FABAD J. Pharm. Sci., 26, 119-123, 2001

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Synthesis of Some New Aminoalkylguanidine Derivatives and Their In Vitro Antimicrobial Activities

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Summary : *In this study, the antimicrobial activities of seven compounds of aminoalkylguanidine derivatives were tested. Five of these compounds (Compound 1-5) were synthesized previously. Two of the compounds included in this study to evaluate antimicrobial efficiency (Compound 6,7) were synthesized originally by the reaction of S-methylthiourea and appropriate amines. The structures of these two compounds were confirmed by IR, ^{1}H-NMR and elementary analysis. The antimicrobial activity of all of the compounds was investigated by broth microdilution method by using two Gram positive (*Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212) and two Gram negative (*Escherichia coli *ATCC 25922,* Pseudomonas aeruginosa *ATCC 27853) bacteria and yeast-like fungi (*Candida albicans *ATCC 90028,* Candida krusei *ATCC 6258,* Candida parapsilosis *ATCC 22019). Among the compounds tested N-[3-(2-Adamantylamino)1-propyl]guanidine sulfate showed the most favorable activity.*

Key Words: *Aminoalkylguanidine, synthesis, antimicrobial activity, antibacterial activity, antifungal activity*

Bazı Yeni Aminoalkilguanidin Türevlerinin Sentezi ve In Vitro Antimikrobiyal Aktiviteleri

Özet : *Bu çalışmada 5 tanesi (Bileşik 1-5) önceden sentezi gerçekleştirilmiş olan 7 adet aminoalkilguanidin türevi bileşiğin antimikrobial etkinliği araştırılmıştır. Çalışmaya katılan bileşiklerden ikisi (Bileşik 6,7) S-metiltiyoüre ve uygun aminlerin reaksiyonu ile orijinal olarak sentezlenmiş ve bu yeni bileşiklerin de yapıları IR, ^{1}H-NMR ve elementel analiz ile aydınlatılmıştır. Bileşiklerin antimikrobiyal aktiviteleri ise ikisi Gram pozitif (*Staphylococcus aureus *ATCC 25923,* Enterococcus faecalis *ATCC 29212) ve ikisi Gram negatif (*Escherichia coli *ATCC 25922,* Pseudomonas aeruginosa *ATCC 27853) olmak üzere dört çeşit bakteri ve maya benzeri mantarlar (*Candida albicans *ATCC 90028,* Candida krusei *ATCC 6258,* Candida parapsilosis *ATCC 22019) kullanılarak mikrodilüsyon yöntemi ile değerlendirilmiştir. Deneye alınan bileşikler içerisinde; N-[3-(2-Adamantilamino)1-propil]guanidin sülfat en iyi aktiviteyi göstermiştir.*

Anahtar kelimeler : *Aminoalkilguanidin, sentez, antimikrobiyal aktivite, antibakteriyal aktivite, antifungal aktivite*

Received : 23.2.2001
Revised : 21.5.2001
Accepted : 29.5.2001

INTRODUCTION

It is well known that an examination of the structures of many commercially available antihypertensive drugs reveals a common functionality in the guanidine group. This group is present in compounds representing diverse classes of antihypertensives, including adrenergic neuron blockers (*guanethidine*), central α_2 agonists (*clonidine, guanabenz, guanfacine*), α_1 antagonists (*prazosin*), vasodilators (*minoxidil*) and diuretics (*amiloride, triamterene*), in addition to antibiotics (*dihydrostreptomycin sulfate*) and antiseptics (*chlorhexidine*). This widespread nature of guanidine functionality is well documented in the literature[1-8]. It has also been reported that different guanidine derivatives (I) have analgesic[6] and antimicrobial activities[6,9-15]. Thus, dihydrostreptomycin sulfate (II) and chlorhexidine (III), which have anti-

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septic activities, contain guanidine moiety in their structures as shown (Fig. 1).

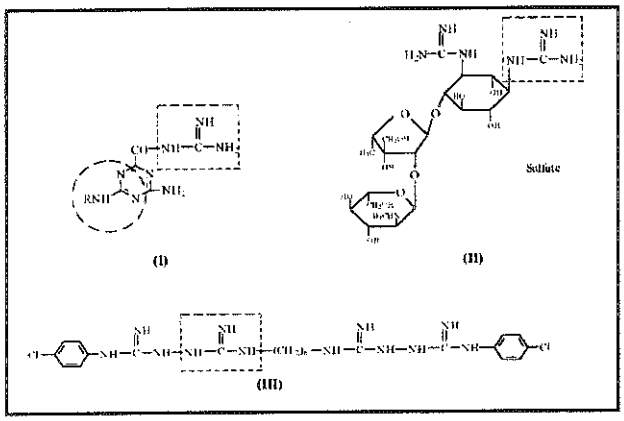
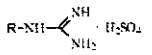
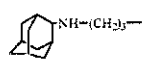
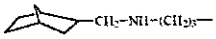
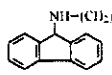
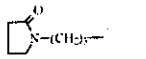
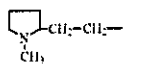


Figure 1. Structures of some antimicrobial agents containing guanidine moiety.

In view of this fact,we have synthesized several compounds having the guanidine functional group to investigate their antimicrobial activities. In this present study, the antimicrobial activity of seven compounds (Table 1) were tested, five of these compounds (Compound 1-5) were synthesized previously to evaluate their antihypertensive activities[8]. These compounds (Compound 1-5) having been synthesized again to check their antimicrobial activity, according to the method used previously[8], while two of the compounds (Compound 6,7) were synthesized specifically for this study. The seven aminoalkylguanidine derivatives used in this study, had not been evaluated from the microbiological aspect previously in literature.

Table 1. Yields and melting points of the compounds.



Compound number	R	Yield (%)	M.P.(°C)
1		93	105-7
2		96	287-8(dec.)
3		94	284-5(dec.)
4		93	273-4(dec.)
5		76	276-7
6		91	232-3
7		89	274-5(dec.)

EXPERIMENTAL SECTION

Chemistry

All chemicals used in this study were supplied by E. Merck (Darmstadt, Germany) and Aldrich (Steinheim, Germany). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA) and were uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer 1720 X (Überlingen, Germany) as Nujol mulls, liquid films or KBr disc (γ, cm^{-1}). ^{1}H-NMR spectra in DMSO-d_6 were recorded on a Bruker AC 80 MHz spectrophotometer (Karlsruhe, Germany) by using tetramethylsilane (TMS) as an internal standard (chemical shift in δ, ppm). Elemental analyses were performed by the Perkin-Elmer Model 240C and Leco CHNS-932 (Überlingen, Germany) at The Scientific and Technical Research Council of Turkey and were within ± 0.4 % of the theoretical values. The purity of the compounds was assessed by TLC on silica gel HF 254 +366 (E. Merck, Darmstadt, Germany).

The compounds were synthesized according to the well-known procedure of guanidine functionality by the reaction of amine and S-methylthiourea[5,6,8]. N-Substituted amines **1c-4c** which were used as starting materials in guanidine synthesis were prepared by refluxing of the appropriate amine or alcohol and acrylonitrile and then reduction with $LiAH_4$[16,17]. In contrast, compound **5d** was synthesized by using Çalış and et. al's method[8]. Figure 2 shows the synthesis pathway of the compounds.

General procedure for the preparation of N-[3-(Substituted)-1-propyl] guanidine sulfate (Compounds 1-7)

To a solution of appropriate amine (**1c-4c, 5d, 6a, 7a**) (0.05 mol) in 50 ml of water, S-methylisothiourea hemisulfate (0.025 mol) was added at room temperature. The mixture was heated at 50°C for 5 h under a continuous nitrogen purge. The existing gases

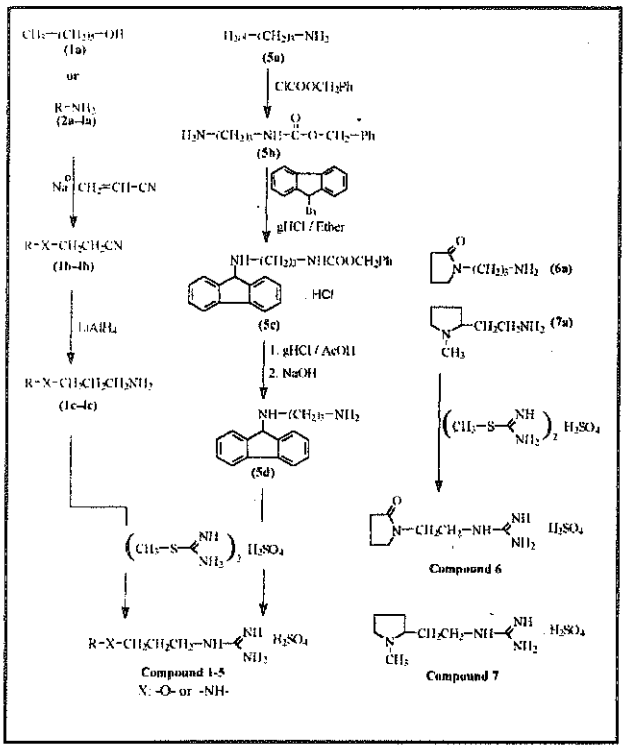


Figure 2. Synthesis of the compounds[1-7].

were passed through two flasks, each containing a saturated ethanolic solution of KOH to trap the CH_3SH by product. The mixture was then concentrated and a suitable amount of acetone was added. The precipitate was filtered and recrystallized from water to give the title compound.

Microbiology

Minimal inhibitory concentrations (MICs) were determined by broth microdilution method following the procedures recommended by the National Committee for Clinical Laboratory Standards[18,19]. Two Gram positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212) and two Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria were used as quality control strains. For testing antifungal activities of the compounds, these reference strains were tested: *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019.

Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) was used when testing bacterial strains. For Candida species, RPMI-1640 medium with L-Glutamin, buffered with MOPS (ICN, FLOW; Aurora, OH, USA) was used. The inoculum densities were 5 x 10^5 cfu/ml for bacteria and fungi, respectively. The compounds under the test were dissolved in 100 % dimethylsulfoxide and the final two fold concentrations were prepared from 512 µg/ml to 0.5 µg/ml. Amikacin and Fluconazole were used as reference powders for bacteria and fungi, respectively. Two fold dilutions were prepared from 64 µg/ml to 0.0625 µg/ml for each of these antibiotics.

MICs were determined after incubation for 24 h at 35°C. Minimal inhibitory concentrations were defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganisms.

RESULTS AND DISCUSSION

Chemistry

The aminoalkylguanidine derivatives (Table 1) were obtained as outlined in Fig2. The general procedure in fig. 2 is that of Maxwell and Ueda at al[5,6]. It was used successfully to synthesize two novel compounds (Compounds 6,7) in high yields (>89 %) as shown in **Table 1**. ^{1}H-NMR spectrum assignments for each compound were made by comparison with their previously recorded ^{1}H-NMR spectrums[8]. In the structure of the guanidine, each nitrogen atom has protones and in this structure, maximum delocalization of the positive charge is the situation, thus, the absence of NH coupling (in the absence of NH signal) is a consequence of the rate of NH exchange in ^{1}H-NMR spectrums of guanidine derivatives[20]. Therefore, NH signal regarding guanidine structure could not be observed in ^{1}H-NMR spectrums of the synthesized compounds. IR and ^{1}H-NMR spectra and elementary analysis data of the synthesized two novel compounds (Compounds 6,7) are as following.

N-[3-(2-Oxopyrrolidinyl)-1-propyl]guanidine sulfate **(Compound 6)**

This was synthesized by the general procedure using 3-(2-Oxopyrrolidinyl)propylamine and S-methylisothiourea, and crystallized from water. Yield: 91 %.

M.p.: 232-3 °C. IR (KBr, cm^{-1}) 1675 guanidinium, 3333 ^{+}NH; ^{1}H NMR (DMSO-d_6, δ, ppm) 1.80-2.60 (t, 6H, CH_2), 3.20-3.60 (t, 6H, CH2-pyrrolidine); Anal. Cal. for $C_8H_{16}N_4O.1/2H_2SO_4$ (M.W.:233.26): C, 41.20; H, 6.87; N, 24.03; Found: C, 41.12; H, 6.91; N, 24.18.

N-[2-(1-Methyl-2-pyrrolidinyl)-1-ethyl]guanidine sulfate **(Compound 7)**

This was synthesized by the general procedure using 2-(1-methyl-2-pyrrolidinyl)ethylamine and S-methylisothiourea, and crystallized from water. Yield: 89 %. M.p.: 274-5°C (dec.). IR (KBr, cm_{-1}) 1666 guanidinium, 3349 ^{+}NH; ^{1}H NMR (DMSO-d_6, δ, ppm) 1.80-2.60 (t, 4H, CH_2), 3.00 (s, 3H, CH_3), 3.20-3.60 (t, 6H, CH_2-pyrrolidine); Anal. Cal. for $C_8H_{18}N_4.H_2SO_4$ (M.W.:268.30): C, 35.81; H, 7.51; N, 20.88; Found: C, 35.92; H, 7.36; N, 20.73.

Microbiology

Antibacterial activity results of the compounds against Gram positive and Gram negative bacteria are shown in Table 2. When antibacterial activity results were investigated, none of the tested compounds were found to be active. The results of screening for antifungal activity of the compounds are reported in Table 3. As the N-[3-(1-adamantylamino)-1-propyl]-guanidine sulfate (Compound 2) was not soluble in the 100% dimethylsulfoxide solvent used, it was not possible to measure its antimicrobial activity. MIC values showed that N-[3-(2-adamantylamino)-1-propyl]-guanidine sulfate (Compound 3) was the most active compound to fluconazole against three fungi used among the tested compounds. N-[3-(2-Oxopyrrolidinyl)-1-propyl]guanidine sulfate (Compound 6) had activites similar to fluconazole against *Candida krusei*. N-[3-(2-adamantylamino)1-propyl]guanidine sulfate (Compound 3) was more effective than fluconazole against *Candida krusei* among the aminoalkylguanidine derivatives, tested.

Table 2. Bacteriostatic activity results of the compounds (MIC µg/ml.)

Compound no	*S.aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	>64	>64	>64	>64
2	–	–	–	–
3	>64	>64	>64	>64
4	>64	>64	>64	>64
5	>64	>64	>64	>64
6	>64	>64	>64	>64
7	>64	>64	>64	>64
Amikacin	2	64	1	4

Table 3. Antifungal activity results of the compounds (MIC µg/ml.)

Compound no	*C. albicans*	*C. krusei*	*C. parapsilosis*
1	>64	>64	>64
2	–	–	–
3	2	16	32
4	>64	>64	>64
5	>64	>64	>64
6	8	64	>64
7	>64	>64	>64
Flucanazole	0.25	64	2

CONCLUSION

Among the synthesized aminoalkylguanidine derivatives, N-[3-(2-adamantylamino)1-propyl] guanidine sulfate (Compound 3) were found to have significantly high antifungal activity against *Candida krusei*. We assume that Compound 3 is the most important compound in the series regarding its antifungal activity.

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