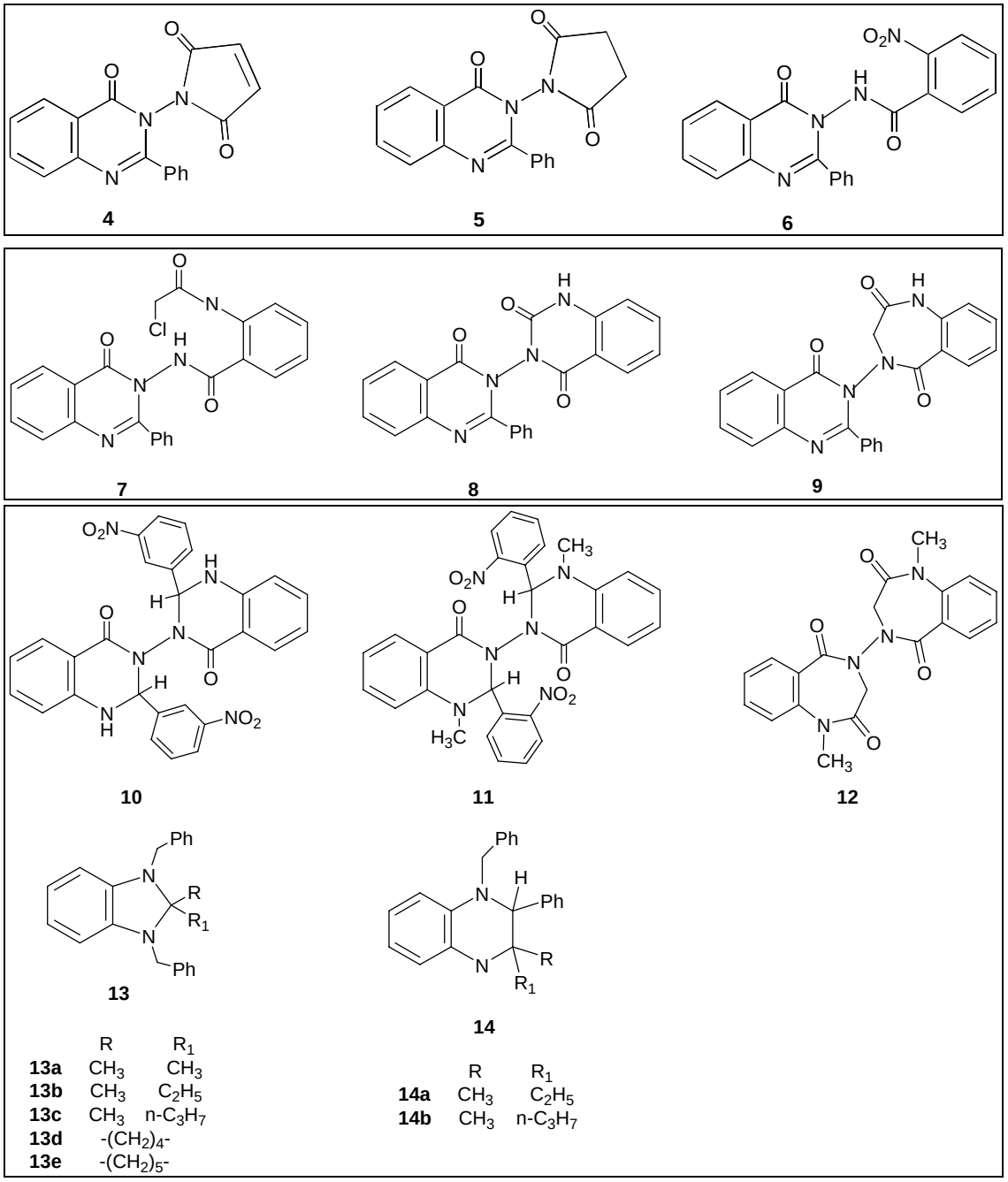
Compound No.	*S. aureus* MTCC96						*E. coli* MTCC119					
	Concentration*						Concentration*					
	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25
Control	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	+	-	+	+	+	+	+
4	-	-	-	-	+	+	-	-	-	-	-	+
5	-	-	-	+	+	+	-	-	-	-	-	-
6	-	-	-	-	-	+	-	-	-	-	-	+
7	-	-	-	-	+	+	-	-	-	+	+	+
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	+	+	-	-	-	-	+	+
10	-	-	-	+	+	+	+	+	+	+	+	+
11	-	-	-	+	+	+	+	+	+	+	+	+
12	-	-	-	+	+	+	-	-	-	-	-	-
13a	-	-	-	-	-	+	-	-	-	-	-	-
13b	-	-	-	-	-	+	-	-	-	-	-	+
13c	-	-	-	-	-	+	-	-	-	-	-	-
13d	-	-	-	-	-	+	-	-	-	-	-	+
13e	-	-	-	-	-	-	-	-	-	-	-	+
14a	-	+	+	+	+	+	-	+	+	+	+	+
14b	+	+	+	+	+	+	-	+	+	+	+	+

* Concentration expressed in μg/cm^2 dissolved in DMSO

(-) = No growth observed (i.e., compound was considered active against the organism)

(+) = Growth observed (i.e., the compound has no antibacterial activity)

Chart-1



	R	R_1
13a	CH_3	CH_3
13b	CH_3	C_2H_5
13c	CH_3	n-C_3H_7
13d	-$(CH_2)_4$-	
13e	-$(CH_2)_5$-	

	R	R_1
14a	CH_3	C_2H_5
14b	CH_3	n-C_3H_7

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