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Synthesis, Characterization and Biomedical Perspectives of Hetero-Cyclic Biginelli Di-Hydropyrimidiones (DHPMs) Analogous Against Selected Pathogenic Bacterial Species

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ABSTRACT

A simple and efficient method has been developed for the synthesis of four Biginelli type heterocyclic compounds of dihydropyrimidinones analogous **4a-d** by a one-pot, three-component cyclo condensation reaction of substituted benzaldehydes **1a-d**, thiourea **3** and ethyl acetoacetate **2** using ammonium dihydrogen phosphate as catalyst. The structures of the products were confirmed by IR and 1H-NMR spectroscopy, as well as by elemental analysis. Representative samples were screened for their anti-microbial activity against five human pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus* by using well diffusion method on Muller Hinton agar plates. The zone of inhibition after 24 h incubation was measured in mm and the potency was compared with standard drug.

INTRODUCTION

Globally, drug resistance by pathogenic bacteria is becoming an alarming area of research which leads to discover new antibacterial drugs for new therapeutic agents with novel modes of action. In organic and medicinal chemistry, the search and discovery for new Multicomponent reactions (MCRs) and the full exploitation of already known multicomponent reactions have gained global importance now in drug designing [1]. Similarly, the traditional Structure Activity Relationship (SAR) is a useful tool in the search for new drugs. SAR is usually determined by making minor changes to the structure of the existing compound and assessing the effect on its biological activity [2].

Pietro Biginelli reported on the acid-catalyzed cyclo condensation reaction of ethyl acetoacetate (**1**), benzaldehyde (**2**) and urea (**3**) which was carried out simply by heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified correctly by Biginelli as 3,4-dihydropyrimidin-2(1*H*)-one (**4**) (Fig.1) [3].

The synthetic potential of this new heterocycle synthesis (now known as Biginelli reaction) remained unexplored for quite some time. In the 1970's and 1980's interest slowly increased, and the scope of the original cyclo condensation reaction shown in Fig. 1 was gradually extended by variation of all three building blocks, allowing access to a large number of multi functionalized dihydropyrimidines (Fig.2). On this basis, several dihydropyrimidinones have been synthesized by using the art of combinatorial chemistry [4].

Over many years, the heterocyclic compounds like dihydropyrimidin-2(1*H*)-ones (DHPMs) and their derivatives have attracted considerable attention, as the dihydropyrimidine scaffold displays a fascinating array of pharmacological and therapeutic properties. More recently, appropriately functionalized DHPMs have emerged as *e.g.* orally active antihypertensive agents **5**, **6** or α1a adrenoceptor-selective antagonists **7**.

A very recent highlight in this context has been the identification of the structurally rather simple DHPM monastrol **8** as a mitotic kinesin Eg5 motor protein inhibitor and potential new lead for the development of anticancer drugs [5]. Apart from synthetic DHPM derivatives, several marine natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate core have recently been isolated [6]. Most notably among

these are the batzelladine alkaloids A and B (e.g. **9**) which inhibit the binding of HIV envelope protein gp-120 to human CD4 cells and, therefore, are potential new leads for AIDS therapy [7].

Although the most straightforward protocol to synthesize DHPMs **4** is the one-pot acid-catalyzed Biginelli condensation shown earlier (Fig.1), this protocol – using ethanol and catalytic amounts of HCl – often provides only low to moderate yields of the desired target molecules of type **4**, in particular, when substituted aromatic aldehydes or thioureas are employed. This has led to the recent disclosure of several improved reaction protocols for the synthesis of DHPMs, either by modification of the classical one-pot Biginelli approach itself [Fig.3] [8], or by the development of novel, but more complex multistep strategies. In addition, several combinatorial approaches towards DHPMs **4** have been advanced, using *e.g.* solid phase, or fluorous phase reaction conditions [9]. Moreover, the applications of more efficient catalysts to improve the yields such as Lewis acids, zinc chloride, cobalt hydrogen sulphate and copper chloride ($CuCl_2$) have also achieved considerable success [10-11].

Since introducing a suitable and efficient catalyst to facilitate the synthesis of various dihydropyrimidiones is highly interesting, the present study is aimed to introduce ammonium dihydrogen phosphate as catalyst for the synthesis of DHPM analogs. Moreover, though many of the pyrimidione products have been reported to possess biological activities such as anti-viral, anti-bacterial, anti-hypertensive and anti-tumor [12-13], the present investigation was also focused to study the antibacterial activity of four Biginelli analogous compounds synthesized by the mentioned scheme I.

MATERIALS AND METHODS

2.1 General procedure for the synthesis of compounds

A mixture of *m*-nitro benzaldehydes **1a**, *p*-methoxy benzaldehydes **1b**, benzaldehydes **1c**, *o*-chloroben- zaldehyde **1d**, ethyl acetoacetate **2** and thiourea **3** in presence of ammonium dihydrogen phosphate was heated at 85 ºC for appropriate time. After completion of the reaction indicated by TLC (*n*-hexane: ethyl acetate, 3:1), the mixture was cooled to room temperature and water was added. Stirring was continued for several minutes for dissolving the catalyst and the excess of thiourea. The solid products were filtered and washed with water. The pure products were obtained by recrystallization from ethanol. The products have

been characterized by comparison of their physical and spectroscopic data with those of the authentic samples.

2.2 Elemental and Spectroscopic Analysis

Carbon, hydrogen and nitrogen analyses of the complexes were carried out on a CHN analyzer Calrlo Erba 1108, Heraeus. The infrared spectra (KBr discs) of the samples were recorded on a Perkin–Elmer 783 series FTIR spectrophotometer in 4000-400 cm^{-1} range. The electronic absorption spectra in the 200-1100 nm were obtained on a Shimadzu UV-1601 spectrophotometer. ^{1}H spectra (300 MHz) were recorded on a Bruker Avance DRX 300 FT-NMR spectrometer using tetramethylsilane (TMS) as the internal standard. The fast atom bombardment (FAB) mass spectra of the complexes were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzylalcohol (NBA) as the matrix. The purity of compounds was evaluated by thin layer chromatography.

The physical and spectroscopic data of the new compounds were reported below:

Data of 4a: M.P. 186°C; Anal. Calc. for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.67; N, 13.08 %. Found: C, 52.28; H, 4.62; N, 13.05 %. **Data of 4b**: M.P 208°C; Anal. Calc. for $C_{15}H_{18}N_2O_3S$: C, 58.82; H, 5.88; N, 9.15%. Found: C, 58.78; H, 5.83; N, 9.13 %. **Data of 4c**: M.P 195°C; Anal. Calc. for $C_{14}H_{16}N_2O_2S$: C, 60.87; H, 5.80; N, 10.14%. Found: C, 60.83; H, 5.75; N, 10.10%. **Data of 4d**: M.P 178°C; Anal. Calc. for $C_{14}H_{16}N_2O_3S$: C, 54.54; H, 5.19; N, 9.09%. Found: C, 54.50; H, 5.15; N, 9.04%.

2.3 Antibacterial activity

Escherichia coli (MTCC 443), *Bacillus subtilis* (MTCC 441), *Salmonella typhi* (MTCC 474), *Klebsiella pneumoniae* (MTCC 109) and *Staphylococcus aureus* (MTCC 96) pure cultures were procured from Institute of Microbial Technology, Chandigarh, India. Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiment were prepared by transferring a loopful of cells from the stock cultures of test tubes of Muller Hinton Broth (MHB). These tubes were incubated with agitation for 24 h at 37°C.

Agar well diffusion assay [10] was used for antibacterial activity of the test compounds. 100 µL of adjusted culture (108cfu/ml) of bacteria was mixed with 100 mL of Muller Hinton Agar (MHA) and poured 25 mL each into sterile petriplates (90 mm) and allowed to solidify and the individual plates were marked for the organisms inoculated. After solidification plates were punched to make well of 9 mm diameter with the help of sterile borer and 50 µL of respective compounds at concentration of 100 µg.mL-1 were pipetted into the well in assay plates. 50 µL of Ciprofloxacin at 50 µg.mL-1 concentration was poured to the well in assay plate and used as a standard antibacterial agent. Plates were incubated at 25°C for 5 days and all the plates were observed for the zone of inhibition, diameter of these zones were measured in mm. All the tests were performed under sterile conditions and repeated three times. The growth of microorganisms was determined visually and the lowest concentration that inhibits the growth of microorganisms for 24 hours at 37^0C was taken as the MIC.

RESULTS AND DISCUSSION

In the present investigation, four Biginelli type heterocyclic compounds of dihydropyrimidinones analogous were synthesized using substituted benzaldehydes, thiourea and ethyl acetoacetate in presence of ammonium dihydrogen phosphate as catalyst. All products were identified using Elemental, IR and ^{1}H-NMR spectral techniques. The data obtained on the structure of the new compounds coincided very well with our earlier work [14].

In the presence of the catalyst ammonium dihydrogen phosphate reaction proceeds smoothly giving desired products in short time (within 2Hrs) and in a quantitative yield. The yields and melting points are given in Table 1. Hamouly *et al.* [15] have reported a similar type of Biginelli reaction for the synthesis of DHPMs using substituted aldehydes and methyfurfural. Similarly, El-Fattah et al. [16] succeeded in the synthesis of novel DHPMs using a mixture of compound, malononitrile, anhydrous ammonium acetate and the appropriate aldehydes namely: *p*-hydroxybenzaldehyde, 4-hydroxy-3-methoxy benzaldehyde (vaniline) and *o*-hydroxybenzaldehyde (salicylaldehyde) in butanol.

3.1 Spectroscopic analysis

The IR spectra provide some information regarding the skeleton of the compounds and were analyzed by a careful comparison with that of the parent compounds. The selected IR absorption bands are discussed here. The compounds show characteristic band for ν(N-H) at 3320 cm^{-1}. The sharp bands in the 750-790 and 1520-1540 cm^{-1} regions are due to aromatic υC-H and υC=C, respectively. The band observed at 1165-1175 cm^{-1} is due to υ C-N. The broad band in the 3000-2800 cm^{-1} region is due to an -OH group. The band appearing at 1710 cm^{-1} is assigned to the carbonyl group of the ethyl acetoacetate moiety ν(C=O) of the compound. The IR spectrum of **4b** compound is shown below (Fig.4):

3.2 ^{1}H-NMR spectra

The comparison of the ^{1}H-NMR spectrum of the compound (**4b**) recorded in $CDCl_3$ at room temperature reinforces the conclusions drawn from the IR spectra. The signals shown by the compound (**4b**) are shown below: The signals at δ 10.02 ppm (-NH), δ 8.15 ppm (-PhNH), δ 7.50 ppm(-NH), δ 4.1 (-CH), δ 3.12 ppm(-OCH_3), δ 2.44 ppm (-CH_2), δ 1.98 ppm (-CH_3), δ 1.50 ppm (-CH_3). Moreover, ^{1}H-NMR spectra of other compounds support the proposed skeleton of the compounds. The ^{1}H-NMR spectrum of compound **4b** is given in Fig.5.

3.3 Antibacterial activity

For evaluating antibacterial activity of the different compounds **4a-d,** Ciprofloxacin was used as the standard drug. The MIC calculated were 3.65µg/mL for compounds **4a**, 3.85 µg/mL for compounds **4b,** 3.25 µg/mL for compounds **4c** and 3.68 µg/mL for compounds **4d**. As depicted in **Table 2-5**, all the synthesized compounds exert a wide range of antibacterial activity against five human pathogenic bacteria. Compounds **4b** with zone of inhibition of 12 and 16 mm against *S. typhi* and *K. pneumoniae*, respectively possessed very good antibacterial activity. This was followed by compound **4c** with 16 mm zone of inhibition against *E. coli*. The remaining compounds **4a** and **4d** with zone of inhibition 12 mm and 11 mm against *B. subtilis* and *S. aureus*, respectively showed moderate antibacterial activity. El-Fattah et al. [16] and Singh et al. [17] have reported moderate activity of some DHPMs analogs synthesized in a Biginelli condensation reaction of a mixture containing substituted aldehydes against human pathogenic bacteria.

CONCLUSION

Four novel Biginelli type heterocyclic compounds of dihydropyrimidinones analogous were synthesized and characterized. These compounds were screened for their antibacterial activity. As seen from the results, the tested compounds showed variable antimicrobial activities which range from moderate to high. Compounds **4b** and **4d** were found potent when compared with that of the standard drug ciprofloxacin.

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Figure No. 1: The synthesis of 3,4-dihydropyrimidin-2(1H)-one

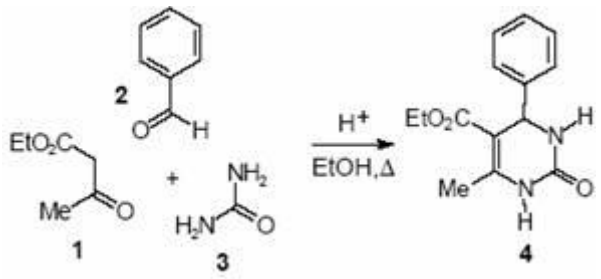


Figure No. 2: Examples of biologically active DHPMs

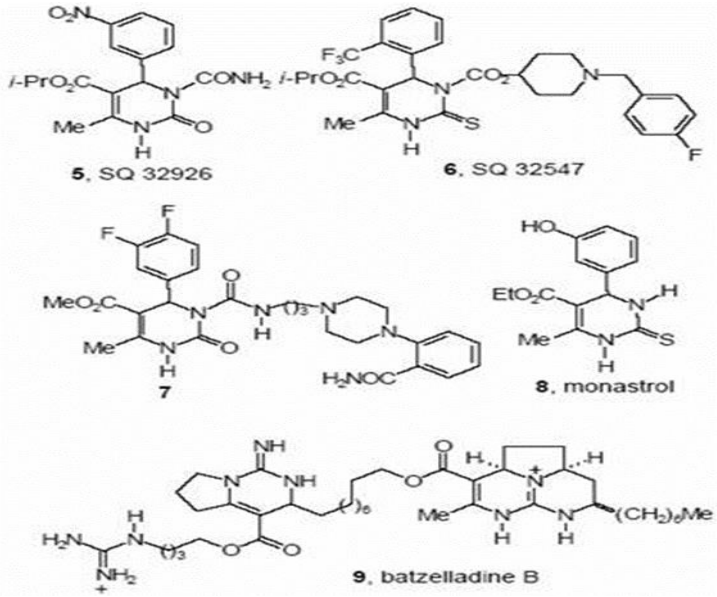
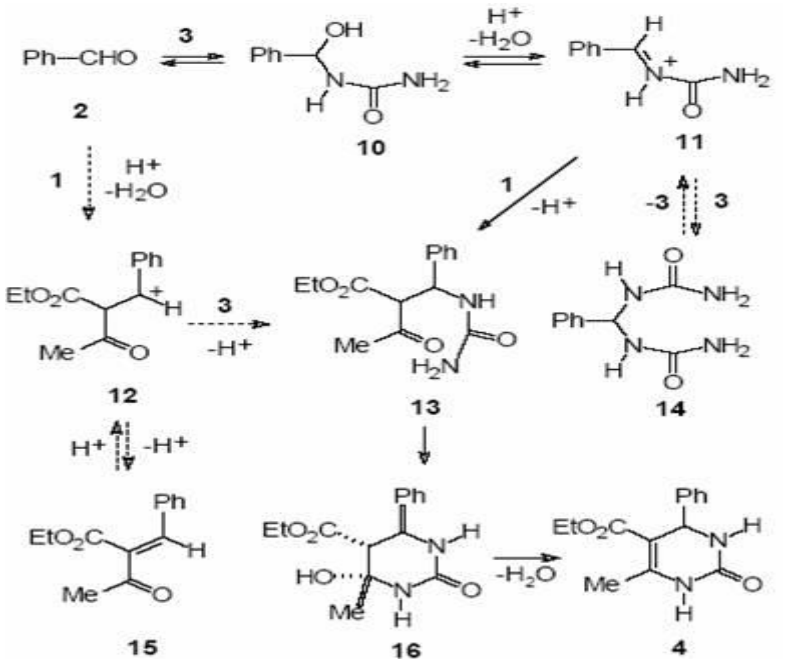
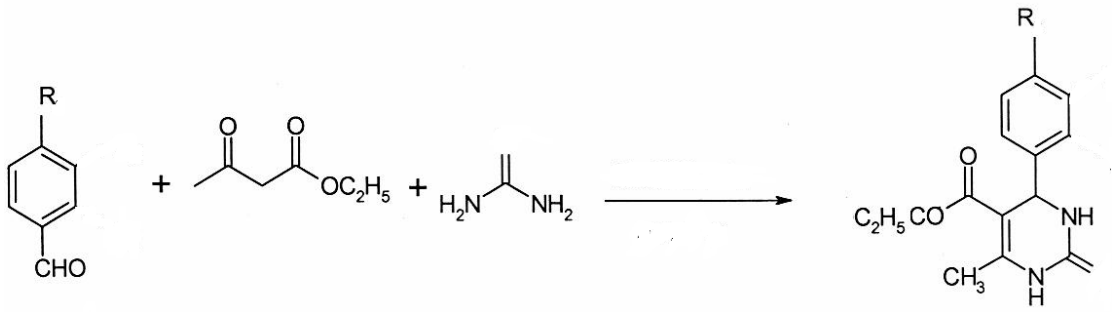


Figure No. 3: The mechanism of the Biginelli reaction



Scheme I: Biosynthesis of Biginelli analogs



Where R = NO_2 (4a); -OCH_3(4b); -H(4c); -OH(4d)

Fig. 4. IR spectrum of compound 4b

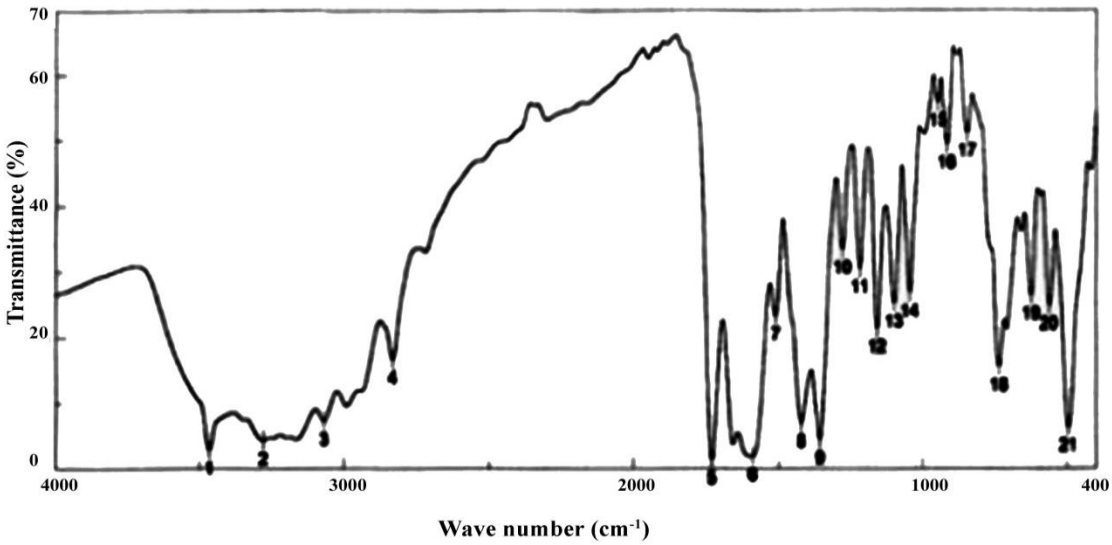


Fig. 5. 1H-NMR spectrum of compound 4b

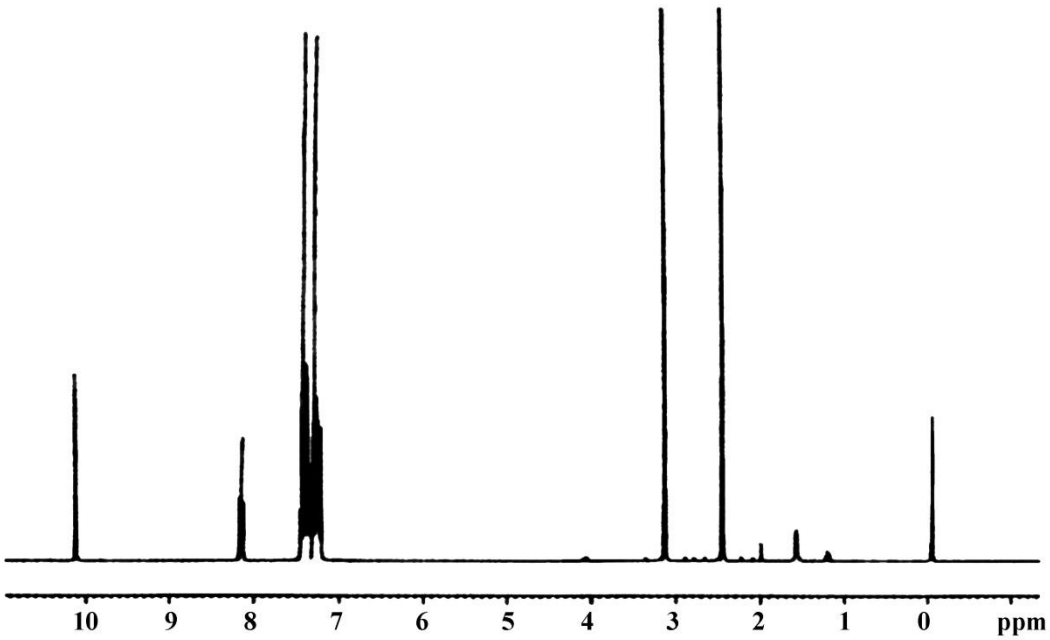


Table No. 1: Physical data of the synthesized heterocyclic analogous

S. No.	R	Yield (%)	Mass	M.P.
4a	NO_2	95	321	186
4b	OCH_3	90	306	208
4c	H	94	276	195
4d	OH	89	308	178

Table No. 2: Antibacterial activity of the compound 4a

Name of the organisms	Diameter of the Zone of inhibition (mm)							
	Compound 4a(in µg/mL)				Ciprofloxacin (in µg/mL)			
	274	**27.4**	**5.48**	**3.65**	**323**	**32.3**	**6.46**	**4.61**
E. coli	17	14	12	11	31	24	23	22
B, subtilis	17	15	13	12	32	31	29	28
S. typhii	13	12	11	9	30	24	21	20
K. pnemoniae	18	15	14	12	25	21	18	17
S. aureus	14	13	11	10	21	18	17	14

Table No. 3: Antibacterial activity of the compound 4b

Name of the organism	Diameter of the Zone of inhibition (mm)							
	Compound 4b (in µg/mL)				Ciprofloxacin (in µg/mL)			
	289	**28.9**	**5.78**	**3.85**	**323**	**32.3**	**6.46**	**4.61**
E. coli	13	12	11	9	31	24	23	22
B, subtilis	16	12	11	8	32	31	29	28
S. typhii	19	16	14	12	30	24	21	20
K. pnemoniae	20	18	17	16	25	21	18	17
S. aureus	14	12	11	10	21	18	17	14

Table No. 4: Antibacterial activity of the compound 4c

Name of the organisms	Diameter of the Zone of inhibition (mm)							
	Compound 4c (in μg/mL)				Ciprofloxacin (in μg/mL)			
	244	**24.4**	**4.88**	**3.25**	**323**	**32.3**	**6.46**	**4.61**
E. coli	21	18	17	16	31	24	23	22
B, subtilis	14	12	11	10	32	31	29	28
S. typhii	14	13	12	10	30	24	21	20
K. pnemoniae	13	12	11	10	25	21	18	17
S. aureus	15	13	12	11	21	18	17	14

Table No. 5: Antibacterial activity of the compound 4d

Name of the organisms	Diameter of the Zone of inhibition (mm)							
	Compound 4d (in μg/mL)				Ciprofloxacin (in μg/mL)			
	276	**27.6**	**5.52**	**3.68**	**323**	**32.3**	**6.46**	**4.61**
E. coli	20	17	14	12	31	24	23	22
B, subtilis	21	16	14	13	32	31	29	28
S. typhii	13	12	11	10	30	24	21	20
K. pnemoniae	17	15	13	11	25	21	18	17
S. aureus	16	14	12	11	21	18	17	14