Microwave-assisted Three-component Synthesis and *In Vitro* Antifungal Evaluation of 6-Cyano-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-ones

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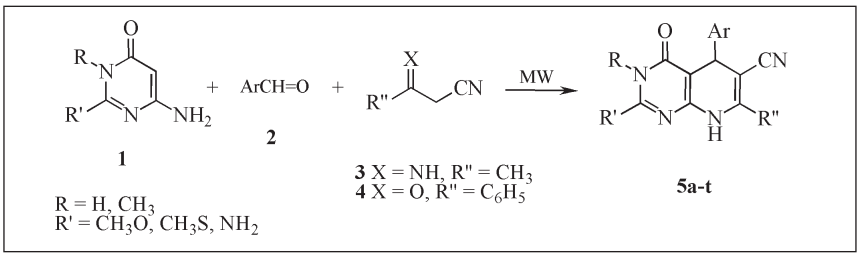

The reaction of 6-aminopyrimidin-4-ones **1** with benzaldehydes **2** and *β*-aminocrotononitrile **3** or benzoylacetonitrile **4** under microwave irradiation in dry media yields the 6-cyano-5,8-dihydropyrido[2,3-*d*]-pyrimidinones **5a-t**. The structure of the synthesized compounds was determined on the basis of nmr measurements, especially by ^{1}H,^{1}H-, ^{1}H,^{13}C COSY, DEPT and NOESY experiments. In contrast with other pyrido-[2,3-*d*]pyrimidine derivatives, these compounds did not show any antifungal *in vitro* activity up to 250 μg/mL.

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Introduction.

The so-called Multi-component reactions (MCRs), in which more than two starting materials together in a reaction vessel combine to form a final product, have been efficiently used to generate chemical diversity in a few reaction steps [1]. Compared to conventional organic reactions, MCRs show the advantages of being highly convergent and easy to perform. In particular, MCRs have been fruitful for the versatile synthesis of heterocycles by a sequence of an initial MCR followed by ring closure reaction [1].

In turn, high-speed syntheses with microwave have attracted considerable attention in recent years [2]. The potential application of microwave technology in organic synthesis [3], particularly in free-solvent conditions, is increasing at a good rate because of its reaction simplicity, less pollution, and minimum reaction time providing rapid access to large libraries of diverse molecules.

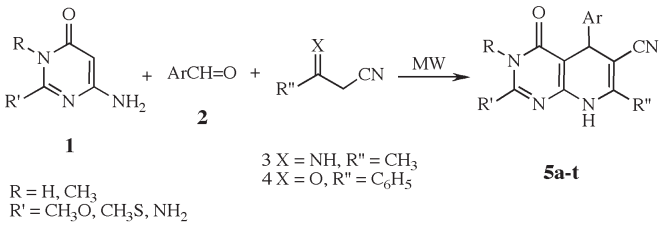
The synthesis of pyridopyrimidine and their derivatives is of high interest in medicinal chemistry, because some of them possess biological and pharmacological activities, such as antifolate [4], antibacterial [5], tyrosine kinase [6], antimicrobial [7], calcium channel antagonists [8], anti-inflammatory and analgesic [9], antileishmanial [10], tuberculostatic [11], anticonvulsants [12], diuretic and potassium-sparing [13], antiaggressive [14] and antifungal [15] activities. In addition, pyridopyrimidines are used as growth promoters, herbicides, agricultural fungicides and UV absorbants [16].

Results and Discussion.

In this paper, we describe a facile three-component, one-spot condensation induced by microwave, between 2,3-substituted-6-aminopyrimidines **1** with R = H, CH_3 and R' = CH_3O, CH_3S, NH_2, 4-substituted benzaldehydes **2** and *β*-aminocrotononitrile **3** or benzoylacetonitrile **4**, which gave 6-cyanopyridopyrimidines **5a-t** (Scheme 1; yield, time of reaction and melting points of compounds **5a-t** are recorded in Table 1). The equimolar amounts of starting

compounds such as amines, nitriles and aldehydes, were placed into pyrex-glass open vessels and irradiated in a microwave oven (600 W) for 2-15 minutes.

Scheme 1



The structures of all obtained compounds were assigned by ^{1}H- and ^{13}C-NMR spectra (Table 2 and 3) and mass spectrometry (see experimental part). Based on ^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC and NOESY techniques, it was possible to assign all protons and carbon atoms of new products.

For example, compound **5b** shows five singlets at 2.04, 2.47, 4.57, 9.91 and 12.36 ppm corresponding to CH_3 at position 7, CH_3S at position 2, methynic proton (H-5), NH (H-8, deuterium exchangeable proton) and NH (H-3, deuterium exchangeable proton), respectively; two doublets at 7.22 and 7.40 ppm (^3J = 8.2 Hz), corresponding to H_o and H_m of 4-ClC_6H_4- substituent at position 5 of pyridine ring (Table 2).

Multiplicity of carbon atoms was determined by ^{13}C-NMR (DEPT-135 experiment) spectroscopy where mainly the signals at 12.7, 17.9 and 38.1 ppm corresponding to methyl-

Table 1

Microwave-assisted Synthesis of 6-Cyanopyridopyrimidines **5a-t**

Comp.	R	R'	R''	Ar	mp, °C	Yield, %	Time of reaction (min)
5a	CH_3	CH_3S	CH_3	4-ClC_6H_4	257-258	72	2
5b	H	CH_3S	CH_3	4-ClC_6H_4	263-265	60	4
5c	H	CH_3O	CH_3	4-ClC_6H_4	260-261	88	3
5d	H	NH_2	CH_3	4-ClC_6H_4	>360	77	2
5e	H	CH_3S	CH_3	3,4-$OCH_2OC_6H_3$	286-288	61	8
5f	H	NH_2	CH_3	4-FC_6H_4	>360	77	3
5g	H	NH_2	CH_3	$CH_3OC_6H_4$	>360	57	8
5h	H	CH_3S	CH_3	4-FC_6H_4	310-312	80	5
5i	H	CH_3O	CH_3	4-FC_6H_4	281-282	70	5
5j	H	CH_3S	CH_3	4-$O_2NC_6H_4$	308-310	82	2
5k	H	CH_3O	C_6H_5	4-ClC_6H_4	181-183	78	9
5l	H	CH_3S	C_6H_5	4-ClC_6H_4	284-285	80	9
5m	CH_3	CH_3S	C_6H_5	4-ClC_6H_4	283-285	79	4
5n	H	CH_3O	C_6H_5	4-$CH_3OC_6H_4$	182-184	60	4
5o	CH_3	CH_3O	C_6H_5	4-$CH_3OC_6H_4$	235-236	80	10
5p	H	CH_3S	C_6H_5	4-$CH_3OC_6H_4$	271-273	85	10
5q	CH_3	CH_3S	C_6H_5	4-$CH_3OC_6H_4$	242-244	76	10
5r	H	CH_3S	C_6H_5	C_6H_5	291-292	50	9
5s	CH_3	CH_3S	C_6H_5	C_6H_5	255-256	67	15
5t	H	CH_3O	C_6H_5	C_6H_5	281-283	75	9

Table 2

^{1}H NMR chemical shifts (δ in ppm) of compounds **5a-t**

Comp.	R (s)	R' (s)	7-CH_3 (s)[a]	5-H (s)	8-H (s)	5-Aryl (dd)[b] *o*	*m*
5a	3.33	2.57	2.11	4.59	9.89	7.23	7.36
5b	12.36	2.47	2.04	4.57	9.91	7.22	7.40
5c	11.95	3.87	2.17	4.54	9.88	7.21	7.39
5d	10.39	6.42	2.06	4.47	9.45	7.20	7.35
5e	11.98	2.47	2.10	4.48	9.84	6.66-6.84	
5f	10.41	6.37	2.05	4.45	9.41	7.05	7.23
5g	10.39	6.35	2.05	4.37	9.35	6.81	7.10
5h	12.37	2.49	2.09	4.56	9.88	7.08	7.27
5i	11.98	3.87	2.09	4.54	9.86	7.08	7.26
5j	12.42	2.41	2.05	4.75	10.00	7.49	8.19
5k	12.11	3.86	7.50-7.53	4.70	10.20	7.34	7.41
5l	12.50	2.49	7.52-7.54	4.73	10.23	7.34	7.43
5m	3.36	2.57	7.51-7.59	4.76	10.24	7.35	7.42
5n	11.49	3.88	7.15-7.22	4.60	10.11	6.73	6.93
5o	3.19	3.96	7.48-7.56	4.63	10.13	6.88	7.26
5p	12.45	2.53	7.49-7.56	4.63	10.17	6.89	7.25

Table 2 (continued)

Comp.	R (s)	R' (s)	7-CH_3 (s)[a]	5-H (s)	8-H (s)	5-Aryl (dd)[b] *o*	*m*
5q	3.35	2.56	7.53-7.55	4.66	10.17	6.88	7.26
5r	12.45	2.47	7.40-7.57	4.70	10.19	7.23-7.38	
5s	3.30	2.57	7.53-7.56	4.73	10.20	7.34-7.39	
5t	12.09	3.99	7.46-7.55	4.67	10.16	7.22-7.38	

[a] Multiplet for **5k-t**; [b] Multiplet for **5e**, **r-t**; [c] δ for OCH_2O-group of compound **5e** 5.97; [d] ppm; for OCH_3 of compounds **5g**, **n-q** 3.71, 3.83, 3.73, 3.73 and 3.74 ppm, respectively.

Table 3
^{13}C NMR chemical shifts (δ in ppm) of compounds **5a-t**[a].

	R	R'	C-2	C-4	C-4a	C-5	C-6	C-7	C-8a	7-R"	CN
5a	-	12.6	150.9	161.5	94.3	38.4	83.1	148.2	144.1	17.8	120.0
5b	28.1	12.7	151.7	161.2	94.4	38.1	83.2	148.1	144.0	17.9	119.7
5c	-	54.8	157.1	161.9	92.4	38.3	83.3	148.0	144.3	17.8	119.7
5d	-	-	153.6	162.9	88.3	38.1	82.6	148.2	144.3	17.8	120.1
5e	-	12.6	151.1	163.1	94.8	38.1	83.6	147.1	147.6	17.1	119.7
5f	-	-	153.7	162.5	88.7	38.0	83.0	148.1	147.8	17.9	120.2
5g	-	-	154.2	161.3	89.0	37.7	83.3	153.4	147.5	17.7	120.3
5h	-	12.7	159.5	162.7	94.7	37.9	83.4	151.7	147.9	17.8	119.7
5i	-	54.7	152.1	162.7	92.7	37.9	83.5	152.1	147.8	17.7	119.7
5j	-	12.7	152.1	161.7	93.8	38.8	82.4	151.9	148.7	17.9	119.4
5k	-	54.7	156.9	161.9	92.6	39.3	83.7	149.9	152.3		119.9
5l	-	12.5	156.5	161.5	94.5	38.8	83.6	149.9	152.0		119.8
5m	29.8	14.3	160.1	161.9	93.6	40.3	83.7	149.9	150.0		119.8
5n	-	54.9	157.4	161.9	90.8	39.8	80.7	149.3	155.4		120.2
5o	27.3	55.7	158.2	160.7	92.6	39.1	84.4	149.4	150.3		120.2
5p	-	12.5	158.2	161.5	95.1	38.3	84.3	149.3	151.5		120.1
5q	29.7	14.2	160.1	161.5	94.3	39.0	84.3	149.3	149.8		120.0
5r	-	12.5	156.9	161.3	94.9	39.7	84.0	149.7	151.8		120.0
5s	29.8	14.3	160.1	161.7	94.0	39.9	84.1	149.7	150.1		120.0
5t	-	54.6	156.8	162.0	92.9	39.3	84.1	149.7	152.3		120.1

[a] δ for OCH_2O-group of compound **5e** 100.9 ppm and for CH_3O of compounds **5g**, **n-q** 54.9, 54.6, 54.9, 55.9 and 54.9 ppm, respectively.

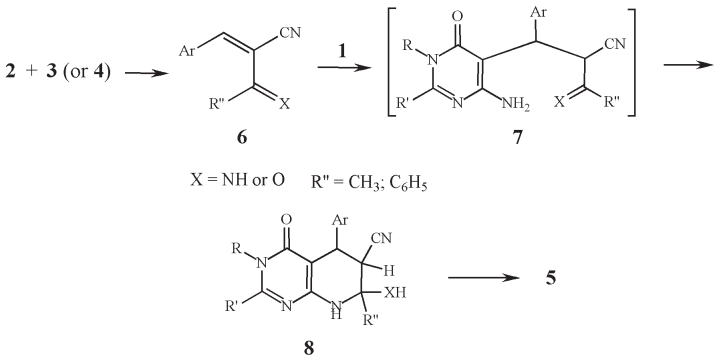
groups (CH_3S and CH_3 at position 7) and C-5, respectively were observed. Complete assignments of the carbon atoms using HSQC, HMBC and NOESY experiments allowed us the structure-elucidation of compounds **5a-t** (Table 3).

We assume that the synthesis of **5a-t** as following: 1) addition of the most basic ring carbon atom in aminopyrimidine **1** to the activated double bond of intermediate **6** *via* the Michael type reaction (the latter **6** is formed by Knovenagel condensation between benzaldehyde **2** and *β*-aminocrotononitrile **3** or benzoylacetonitrile **4**), 2) posterior enamine-imine or enamine-ketone cyclization of **7** and 3) elimination of ammonia or water molecule, respectively, to form the dihydropyridine nucleus (Scheme 2) [17].

formation of the benzylidene derivative of *β*-aminocrotononitrile or benzoylacetonitrile **6** as an intermediate of the reaction in study, was confirmed by the direct interaction of amines **1** with the previously synthesized benzylidene derivatives **6** using microwave irradiation. Under the same conditions, this reaction led to the same 5-aryl-6-cyano-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-ones **5**.

It is important to point out that when the mixture of aminopyrimidines **1**, benzaldehydes **2** and 4,4-dimethyl-3-oxopentanonitrile **9** was irradiated during 8-12 minutes, the reaction leads in all cases to the stable products **10** (Scheme 3) with traces, in some cases, of compound **11**. Nevertheless, a prolonged irradiation does not lead to the

Scheme 2



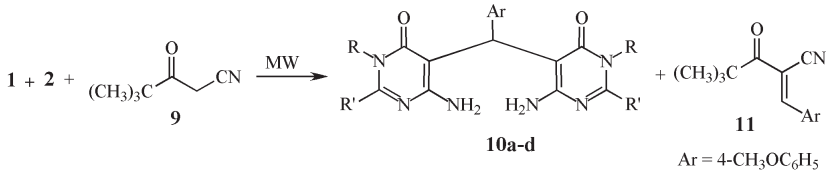
As an evidence of the proposed reaction route we isolated the intermediate **8** (R = H, R' = OCH_3, X = O; Ar = C_6H_5, R" = C_6H_5), which upon a new irradiation time (6-10 minutes) lose a water molecule yielding 5-aryl-6-cyano-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-ones **5**. The probable for-

formation of the target pyridopyrimidines analogues to **5a-t**. We consider that the presence of the bulky *tert*-butyl group hinder first, the effective formation of compound **11** and afterwards the Michael addition of the basic ring carbon atom of aminopyrimidine **1** to the double bond of the

benzylidene derivative **11** (Ar = 4-$CH_3OC_6H_4$). A similar process with analogues of compound **10** has been described in the literature [18].

gal strains comprising human opportunistic pathogenic yeasts, hialohyphomycetes as well as dermatophytes with the broth dilution method [19]. Results showed that none of

Scheme 3



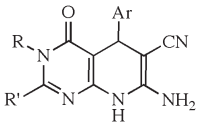
	R	R'	Ar	t, min
10a	CH_3	CH_3O	4-ClC_6H_4	6
10b	H	CH_3O	4-ClC_6H_4	4
10c	H	NH_2	4-ClC_6H_4	3
10d	CH_3	CH_3O	4-$CH_3OC_6H_5$	4

Some compounds of series **5a-t** together with the analogues **12a-d** (Table 4), previously synthesized by conventional procedures (ethanol reflux) [17b], were *in vitro* assayed for antifungal properties against a panel of 10 fun-

the tested compounds displayed any activity against yeasts and hialohyphomycetes up to 250 μg/mL. In contrast compounds **5a-f** and **12a** showed activity against dermatophytes with MIC values between 100-200 μg/mL (Table 4).

Table 4

MIC values (μg/mL) of pyrido[2,3-*d*]pyrimidines **5a-c, e, f**, **l**, **n**, **q**, **s** and **12a-d** against a panel of yeasts, hialohyphomycetes and dermatophytes



12a R = CH_3; R' = CH_3O; Ar = C_6H_5
12b R = CH_3; R' = CH_3O; Ar = 4-ClC_6H_4
12c R = CH_3; R' = CH_3O; Ar = 4-$O_2NC_6H_4$
12d R = H; R' = CH_3S; Ar = 4-$O_2NC_6H_4$

	C.a.	C.t.	S.c.	C.n.	A.f.	A.fl.	A.n.	M.g.	T.r.	T.m.
5a	>250	>250	>250	>250	200	200	200	200	200	200
5b	>250	>250	>250	>250	>250	>250	>250	200	200	100
5c	>250	>250	>250	>250	>250	>250	>250	200	100	200
5e	>250	>250	>250	>250	>250	>250	>250	200	200	100
5f	>250	>250	>250	>250	>250	>250	>250	100	200	200
5l	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
5n	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
5q	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
5s	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
8	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
12a	200	200	200	200	200	200	>250	100	50	50
12b	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
12c	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
12d	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
Keto	0.5	0.125	0.5	0.25	0.125	0.5	0.25	0.05	0.025	0.025
Amp	1.0	0.5	0. 5	0.25	0.5	0.5	0.5			
Terbin								0.04	0.01	0.04

C.a.: *Candida albicans* ATCC 10231; *C.t.*: *Candida tropicalis* C131; *S.c.*: *Saccharomyces cerevisiae* ATCC 9763; *C.n.*: *Cryptococcus neoformans* ATCC 32264; *A.f.*: *Aspergillus fumigatus* ATCC 26934; *A.fl.*: *Aspergillus flavus* ATCC 9170, *A.n.*: *Aspergillus niger* ATCC 9029; *M g:Microsporum gypseum* C 115; *T.r.: Trichophyton rubrum* C113; *T.m: Trichophyton mentagrophytes* ATCC 9972. Keto= Ketoconazole; Amp= Amphotericin B. Terbin = Terbinafine.

Conclusions.

In conclusion, we have described in this paper the preparation of novel 5-aryl-6-cyano-7-methyl-5,8-dihydropyrido[2,3-*d*]pyrimidines by multicomponent reaction between 6-amino-4-pyrimidinones, benzaldehydes and β-aminocrotononitrile or benzoylacetonitrile under the microwave irradiation. Regarding the antifungal properties, some compounds of the series show activity against dermatophytes, fungi causing the most important superficial mycoses in human beings. The antifungal activity showed by compounds **5a-f** and **12a**, although marginal, could open new avenues for the design of new series of analogues of 6-cyano-6,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-ones with better antifungal properties than this series. They could lead to the development of new antifungals useful for the treatment of dermatophytoses, mycoses very difficult to eradicate.

EXPERIMENTAL

Melting points were determined in a Büchi Melting Point Apparatus and are uncorrected. The ^{1}H- and ^{13}C NMR spectra were run on a Bruker DPX 300 spectrometer operating at 300 MHz and 75 MHz respectively, using dimethyl sulfoxide-d_6 as solvent and tetramethylsilane as internal standard. The mass-spectra were scanned on a Hewlett Packard HP Engine-5989 spectrometer (equipped with a direct inlet probe) and operating at 70 eV. The elemental analyses have been obtained using a LECO CHNS-900 equipment.

General Procedure of Preparation of Pyrido[2,3-*d*]pyrimidin-4(3*H*)-ones (**5a-t**).

Equimolar amounts of amine **1**, aldehyde **2** and β-aminocrotononitrile **3** or benzoylacetonitrile **4** were placed into pyrex-glass open vessels and irradiated in a domestic microwave oven for 2-15 min. (at 600 watts) as indicated in Table 1. The solid was crushed with ethanol and filtered. The products **5** were recrystallized from absolute ethanol and analyzed (see also Tables 1-3 for some physical and chemical data).

5-(4-Chlorophenyl)-6-cyano-3,7-dimethyl-2-methylsulfanyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5a**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 360/358 (5/12, M$^+$), 247 (100, M$^+$- 4-ClC_6H_4), 199 (25), 171 (5), 88 (8).

Anal. Calcd. for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61. Found: C, 56.81; H, 4.14; N, 15.73.

5-(4-Chlorophenyl)-6-cyano-7-methyl-2-methylsulfanyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5b**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 346/344 (4/9, M$^+$), 233 (100, M$^+$- 4-ClC_6H_4), 185 (21).

Anal. Calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25. Found: C, 55.65; H, 3.72; N, 16.31.

5-(4-Chlorophenyl)-6-cyano-7-methyl-2-methoxy-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5c**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 330/328 (3/8, M$^+$), 217 (100, M$^+$- 4-ClC_6H_4), 185 (12), 174 (6), 130 (5), 75 (7).

Anal. Calcd. for $C_{16}H_{13}ClN_4O_2$: C, 58.46; H, 3.99; N, 17.04. Found: C, 58.33; H, 3.91; N, 17.15.

2-Amino-5-(4-chlorophenyl)-6-cyano-7-methyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5d**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 315/313 (2/5, M$^+$), 203 (14), 202 (100, M$^+$- 4-ClC_6H_4), 185 (17), 126 (28), 111 (10), 98 (12), 75 (19), 68 (14), 55 (10), 44 (14), 43 (74).

Anal. Calcd. for $C_{15}H_{12}ClN_5O$: C, 57.42; H, 3.86; N, 22.32. Found: C, 57.35; H, 3.95; N, 22.23.

5-(3,4-Dioxomethylenphenyl)-6-cyano-7-methyl-2-methylsulfanyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5e**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 355 (8), 354 (34, M$^+$), 234 (16), 233 (100, M$^+$- 3,4-OCH_2O-C_6H_5), 185 (22), 122 (8), 65 (8).

Anal. Calcd. for $C_{17}H_{14}N_4O_3S$: C, 57.62; H, 3.98; N, 15.81. Found: C, 57.70; H, 3.86; N, 15.85.

2-Amino-5-(4-fluorophenyl)-6-cyano-7-methyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5f**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 297 (6, M$^+$), 203 (14), 202 (100, M$^+$- 4-FC_6H_4), 185 (21), 158 (5), 132 (8), 95 (13), 75 (18), 43 (30).

Anal. Calcd. for $C_{15}H_{12}FN_5O$: C, 60.60; H, 4.07; N, 23.56. Found: C, 60.53; H, 4.13; N, 23.49.

2-Amino-5-(4-methoxyphenyl)-6-cyano-7-methyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5g**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 309 (13, M$^+$), 307 (6), 203 (15), 202 (100, M$^+$- 4-$CH_3OC_6H_4$), 185 (14), 108 (10), 92 (13), 77 (13), 64 (12), 44 (12), 43 (37), 42 (19).

Anal. Calcd. for $C_{16}H_{15}N_5O_2$: C, 62.13; H, 4.89; N, 22.64. Found: C, 62.20; H, 4.77; N, 22.59.

6-Cyano-5-(4-fluorophenyl)-3,7-dimethyl-2-methylsulfanyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5h**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 329 (10), 328 (15, M$^+$), 234 (15), 233 (100, M$^+$- 4-FC_6H_4), 185 (17).

Anal. Calcd. for $C_{16}H_{13}FN_4OS$: C, 58.52; H, 3.99; N, 17.06. Found: C, 58.46; H, 4.04; N, 17.15.

6-Cyano-5-(4-fluorophenyl)-3,7-dimethyl-2-methoxy-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5i**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 312 (13, M$^+$), 218 (16), 217 (100, M$^+$- 4-FC_6H_4), 185 (14), 174 (7).

Anal. Calcd. for $C_{16}H_{13}FN_4O_2$: C, 61.54; H, 4.20; N, 17.94. Found: C, 61.61; H, 4.25; N, 17.83.

6-Cyano-3,7-dimethyl-2-methylsulfanyl-5-(4-nitrophenyl)-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5j**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 355 (6, M$^+$), 353 (4),

234 (13), 233 (100, M^+- 4-$O_2NC_6H_4$), 185 (22), 157 (8), 130 (8), 76 (11), 74 (10), 50 (9), 42 (8).

Anal. Calcd. for $C_{16}H_{13}N_5O_3S$: C, 54.08; H, 3.69; N, 19.71. Found: C, 54.15; H, 3.61; N, 19.82.

5-(4-Chlorophenyl)-6-cyano-2-methoxy-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5k**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 392/390 (3/7, M^+), 295/293 (1/19), 280 (19), 279 (100, M^+- 4-ClC_6H_4), 247 (8), 236 (5), 192 (6), 77 (4), 75 (5).

Anal. Calcd. for $C_{21}H_{15}ClN_4O_2$: C, 64.54; H, 3.87; N, 14.34. Found: C, 64.60; H, 3.75; N, 14.24.

5-(4-Chlorophenyl)-6-cyano-2-methylsulfanyl-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5l**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 408/406 (3/8, M^+), 298/296 (5/20), 297 (7), 295 (100, M^+- 4-ClC_6H_4), 247 (13), 192 (7), 77 (4), 75 (5).

Anal. Calcd. for $C_{21}H_{15}ClN_4OS$: C, 61.99; H, 3.72; N, 13.77. Found: C, 61.92; H, 3.75; N, 13.82.

5-(4-Chlorophenyl)-6-cyano-2-methylsulfanyl-3-methyl-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5m**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 422/420 (3/8, M^+), 309 (100, M^+- 4-ClC_6H_4), 261 (16), 192 (12), 111 (5), 88 (19), 51 (4).

Anal. Calcd. for $C_{22}H_{17}ClN_4OS$: C, 62.78; H, 4.07; N, 13.31. Found: C, 62.73; H, 4.14; N, 13.26.

6-Cyano-2-methoxy-5-(4-methoxyphenyl)-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5n**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 386 (13, M^+), 309 (51), 279 (100, M^+- 4-$CH_3OC_6H_4$), 261 (15), 247 (12), 192 (18), 141 (16), 121 (17), 111 (10), 77 (48), 58 (27), 39 (16).

Anal. Calcd. for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.31; H, 4.66; N, 14.62.

6-Cyano-2-methoxy-5-(4-methoxyphenyl)-3-methyl-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5o**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 400 (17, M^+), 293 (100, M^+- 4-$CH_3OC_6H_4$), 236 (10), 192 (7), 77 (7).

Anal. Calcd. for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99. Found: C, 68.88; H, 5.11; N, 13.91.

6-Cyano-5-(4-methoxyphenyl)-2-methylsulfanyl-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5p**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 402 (17, M^+), 295 (100, M^+- 4-$CH_3OC_6H_4$), 247 (14), 192 (10), 77 (11).

Anal. Calcd. for $C_{22}H_{18}N_4O_2S$: C, 65.65; H, 4.51; N, 13.92. Found: C, 65.61; H, 4.46; N, 13.84.

6-Cyano-5-(4-methoxyphenyl)-3-methyl-2-methylsulfanyl-7-phenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5q**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 416 (18, M^+), 309 (100, M^+- 4-$CH_3OC_6H_4$), 261 (19), 192 (12), 88 (16).

Anal. Calcd. for $C_{23}H_{20}N_4O_2S$: C, 66.33; H, 4.84; N, 13.45. Found: C, 66.40; H, 4.79; N, 13.55.

6-Cyano-2-methylsulfanyl-5,7-diphenyl-5,8-dihydropyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (**5r**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 372 (8, M^+), 295 (100, M^+- C_6H_5), 247 (14), 192 (10), 77 (24), 51 (17).

Anal. Calcd. for $C_{21}H_{16}N_4OS$: C, 67.72; H, 4.33; N, 15.04. Found: C, 67.65; H, 4.26; N, 15.11.

6-Cyano-3-methyl-2-methylsulfanyl-5,7-diphenyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**5s**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 386 (8, M^+), 309 (100, M^+- C_6H_5), 261 (16), 192 (11), 88 (15), 77 (18), 51 (10).

Anal. Calcd. for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 68.46; H, 4.61; N, 14.66.

6-Cyano-2-methoxy-5,7-diphenyl-5,8-dihydropyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (**5t**).

This compound was obtained according to general procedure as white crystals. MS: (70 eV) m/z (%) = 356 (9, M^+), 280 (19), 279 (100, M^+- C_6H_5), 247 (8), 192 (8), 77 (11), 51 (10).

Anal. Calcd. for $C_{21}H_{16}N_4O_2$: C, 70.78; H, 4.53; N, 15.72. Found: C, 70.71; H, 4.44; N, 15.83.

6-Cyano-7-hydroxy-2-methoxy-5,7-diphenyl-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-4(3*H*)-one **(8)**.

This compound was obtained by the same procedure as described for compounds **5**. Irradiation time 8 min; white crystals; mp 244 °C; yield 75%; MS: (70 eV) m/z (%) = 374 (1, M^+), 356 (3, M^+- H_2O), 280 (8), 279 (37), 233 (28), 232 (12), 141 (13), 106 (9), 105 (100), 78 (8), 77 (80), 51 (41), 50 (14), 39 (7); δ_H (300 MHz, dimethyl sulfoxide-d_6) 3.84 (s, 3H, CH_3O), 3.14 (d, 1H, 6-H), 4.02 (d, 1H, 5-H), 6.79 (s, 1H, 7-OH), 7.73 (s, 1H, 8-NH), 11.45 (s, 1H, 3-NH); δ_C (75 MHz, dimethyl sulfoxide-d_6) 40.6 (C-5), 54.1 (CH_3O), 49.4 (C-6), 80.7 (C-7), 89.7 (C-4a), 118.6 (CN), 157.5 (C-8a), 156.6 (C-2), 161.0 (C=O).

Anal. Calcd. for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96. Found: C, 67.46; H, 4.78; N, 14.89.

General Procedure to Accede to Aryl-bis(pyrimidin-5-yl)-methanes **10a-d**.

Equimolar amounts of amine **1** and 4-substituted benzaldehyde **2** were placed into pyrex-glass open vessels and irradiated in a domestic microwave oven for 3-6 min. at 600 watts. The solid was crushed with ethanol and collected by filtration. The products **10a-d** were recrystallized from absolute ethanol.

Bis(6-amino-2-methoxy-3-methyl-4-oxopyrimidin-5-yl)-4-chlorophenylmethane (**10a**).

This compound was obtained according to general procedure as white crystals; yield 60 %; mp 232-4 °C; MS: (70 eV) m/z (%) = 434/432 (40/100, M^+), 278/276 (17/45, M^+- (6-aminopyrimidine)), 72 (39), 58 (24), 42 (13); δ_H (300 MHz, dimethyl sulfoxide-d_6) 3.20 (s, 6H, 3-CH_3N), 3.92 (s, 6H, 2-CH_3O), 5.52 (d, 1H, CH), 6.96 (s, 4H, 6-NH_2), 7.03 (d, 2H, H_o), 7.25 (d, 2H, H_m); δ_C (75 MHz, dimethyl sulfoxide-d_6) 27.4 (CH_3N), 34.7 (C-methynic), 55.2 (CH_3O), 90.1 (C-5), 127.4, 128.5, 129.2 and 138.8 (aromatic), 154.7 (C-2), 160.5 (C-6), 162.9 (C=O).

Anal. Calcd. for $C_{19}H_{21}ClN_6O_4$: C, 52.72; H, 4.89; N, 19.41. Found: C, 52.85; H, 4.76; N, 19.53.

Bis(6-amino-2-methoxy-4-oxopyrimidin-5-yl)-4-chlorophenylmethane (**10b**).

This compound was obtained according to general procedure as white crystals; yield 63 %; mp 269-71 °C; MS: (70 eV) m/z (%) = 406/404 (11/31, M^+), 266/264 (6/24, M^+- (6-aminopyrimidine)), 141 (47), 127 (12), 111 (18), 75 (42), 58 (45), 40 (100); δ_H (300 MHz, dimethyl sulfoxide-d_6) 3.83 (s, 6H, 2-CH_3O), 5.43 (d, 1H, CH), 6.75 (s, 4H, 6-NH_2), 6.80 (d, 2H, H_o), 7.25 (d, 2H, H_m), 11.55 (s, 2H, NH); δ_C (75 MHz, dimethyl sulfoxide-d_6) 33.0 (C-methynic), 54.1 (CH_3O), 90.2 (C-5), 127.3, 128.5, 129.1 and 139.0 (aromatic), 155.6 (C-2), 162.4 (C-6), 163.9 (C=O).

Anal. Calcd. for $C_{17}H_{17}ClN_6O_4$: C, 50.44; H, 4.23; N, 20.76. Found: C, 50.36; H, 4.15; N, 20.65.

Bis(2,6-diamino-4-oxopyrimidin-5-yl)-4-chlorophenylmethane (**10c**).

This compound was obtained according to general procedure as white crystals; yield 65 %; mp 272-4 °C; MS: (70 eV) m/z (%) = 376/374 (5/16, M^+), 252/250 (4/19, M^+- (6-aminopyrimidine)), 126 (13), 98 (8), 68 (10), 43 (100); δ_H (300 MHz, dimethyl sulfoxide-d_6) 5.34 (d, 1H, CH), 6.14 (s, 4H, 2-NH_2), 6.97 (s, 4H, 6-NH_2), 7.01 (d, 2H, H_o), 7.21 (d, 2H, H_m), 9.96 (s, 2H, NH); δ_C (75 MHz, dimethyl sulfoxide-d_6) 35.8 (C-methynic), 90.1 (C-5), 127.1, 128.7, 128.8 and 140.6 (aromatic), 153.3 (C-2), 162.3 (C-6), 163.3 (C=O).

Anal. Calcd. for $C_{15}H_{15}ClN_8O_2$: C, 48.07; H, 4.03; N, 29.90. Found: C, 48.18; H, 4.13; N, 29.79.

Bis(6-amino-2-methoxy-3-methyl-4-oxopyrimidin-5-yl)-4-methoxyphenylmethane (**10d**).

This compound was obtained according to general procedure as white crystals; yield 64 %; mp 199-201 °C; MS: (70 eV) m/z (%) = 428 (100, M^+), 413 (7), 339 (7), 274 (92, M^+- (6-aminopyrimidine)), 258 (16), 89 (11), 72 (26), 42 (7); δ_H (300 MHz, dimethyl sulfoxide-d_6) 3.09 (s, 6H, 3-CH_3N), 3.88 (s, 3H, 4-CH_3O-C_6H_4), 3.92 (s, 6H, 2-CH_3O), 5.49 (d, 1H, CH), 6.37 (s, 4H, NH_2-groups), 6.73 (d, 2H, H_o), 7.14 (d, 2H, H_m); δ_C (75 MHz, dimethyl sulfoxide-d_6) 30.6 (CH_3N), 34.3 (C-methynic), 54.8 (CH_3O-C_6H_4), 55.1 (CH_3O), 90.7 (C-5), 112.9, 127.5, 131.3 and 131.7 (aromatic), 154.6 (C-2), 162.1 (C-6), 162.8 (C=O).

Anal. Calcd. for $C_{20}H_{24}N_6O_5$: C, 56.07; H, 5.65; N, 19.61. Found: C, 56.15; H, 5.77; N, 19.52.

2-(4-Methoxybenzyliden)-4,4-dimethyl-3-oxopentanonitrile (**11**).

This compound was obtained as a subproduct in the synthesis of compound **10**. Also, **11** was obtained by irradiation at 600 watts in a domestic microwave oven for 5 min. of equimolar amounts of amine **1** and 4-methoxybenzaldehyde **2**. The crude solid obtained was crushed with ethanol, filtered and recrystallized from absolute ethanol**;** mp 87-89 °C; yield 15 %; MS: (70 eV) m/z (%) = 243 (40, M^+), 244 (30), 187 (22), 186 (100), 159 (29), 158 (24), 144 (6), 143 (7), 116 (4), 115 (8), 114 (5), 103 (4), 89 (7), 88 (4), 77 (5), 63 (4), 57 (23), 41 (20), 39 (9); δ_H (300 MHz, dimethyl sulfoxide-d_6) 1.34 (s, 9H, $(CH_3)_3C$), 3.87 (s, 3H, 4-CH_3O-C_6H_4), 7.13 (d, 2H, H_o), 8.10 (d, 2H, H_m), 8.25 (s, 1H, =C-H); δ_C (75 MHz, dimethyl sulfoxide-d_6) 26.2 ($(CH_3)_3C$), 43.9 ($C(CH_3)_3$), 103.3 (=C), 114.9 (C_o), 118.8 (CN), 124.4 (C_i), 133.6 (C_m), 155.4 (=CH), 163.4 (C_p), 197.7 (C=O).

Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 74.17; H, 7.11; N, 5.67.

Microorganisms and Media.

For the antifungal evaluation, strains from the American Type Culture Collection (ATCC, Rockville, MD, USA) and the Centro de Referencia Micológica (C, CEREMIC), Facultad de Ciencias Bioquímicas y Farmacéuticas, Suipacha 531, 2000, Rosario, Argentina: *Candida albicans* ATCC 10231, *Candida tropicalis* C131, *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATCC 26934, *Aspergillus niger* ATCC 9029, *Trichophyton mentagrophytes* ATCC 9972, *Trichophyton rubrum* C 113, and *Microsporum gypseum* C 115 were used. Strains were grown on Sabouraud-chloramphenicol agar slants for 48 h at 30 °C. The strains were maintained on slopes of Sabouraud-dextrose agar (SDA, Oxoid) and subcultured every 15 days to prevent pleomorphic transformations. Spore suspensions were obtained according to reported procedures [20].

Antifungal Susceptibility Testing.

The Minimal Inhibitory Concentration (MIC) of each extract was determined by using broth microdilution techniques following the guideliness of the National Committee for Clinical Laboratory Standards for yeasts (M27-A2) and for filamentous fungi (M-38A) [19]. MIC values were determined in RPMI 1640 (Sigma, St Louis, Mo, USA) buffered to a pH 7.0 with MOPS. The starting inocula were $1x10^3$ to $5x\ 10^3$ CFU/mL. Microtiters trays were incubated at 35 °C for yeasts and hialophyphomycetes and at 28-30 °C for dermatophyte strains in a moist, dark chamber, and MICs were recorded at 48 h for yeasts, and at a time according to the control fungus growth, for the rest of fungi. The susceptibilities of the standard drugs Ketoconazole (Janssen, Belgium), Terbinafine (Novartis, Argentina) and Amphotericin B (Sigma, St Louis, Mo, USA) were defined as the lowest concentration of drug which resulted in total inhibition of fungal growth.

For the assay, compounds stock solutions were two-fold diluted with RPMI from 250-0.98 µg/ml (final volume = 100 µL) and a final dimethyl sulfoxide concentration ≤ 1%. A volume of 100 µL of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. The MIC was defined as the minimum inhibitory concentration of the extract which resulted in total inhibition of the fungal growth.

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REFERENCESAND NOTES

[1a] C. Hulme, V. Gore, *Curr. Med. Chem.*, **10**, 51 (2003); [b] A. Dömling, I. Ugi, *Angew. Chem., Int. Ed.*, **39**, 3168 (2000); [c] V. Nair, C.

Rajesh, A. U. Vinod, S. Bindu, A. R. Sreekanth, J. S. Mathen, L. Balagopal, *Acc. Chem. Res.*, **36**, 899 (2003); [d] J. Zhu, *Eur. J. Org. Chem*., 1133 (2003); [e] A. Domling, *Curr. Opin. Chem. Biol.*, **6**, 306 (2002); [f] R. V. A. Orru, M. de Greef, *Synthesis*, 1471 (2003).

[2a] D. Adam, *Nature*, **421**, 571 (2003); [b] C. O. Kappe, *Angew. Chem. Int. Ed.*, **43**, 6250 (2004); [c] M. Nüchter, U. Müller, B. Ondruschka, A. Tied, W. Lautenschläger, *Chem. Eng. Technol.*, **26**, 1207 (2003).

[3a] A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault, D. Mathé, *Synthesis*, 1213 (1998); [b] R. S. Varma, *Green Chem.*, 43 (1999); [c] S. Deshayes, M. Liagre, A. Loupy, J.-L. Luche, A. Petit, *Tetrahedron*, **55**, 10851 (1999); [d] R. S. Varma, *Clean Prod. Process.*, **1**, 132 (1999); [e] R. S. Varma, *Pure Appl. Chem.*, **73**, 193 (2001); [f] J. Cossy, C. Willis, V. Bellosta, L. S. Jalmes, *Synthesis*, 951 (2002); [g] A. R. Katritzky, S. K. Sing, *ARKIVOK*, **XIII**, 68 (2003); [h] A. de la Hoz, A. Díaz-Ortíz, A. Moreno, *Chem. Soc. Rev.*, **34**, 164 (2005).

[4] A. Rosowsky, C. E. Mota, S. F. Queener, *J. Heterocyclic Chem.*, **32**, 335 (1995).

[5] L. V. G. Nargund, Y. S. R. Reddy, R. Jose, *Ind. Drugs*, **29**, 45 (1991).

[6] A. M. Thompson, A. J. Bridges, D. W. Fry, A. J. Kraker, W. A. Denny, *J. Med. Chem.*, **38**, 3780 (1995).

[7] I. O. Donkor, C. L. Klein, L. Liang, N. Zhu, E. Bradley, A. M. Clark, *J. Pharm. Sci.*, **84**, 661 (1995).

[8] A. Pastor, R. Alajarin, J. J. Vaquero, J. Alvarez-Builla, M. Fau de Casa-Juana, C. Sunkel, J. G. Priego, I. Fonseca, J. Sanz-Aparicio, *Tetrahedron*, **50**, 8085 (1994).

[9] V. E. Kolla, A. B. Deyanov, F. Y. Nazmetdinov, Z. N. Kashina, L. P. Drovosekova, *Khim-Farm Zh.*, **27**, 29 (1993).

[10] N. K. Satti, K. A. Suri, O. P. Suri, A. Kapil, *Ind. J. Chem., Sect. B*, **32**, 978 (1993).

[11] I. D. Bystryakova, I. A. Burova, G. M. Chelysheva, S. V. Zhilinkova, N. M. Smirnova, T. S. Safonova, *Khim-Farm. Zh.*, **25**, 31 (1991).

[12] A. B. Deyanov, R. K. Niyazov, F. Y. Nazmetdinov, B. Y. Syropyatov, V. E. Kolla, M. E. Konshin, *Khim-Farm. Zh.*, **25**, 26 (1991).

[13] A. Monge, V. Martinez-Merino, C. Sanmartin, F. J. Fernandez, M. C. Ochoa, C. Berllver, P. Artigas, E. Fernandez-Alvarez, *Eur. J. Med. Chem.*, **24**, 209 (1989).

[14] H. Saladowska, A. Bartoszko-Malik, T. Zawisza, *Farmaco*, **45**, 101 (1990).

[15a] V. K. Ahluwalia, R. Batla, A. Khurana, R. Kumar, *Ind. J. Chem. Sect. B*, **29**, 1141 (1990); [b] I. Devi, H. N. Borah, P. J. Bhuyan, *Tetrahedron Lett.*, **45**, 2405 (2004).

[16a] A. L. Beckwith, US Patent US 3947416, 1976; *Chem. Abstr.*, **85**, 33073j (1976); [b] N. Kihara.; I. Tomino; M. Takei, Jpn.Patent 61076488 A2 [76488], 1986; *Chem. Abstr.*, **105**, 208915w (1986).

[17a] J. Quiroga, A. Hormaza, B. Insuasty, M. Nogueras, A. Sánchez, N. Hanold, H. Meier, *J. Heterocyclic Chem.*, **34**, 521 (1997); [b] J. Quiroga, M. Alvarado, B. Insuasty, M. Nogueras, A. Sánchez, J. Cobo, *J. Heterocyclic Chem.*, **35**, 1309 (1998); [c] J. Quiroga, M. Alvarado, B. Insuasty, M. Nogueras, A. Sánchez, M. D. López, *J. Heterocyclic Chem.*, **36**, 113 (1999); [d] J. Quiroga, H. Insuasty, B. Insuasty, R. Abonía, J. Cobo, A. Sánchez, M. Nogueras, *Tetrahedron*, **58**, 4873 (2002); [e] J. Quiroga, C. Cisneros, B. Insuasty, R. Abonía, M. Nogueras, A. Sánchez, *Tetrahedron Lett.*, **42**, 5625 (2001).

[18a] J. Quiroga, J. Portilla, B. Insuasty, R. Abonía, M. Nogueras, M. Sortino, S. Zacchino, *J. Heterocyclic Chem.*, **42**, 61 (2005); [b] A. Esteves- Souza, A. Echevarría, I. Vencato, M. L. Jimeno, J. Elguero, *Tetrahedron*, **57**, 6147 (2001); [c] A. A. S. El-Ahl, S. A. A. El Bialy, M. A. Ismail, *Heterocycles*, **66**, 1316 (2001); [d] H. Hirota, H. Sajiki, K. Kubo, M. Kido, K. Nakagawa, *Tetrahedron*, **52**, 9971 (1996); [e)] W. Pfleiderer, F. Sagi, L. Groezinger, *Chem. Ber.*, **99**, 3530 (1966).

[19a] NCCLS, National Committee for Clinical Laboratory Standards, 2002, Method M27-A2, 2nd ed, Wayne Ed.; Vol. 22 (15), pp 1-29; [b] NCCLS, National Committee for Clinical Laboratory Standards, 2002, Method M-38A, 2nd ed, Wayne Ed.; Vol. **22** (16), pp 1-27.

[20] L. Wright, E. Scott, S. Gorman, *J. Antimicrob. Chemother.*, **12**, 317 (1983).