Synthesis, Characterization and Biological Activity of bis(3-Aryl-1-hexahydropyrimidinyl)methanes. Novel Heterocyclic Polyamine Derivatives.

Juan Á. Bisceglia [a], María B. García [a], Rosana Massa [b], María L. Magri [a], Mariana Zani [a], Gabriel O. Gutkind [b] and Liliana R. Orelli [a]*

[a] Departamento de Química Orgánica, [b] Departamento de Microbiología. Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 (1113), Buenos Aires, Argentina.

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A method is described for the synthesis of bis(3-aryl-1-hexahydropyrimidinyl)methanes **1**, by condensation of *N*-aryl-1,3-propanediamines **2** with formaldehyde. The reaction mechanism involves *N*-arylhexahydropyrimidines **3** as intermediates. Such compounds can also be prepared efficiently by a methylene exchange reaction between bis-hexahydropyrimidines **1** and the corresponding diamines **2**. The antimicrobial activity of compounds **1** was evaluated by the disk diffusion method and some of them showed moderate to good growth inhibition activity.

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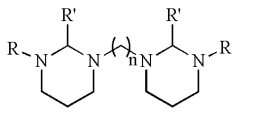
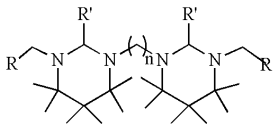
Introduction.

Naturally occurring polyamines are of biochemical interest as they are involved in cellular proliferation and differentiation processes [1], while some synthetic analogues behave as potent antibiotics and antineoplastics [2]. In the last years some linear polyamines containing 1,3-propanediamine moieties were reported that possess interesting pharmacological properties, acting as antineoplastic, antiviral and antispasmodic agents [3]. In this sense, cyclic analogues containing hexahydropyrimidine moieties (Scheme I) were investigated, as they share some pharmacological features of the parent compounds [4]. It is well known that bioreversible transformation of 1,*n*-diamines into the corresponding cyclic aminals increases their lipophylicity, thus improving their bioavailability [5].

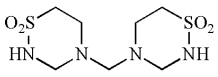
Scheme I



n=3-6; R=C_1-C_{12}; R'=C_1-C_{12}



n=8,9; R=H, CH_3, C_2H_5; R'=H,C_1-C_5, C_6H_5

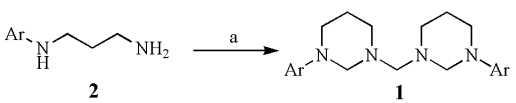


Taurolidine

The method described in the literature for the synthesis of bis(3-alkyl-1-hexahydropyrimidinyl)alkanes involves acylation of the parent heterocyclic polyamine followed by reduction of the resulting *N,N'*-diacyl derivatives [6]. It is therefore restricted to primary *N*-alkyl derivatives where both heterocylic moieties are connected by a polymethylene chain (n≥2, Scheme I). Continuing ongoing research on heterocycles containing the 1,3-propanediamine moiety [7], we present here an easy approach to *N*-aryl substituted bis(1-hexahydropyrimidinyl)methanes **1**, by condensation of *N*-aryltrimethylenediamines with formaldehyde (Scheme II). Experimental evidence indicated that the reaction mechanism involves heterocyclization of precursors **2** to the corresponding 1-arylhexahydropyrimidines **3** followed by intermolecular condensation.

The structure of compounds **1**, bearing both endo and exocyclic N-CH_2-N moieties, is analogous to that of the thiadiazine taurolidine (Scheme I). Taurolidine is a known bactericide [8a] that also inactivates bacterial endotoxins [8b]. It was demonstrated that the mechanism of its bactericidal action involves delivery of formaldehyde as methylene iminium ion ($H_2C{=}N^+$), which originates from both endo and exocyclic N-CH_2-N moieties within its structure [9]. The close structural similarity of taurolidine and compounds **1** under study prompted us to investigate their antibacterial and antifungal activity *in vitro*.

Scheme II



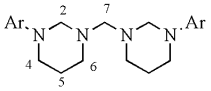
2 **1**

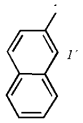
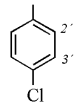
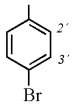
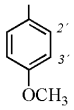
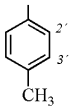
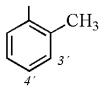
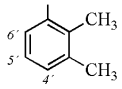
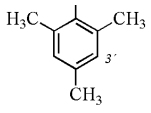
[a] aq. H_2CO excess/methanol, 4h, room temperature.

Compd.	**1,2a**	**1,2b**	**1,2,3c**	**1,2d**	**1,2e**	**1,2f**	**1,2g**	**1,2h**	**1,2i**	**1,2j**	**1,2k**
Ar	C_6H_5	2-$C_{10}H_7$	4-ClC_6H_4	3-ClC_6H_4	2-ClC_6H_4	4-BrC_6H_4	4-$CH_3OC_6H_4$	4-$CH_3C_6H_4$	2-$CH_3C_6H_4$	2,3-$(CH_3)_2C_6H_3$	2,4,6-$(CH_3)_3C_6H_2$

Table I

Bis(3-Aryl-1-hexahydropyrimidinyl)methanes **1a-k**



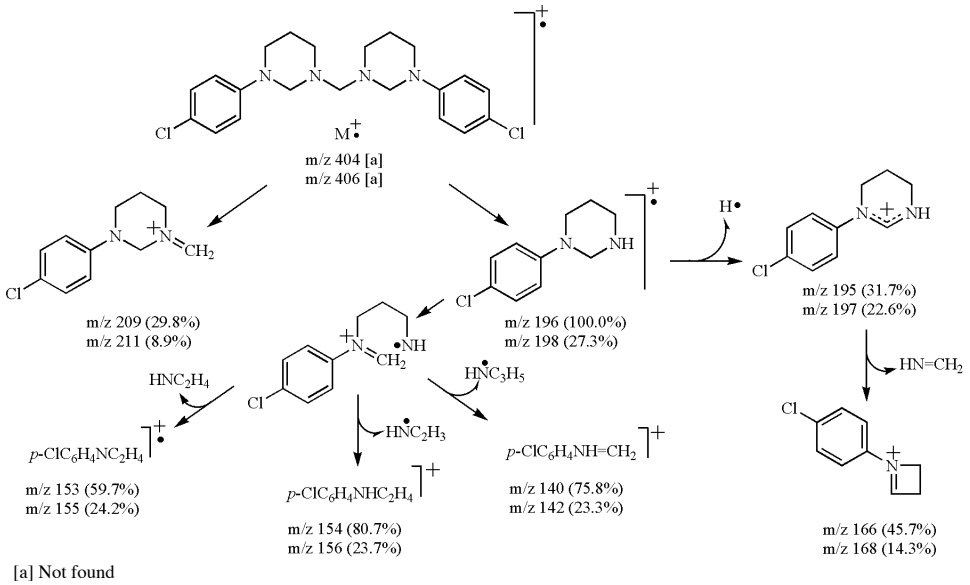
Compd. No.	Ar	Yield (%)	Mp (°C)	Formula	Analyses (Calcd./Found) %C	%H	%N	Mass [M+1]+	^{1}H nmr H2	H4	H5	H6	H7	Ar
1a		82	51-52 [a]	$C_{21}H_{28}N_4$	74.96 74.76	8.39 8.44	16.65 16.70	337	4.12 (s)	3.34 (t)	1.74 (p)	2.83 (t)	3.36 (s)	*2':* 7.23 (m) *3':* 7.26 (m) *4':* 6.82 (m)
1b		61	oil	$C_{29}H_{32}N_4$	79.78 79.92	7.39 7.33	12.83 12.79	437	4.25 (s)	3.47 (t)	1.82 (p)	2.90 (t)	3.44 (s)	*1':* 7.23 (d) *3'-8':* 7.61-7.69 (m), 7.28-7.35 (m)
1c		89	129-130 [a]	$C_{21}H_{26}Cl_2N_4$ 61.93	62.22 6.43	6.46 13.86	13.82	405	4.05 (s)	3.30 (t)	1.71 (p)	2.79 (t)	3.27 (s)	*2':* 6.82 (dd) *3':* 7.15 (dd)
1d		78	oil	$C_{21}H_{26}Cl_2N_4$	62.22 62.54	6.46 6.42	13.82 13.77	405	4.10 (s)	3.27 (t)	1.71 (p)	2.82 (t)	3.33 (s)	*2':* 6.88 (s) *4':* 7.14 (d) *5':* 6.80 (t) *6':* 7.09 (d)
1e		74	oil	$C_{21}H_{26}Cl_2N_4$	62.22 62.48	6.46 6.49	13.82 13.75	405	3.97 (s)	3.22 (t)	1.72 (p)	2.88 (t)	3.64 (s)	*3':* 7.23 (dd) *4':* 6.94 (m) *5':* 7.30 (m) *6':* 7.15 (m)
1f		87	131-132 [a]	$C_{21}H_{26}Br_2N_4$	51.03 51.12	5.30 5.33	11.34 11.34	493	4.06 (s)	3.31 (t)	1.70 (p)	2.80 (t)	3.27 (s)	*2':* 6.78 (d) *3':* 7.29 (d)
1g		79	oil	$C_{23}H_{32}N_4O_2$	69.67 69.54	8.13 8.16	14.13 14.19	397	3.97 (s)	3.20 (t)	1.75 (p)	2.79 (t)	3.37 (s)	*2':* 6.81 (dd) *3':* 6.91 (dd) OCH_3: 3.75 (s)
1h		84	oil	$C_{23}H_{32}N_4$	75.78 75.59	8.85 8.89	15.37 15.43	365	4.05 (s)	3.28 (t)	1.75 (p)	2.81 (t)	3.36 (s)	*2':* 6.86 (d) *3':* 7.04 (d) CH_3: 2.28 (s)
1i		76	oil	$C_{23}H_{32}N_4$	75.78 75.64	8.85 8.91	15.37 15.44	365	3.80 (s)	3.05 (t)	1.75 (p)	2.86 (t)	3.61 (s)	*3'-5':* 7.08-7.17 (m), *6':* 6.98 (dd) CH_3: 2.33 (s)
1j		72	oil	$C_{25}H_{36}N_4$	76.49 76.63	9.24 9.26	14.27 14.17	393	3.75 (s)	2.99 (t)	1.75 (p)	2.84 (t)	3.58 (s)	*4'-6':* 6.88 (d), 6.95-7.05 (m) $C_6H_3CH_3$: 2.23 (s), 2.25 (s)
1k		66	oil	$C_{27}H_{40}N_4$	77.10 77.25	9.58 9.54	13.32 13.24	421	3.93 (s)	3.10 (t)	1.72 (p)	2.83 (t)	3.61 (s)	*3', 5':* 6.78 (s) $C_6H_2CH_3$ *ortho:* 2.25 (s), *meta:* 2.22 (s)

[a] Crystalized from methanol/water.

Results and Discussion.

The method involves treatment of a methanolic solution of *N*-aryl-1,3-propanediamines **2** with an excess aqueous formaldehyde, and leads to bis(3-aryl-1-hexahydropyrimidinyl)methanes **1** as the sole products (Scheme II). Yields, melting points, elemental analyses and ^{1}H NMR data of compounds **1** are given in Table I. ^{13}C NMR spectrum of compound **1c** is described in the experimental section.

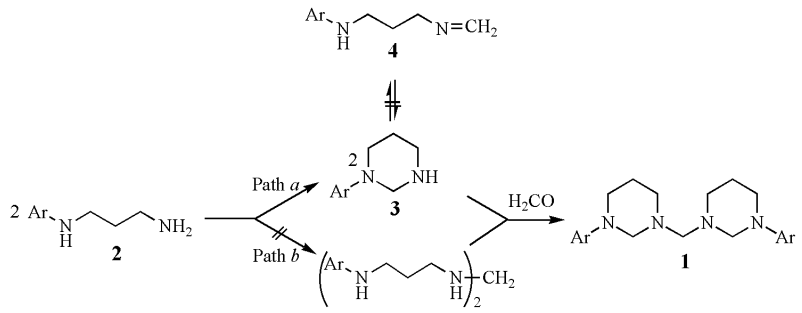
Scheme III



[a] Not found

Most relevant fragments in the mass spectrum (EI, 20 eV) of compound **1c**.

Scheme IV



FAB$^+$ mass spectra of compounds **1** showed the corresponding $[M+1]^+$ peaks, confirming their molecular weights (Table I). The electron impact (20 eV) spectrum of compound **1c** was also analyzed on the basis of literature data on six [10] and five [11] membered cyclic aminals. The most important fragmentation pathways proposed for **1c** are depicted in Scheme III.

The two possible courses that can be expected for the condensation leading to compounds **1** are shown in Scheme IV (Ar=*p*-ClC_6H_4). They involve either condensation affording cyclic aminals **3** followed by intermolecular reaction (Path *a*) or the inverse sequence, *i.e.*, intermolecular aminal formation followed by heterocyclization (Path *b*). In order to investigate both possibilities, we performed the reaction of trimethylenediamine **2c** with an equimolar amount of formaldehyde. ^{1}H nmr analysis of the crude reaction mixture after workup showed the presence of compound **1c**, its precursor **2c** and a third predominant species whose spectral features were consistent with 1-(*p*-chlorophenyl)-hexahydropyrimidine **3c**. To avoid formation of bis-hexahydropyrimidine **1c**, the reaction was repeated with a diamine/formaldehyde molar ratio of 1/0.5. In such conditions, a mixture of compounds **2c** and **3c** was obtained, as disclosed by the ^{1}H nmr spectrum of the crude reaction mixture. An analogous reaction was previously reported by Evans for *N-tert*-butyl-1,3-propanediamine [10]. Attempts of chromatographic purification of compound **3c** on Silica gel or neutral Alumina led to its partial hydrolysis, regenerating **2c**. Synthesis of **3c** was also attempted by a methylene exchange reaction between bis-hexahydropyrimidine **1c** and diamine **2c**. Flores-Parra *et al.* reported an analogous reaction in a bis(1,3-oxazolidine-3-yl)methane derivative, containing N-CH_2-N as well as N-CH_2-O functionalities. Treatment of such compound with an equimolar amount of the aminoalcohol in refluxing toluene originated the corresponding N-H oxazolidine with good yields [12]. In such conditions, reaction between bis-hexahydropyrimidine **1c** and diamine **2c** led quantitatively to hexahydropyrimidine **3c**, indicating complete transference of the more labile exocyclic methylene group in the N-CH_2-N function (Scheme V, Ar=*p*-ClC_6H_4). This reaction represents a high yield synthesis of reactive N-H hexahydropyrimidines **3**, which

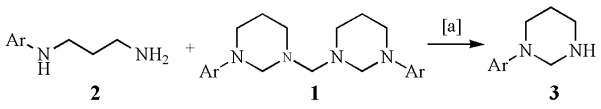
Table II

Growth Inhibition Activity [a] of Bis(3-aryl-1-hexahydropyrimidinyl)methanes **1a-k** against *B. subtilis, E. coli, M. luteus, L. monocytogenes, P. aeruginosa, S. aureus, C. albicans and A. niger* [b] *in vitro*

Compd.	BS		EC		ML		LM		PA		SA		CA		AN	
	AM1	AM11	AM1	AM11	AM1	AM11	AM1	AM11	AM1	AM11	AM1	AM11	AM1	AM11	AM1	AM11
1a	6.0	6.7	8.0	6.0	-	-	9.3	-	-	-	-	-	-	-	-	-
1b	6.6	5.8	7.0	-	-	17.0	7.0	-	-	-	-	-	-	9.0	-	11.5
1c	6.0	-	7.0	-	-	11.6	10.0	-	8.0	8.0	-	-	-	-	-	7.3
1d	6.8	6.0	7.7	6.0	-	13.7	9.0	-	-	-	-	-	-	-	-	9.0
1e	7.7	7.0	9.0	6.8	-	9.0	9.0	-	6.0	6.0	-	-	-	-	-	9.0
1f	6.1	-	6.0	-	-	11.6	-	-	8.0	7.0	-	-	-	7.8	-	8.0
1g	-	-	-	-	-	-	-	-	8.0	7.0	-	-	-	-	-	-
1h	6.7	6.0	7.7	7.0	-	8.3	7.7	-	6.0	-	-	-	-	-	-	6.0
1i	7.0	6.0	8.5	6.0	-	9.8	8.0	-	6.0	-	-	-	-	-	-	-
1j	-	-	-	-	8.0	11.5	-	-	-	-	-	-	-	-	-	-
1k	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Diameter (in mm) of inhibition zones; [b] BS: *B. subtilis*, EC: *E. coli*, ML: *M. luteus*, LM: *L. monocytogenes*, PA: *P. aeruginosa*, SA: *S. aureus*, CA: *C. albicans*, AN: *A. niger*, AM1: antibiotic medium N°1 (pH=6.5), AM11: antibiotic medium N°11 (pH=7.9).

Scheme V



[a] toluene, reflux, 24 h.

cannot be prepared efficiently and directly from the diamine and formaldehyde. Interestingly, the ^{1}H and ^{13}C nmr spectra of compound **3c** did not show any signals attributable to the tautomeric Schiff base **4c** (Scheme IV, Ar=*p*-ClC_6H_4). Previous reports on structurally related *N*-monosubstituted cyclic aminals indicate that such heterocycles are in equilibrium with their open-chain tautomers. Ring-chain tautomerism of five and six membered 1,3-diazaheterocycles represents an area of increasing interest in recent years [13]. This is because it is involved in important biochemical reactions of the natural cofactor N^5, N^{10}-methylenetetrahydrofolic acid, which contains a diazolidine moiety that undergoes ring-chain tautomerism while acting as a methylene transfer agent [14]. From the synthetic point of view, selective monofunctionalization of ethylene and propylenediamines can be achieved on the basis of the tautomeric character of their 2-substituted-1,3-diazaheterocyclic derivatives [15]. Although literature on hexahydropyrimidines is almost restricted to *N*-alkyl derivatives [13a,e], Göblyös *et al.* recently reported the reaction of *N*-phenyl-1,3-propanediamine with several aromatic aldehydes, which leads to an equilibrium between ring and chain tautomers where the latter predominate [13f]. The virtual absence of the open-chain tautomer in the case of compound **3c** may be attributed to the higher stability of hexahydropyrimidines devoid of a 2-substituent, which was previously observed by us in closely related systems [7c].

The structural similarity of taurolidine (Scheme I) and compounds of type **1** under study prompted us to investigate their antibacterial and antifungal activity *in vitro*. For this purpose the disk diffusion method was used. Results, measured as the diameters of inhibition zones for each microorganism, are reported in Table II.

In conclusion, we report an efficient method for the synthesis of bis(3-aryl-1-hexahydropyrimidinyl)methanes **1**, novel heterocyclic polyamine derivatives containing two types of aminal functionalities. The ability of compounds **1** to act as formaldehyde donors was demonstrated in an exchange reaction where the exocyclic N-CH_2-N moiety of **1c** was transferred to the corresponding *N*-aryl-1,3-propanediamine **2c**, yielding *N*-arylhexahydropyrimidine **3c**. Some bis-hexahydropyrimidines **1** showed moderate to good growth inhibition activity against *E. coli*, *M. luteus*, *L. monocytogenes* and *A. niger* in a preliminary screening.

EXPERIMENTAL

Melting points were taken on a Büchi capillary apparatus and are uncorrected. ^{1}H and ^{13}C nmr spectra were recorded on a Bruker MSL 300 MHz spectrometer. Deuteriochloroform was used as the solvent, and the standard concentration of the samples was 20 mg/ml. Chemical shifts are reported in ppm (δ) relative to TMS as an internal standard. Deuterium oxide was employed to confirm exchangeable protons (ex). Splitting multiplicities are reported as singlet (s), broad signal (bs), doublet (d), double doublet (dd), triplet (t), double triplet (dt), quartet (q), pentet (p) and multiplet (m). Electron impact mass spectra were recorded with a GC-MS Shimadzu QP-1000 spectrometer operating at 20 eV. Mass spectra (FAB^+) were taken on a VG ZAB-SEQ4F Mass Spectrometer. FAB^+ desorption was performed using a Cesium ion gun (30 kV). Samples were supported on a glycerol matrix. Tlc analyses were carried out on aluminium sheets Alumina 60 F_{254} using chloroform as the solvent. Column chromatographies were performed on Aluminium Oxide (neutral, grade I, 70-230 mesh), with typically 30-50 g of stationary phase per gram substance. Reagents, solvents and starting materials were purchased from standard sources and purified according to literature procedures.

N-aryl-1,3-propanediamines **2** were obtained according to the literature procedure [16]. Yields, physical data and elemental analyses of new compounds are as follows.

N-(β-Naphthyl)-1,3-propanediamine (**2b**).

This compound was obtained as an oil (67%); ^{1}H nmr: δ 7.66 (1H, d, aromatics), 7.61 (2H, d, aromatics), 7.36 (1H, dt, aromatics), 7.19 (1H, dt, aromatics), 6.88 (1H, dd, aromatics), 6.81 (1H, d, aromatics), 3.31 (2H, t, CH_2NAr), 2.90 (2H, t, CH_2NH$_2$), 1.84 (2H, p, C-CH$_2$-C), 1.42 (2H, bs, ex, NH$_2$); ms: m/z 200 ($M^{+\cdot}$).

Anal. Calcd for $C_{13}H_{16}N_2$: C, 77.96; H, 8.05; N, 13.99. Found: C, 78.07; H, 8.08; N, 13.93.

N-(m-Chlorophenyl)-1,3-propanediamine (**2d**).

This compound was obtained as an oil (74%); ^{1}H nmr: δ 7.05 (1H, t, H5'), 6.61 (1H, dd, H4'), 6.56 (1H, t, H2'), 6.45 (1H, dd, H6'), 3.17 (2H, t, CH_2NAr), 2.85 (2H, t, CH_2NH$_2$), 1.75 (2H, p, C-CH$_2$-C), 1.61 (2H, bs, ex, NH$_2$); ms: m/z 184 ($M^{+\cdot}$), 186 ($M+2^{+\cdot}$).

Anal. Calcd for $C_9H_{13}ClN_2$: C, 58.54; H, 7.10; N, 15.17. Found: C, 58.66; H, 7.07; N, 15.13.

N-(o-Chlorophenyl)-1,3-propanediamine (**2e**).

This compound was obtained as an oil (71%); ^{1}H nmr: δ 7.23 (1H, dd, H3'), 7.13 (1H, dt, H5'), 6.58-6.67 (2H, m, H4' and H6'), 4.55 (1H, bs, ex, N*H*Ar), 3.24 (2H, t, CH_2NAr), 2.87 (2H, t, CH_2NH$_2$), 1.82 (2H, p, C-CH$_2$-C), 1.70 (2H, bs, ex, NH$_2$); ms: m/z 184 ($M^{+\cdot}$), 186 ($M+2^{+\cdot}$).

Anal. Calcd for $C_9H_{13}ClN_2$: C, 58.54; H, 7.10; N, 15.17. Found: C, 58.65; H, 7.07; N, 15.12.

N-(*p*-Bromophenyl)-1,3-propanediamine (**2f**).

This compound was obtained as an oil (73%); ^{1}H nmr: δ 7.10 (2H, d, H3'and H5'), 6.52 (2H, d, H2'and 4'), 3.16 (2H, t, CH_2NAr), 2.85 (2H, t, CH_2NH$_2$), 1.90 (2H, bs, ex, NH$_2$), 1.75 (2H, p, C-CH$_2$-C); ms: m/z 228 ($M^{+\cdot}$), 230 ($M+2^{+\cdot}$).

Anal. Calcd for $C_9H_{13}BrN_2$: C, 47.18; H, 5.72; N, 12.23. Found: C, 47.26; H, 5.70; N, 12.19.

N-(2,3-Dimethylphenyl)-1,3-propanediamine (**2j**).

This compound was obtained as an oil (77%); ^{1}H nmr: δ 7.02 (1H, t, H5'), 6.58 (1H, d, H4'), 6.51 (1H, d, H6'), 3.23 (2H, t, CH_2NAr), 2.88 (2H, t, CH_2NH$_2$), 2.29 (3H, s, CH$_3$C$_6$H$_3$), 2.04 (3H, s, CH$_3$C$_6$H$_3$), 1.82 (2H, p, C-CH$_2$-C), 1.60 (2H, bs, ex, NH$_2$); ms: m/z 178 ($M^{+\cdot}$).

Anal. Calcd for $C_{11}H_{18}N_2$: C, 74.11; H, 10.18; N, 15.71. Found: C, 73.98; H, 10.24; N, 15.76.

N-(2,4,6-Trimethylphenyl)-1,3-propanediamine (**2k**).

This compound was obtained as an oil (73%); ^{1}H nmr: δ 6.82 (2H, s, H3'and H5'), 3.21 (2H, t, CH_2NAr), 2.86 (2H, t, CH_2NH$_2$), 2.25 (6H, s, *ortho* CH_3C$_6$H$_2$), 2.21 (3H, s, *para* CH_3C$_6$H$_2$), 1.81 (2H, p, C-CH$_2$-C), 1.60 (2H, bs, ex, NH$_2$); ms: m/z 192 ($M^{+\cdot}$).

Anal. Calcd for $C_{12}H_{20}N_2$: C, 74.95; H, 10.48; N, 14.57. Found: C, 74.81; H, 10.52; N, 14.61.

Bis(3-aryl-1-hexahydropyrimidinyl)methanes **1**.

General Procedure.

A solution of the corresponding *N*-aryl-1,3-propanediamine **2** (2 mmoles) in methanol (20 ml) was treated with 4 ml of aqueous formaldehyde. The solution was stirred at room temperature until disappearance of the starting material, as disclosed by tlc (neutral Alumina, chloroform). The solvent was then evaporated *in vacuo*. The residue was treated with saturated sodium carbonate solution (5 ml) and extracted with methylene chloride (3 x 20 ml). The organic phases were pooled, washed with water (10 ml) and dried over anhydrous sodium sulphate. The solution was concentrated *in vacuo* and the residue purified by column chromatography (neutral Alumina, chloroform). Yields, melting points, elemental analyses and ^{1}H nmr data of compounds **1** are given in Table I.

Bis(3-*p*-Chlorophenyl-1-hexahydropyrimidinyl)methane (**1c**).

Compound **1c** has ^{13}C nmr: δ 148.7 (C1'), 124.0 (C4'), 128.8 (C3'), 117.8 (C2'), 72.8 (C7), 70.1 (C2), 50.6, 49.4 (C4 and C6), 22.5 (C5).

1-*p*-Chlorophenylhexahydropyrimidine (**3c**).

A solution of *N*-(*p*-chlorophenyl)-1,3-propanediamine **2c** (0.5 mmol) and bis(3-*p*-chlorophenyl-1-hexahydropyrimidinyl)-methane **1c** (0.5 mmol) in toluene was refluxed for 24 h and the solvent was evaporated *in vacuo*. The reaction was monitored by ^{1}H nmr analysis. An analytical sample of **3c** was obtained by recrystallization from cyclohexane, mp 85-86°; ^{1}H nmr: δ 7.20 (2H, d, 2 *meta* H), 6.83 (2H, d, 2 *ortho* H), 4.15 (2H, s, N-CH$_2$-N), 3.32 (2H, t, CH_2NAr), 2.94-2.98 (2H, m, CH_2NH), 1.72 (2H, p, C-CH$_2$-C), 1.64 (1H, bs, ex, NH); ^{13}C nmr: δ 149.1 (C1'), 129.1 (C3'), 125.4 (C4'), 117.9 (C2'), 66.7 (C2), 49.3 (C4), 45.1 (C6), 26.6 (C5); ms: m/z 196 ($M^{+\cdot}$), 198 ($M+2^{+\cdot}$).

Anal. Calcd. for $C_{10}H_{13}ClN_2$: C, 61.07; H, 6.66; N, 14.24. Found: C, 60.98; H, 6.69; N, 14.29.

Antimicrobial Activity of Compounds **1a-k**.

Disk Diffusion Method.

Test disks (6 mm in diameter) impregnated with 100 μg of the appropriate sample were used to test antibacterial and antifungal activity at pH 6.5 and 7.9. Disks were applied on the surface of plates containing each 25 ml of Antibiotic medium N°1 (pH=6.5) or N°11 (pH=7.9), inoculated with 10^6 CFU/ml of the microorganisms. The following strains were used: *Bacillus subtilis* ATCC 6633 CCM-A-10, *Micrococcus luteus* ATCC 9341 CCM-A-45, *Staphylococcus aureus* ATCC 6538P CCM-A-305, *Listeria monocytogenes* ATCC 15313 CCM-A-454, *Escherichia coli* ATCC 11105 CCM-A-424, *Pseudomonas aeruginosa* ATCC 9027 CCM-A-39, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Growth inhibition was tested after a 24 hour incubation at 37°. Results, expressed as the diameter (in mm) of inhibition zones, are shown in Table II.

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[*] Author to whom correspondence should be addressed. E-mail: lorelli@ffyb.uba.ar.

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