Solvent-free Microwave Multicomponent Regiospecific Synthesis of Pyrimido[4,5-*c*]isoquinolines and Evaluation *In Vitro* of their Antifungal Properties

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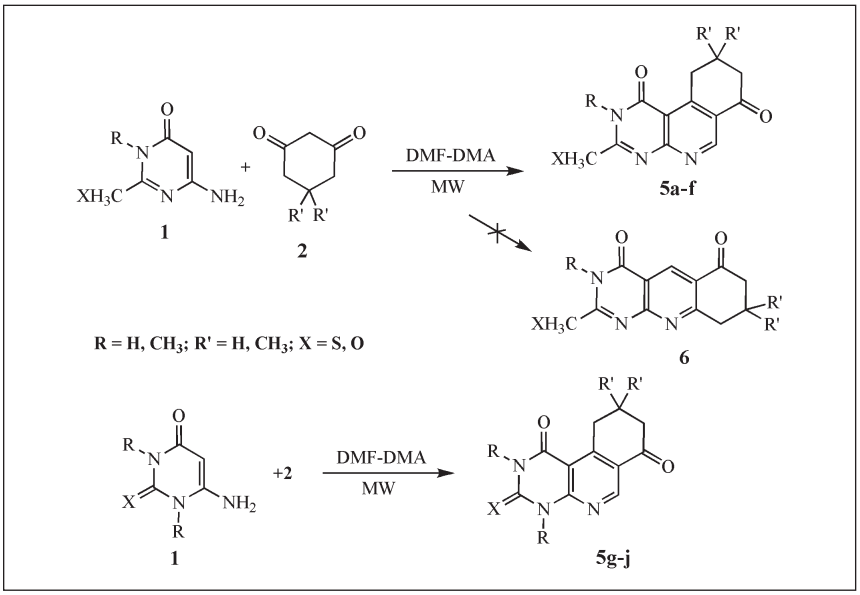

The free-solvent multicomponent reaction under microwave irradiation of 6-aminopyrimidin-4(3*H*)-ones (**1**) with dimedone (**2**) and *N*,*N*-dimethylformamide dimethylacetal yields the pyrimido[4,5-*c*]isoquinolinones (**5a-j**). In this process, the intermediate of cyclization was isolated. The structure of the synthesized compounds was determined on the basis of nmr measurements, especially by ^{1}H,^{1}H-,^{1}H,^{13}C COSY, and DEPT. These compounds showed antifungal *in vitro* activity particularly against dermatophytes.

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Introduction.

As a consequence of the growing population of immunocompromised patients, the frequency of infections caused by fungi has dramatically increased in the last two decades [1], constituting the major cause of morbidity and in some cases of mortality for these individuals [2].

Although their appears to be an array of drugs in clinical use for the treatment of mycoses, there is in fact a limited number of efficacious antifungal drugs [3]. Many of the currently available drugs are either toxic or fungistatic but not fungicide thus producing recurrence, being these problems are compounded by the rapid emergence of resistant organisms which further diminishes therapeutic capabilities [4,5]. Discovery of new effective and safe antifungal drugs for the treatment of opportunistic fungal infections is a major challenge in infectious disease research [6].

In spite of the fact that some antifungal drugs, such as terbinafine [7] or caspofungin [8], have emerged as new therapeutic alternatives, considerable effort is still concentrated in the azole area, since many azole-type compounds such as imidazole- and triazole-containing structures have been and are, at present, drugs of choice for the treatment of deep mycoses [9]. Analogues containing pyrimidine, a one-carbon enlarged imidazole ring, have not been exten-

sively studied as antimycotic agents, although 5-fluorocytosine [10], a pyrimidine-containing structure, has been a useful antifungal agent, alone or in combination with another drugs [11].

In the course of our ongoing program devoted to the synthesis of heterocyclic compounds using multicomponent regiospecific synthesis under microwave, we recently synthesized pyrido[4,5-*b*]quinolines [12] (**4**) in a multicomponent reaction of aminopyrimidines (**1**), dimedone (**2**) and aromatic aldehydes (**3**), (Scheme 1), which are interesting biological molecules [13].

The use of Multicomponent Condensations (MCCs) has attracted considerable attention, particularly in combinatorial chemistry, by virtue of their convergence, productivity, ease of execution and generally high yields of products [14]. Mainly, there has been a tremendous development in three-component reactions in the past decade and great efforts are being made at present to develop new MCCs [15].

In turn, the application of microwave technology in organic synthesis [16], particularly in free-solvent conditions, is growing at a high rate because of its reaction simplicity, lower environmental impact, and minimum reaction time providing rapid access to large libraries of diverse molecules.

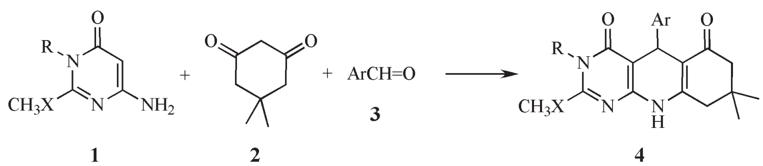
In this paper, we report the synthesis of a series of fused pyrido[2,3-*d*]pyrimidines, novel compounds, using this useful methodology, in order to explore their potential as antifungal agents.

Results and Discussion.

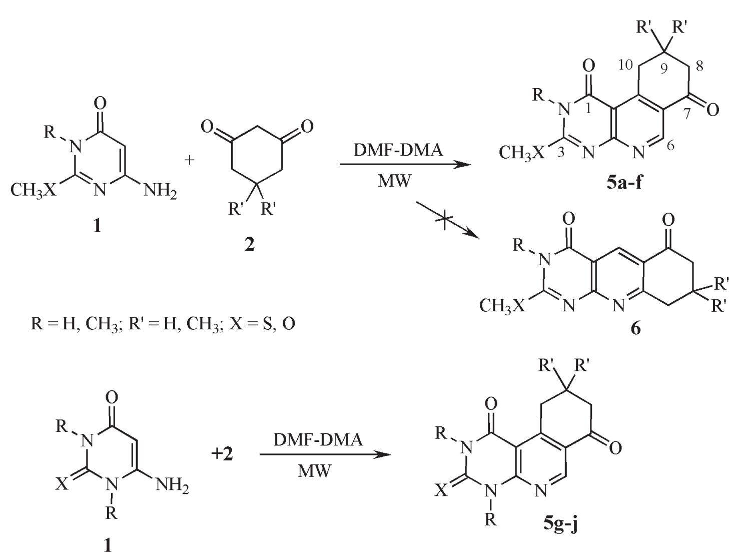
The reaction between aminopyrimidine (**1**), dimedone (**2**) and *N*,*N*-dimethylformamide dimethylacetal (1:1:1.2 molar relation) carried out by microwave irradiation (600 watts) during 2-6 minutes and by heating in ethanol for a longer time (4-6 hours) gave regioespecifically 9,9-dimethyl-9,10-dihydropyrimido[4,5-*c*]isoquinoline-1,7(2*H*,8*H*)-diones **5a-j** in 55-70% and 21-25% yield respectively (Scheme 2). The one-step cyclocondensation reaction can afford angular and/or linear products (**5/6**).

The structure of compounds **5a-j** were assigned by ^{1}H and ^{13}C-nmr spectra and mass spectrometric data (see experimental). The support for the structures **5** was provided from nmr spectra (in particular HSQC and HMBC experiments). In HMBC experiments, absence of correlation between the pyridinic proton and the carbon of carbonilic group at pyrimidine moiety discard linear products **6**.

Scheme 1



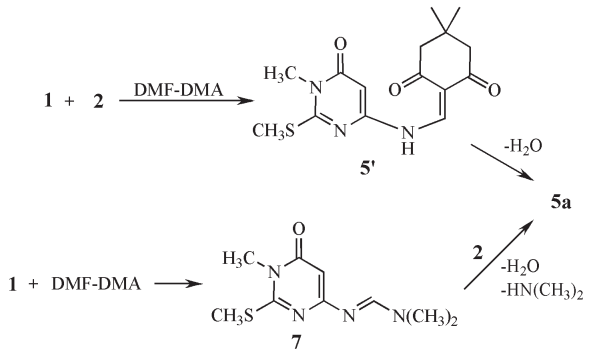
Scheme 2



For example, compound **5a** gave a ^{1}H-nmr spectrum with four relatively sharp singlets at 8.71, 3.51, 2.68 and 1.05 ppm with integrals in the ratio 1:3:3:6 respectively, which were readily assigned to the H-6 isoquinolinic ring, to the methyls of CH_3N and CH_3S groups on pyrimidine nucleus and to the two methyl groups at position 9 respectively. Two broad singlets at 2.62 and 3.07 ppm were assigned to CH_2-groups of positions 8 and 10 respectively.

Regarding ^{13}C-nmr spectra, DEPT experiments allowed us the assignment of the signals belonging to quaternary, tertiary, secondary and primary carbon atoms of compounds **5a-j**.

Scheme 3



The unequivocally assignment of signals in the ^{1}H- and ^{13}C-nmr spectra of compounds **5a-j w**as deduced from the concerted application of both direct and long range heteronuclear chemical shift correlation experiments. One-bond proton-carbon chemical shift correlations were established using the HSQC [17] sequence and $(CH)_n$ groups were unambiguously characterized from the analysis of long-range correlation responses over to two and three bonds (^{2}J or ^{3}J couplings) using the HMBC [18] technique.

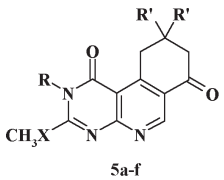
It is noteworthy that when aminopyrimidine **1** (R = CH_3, X = S), dimedone **2** and *N*,*N*-dimethylformamide dimethylacetal were irradiated during only 2 min, the reaction led to the formation of the stable intermediate **5'**, which was isolated, characterized and cycled by subsequent irradiation for 4.5 min to the corresponding compound **5a** (Scheme 3).

The isolation of intermediate **5'** allowed us to assume that the formation of **5** probably occurs by the attack of the nucleophilic methylene-group of dimedone to the double bond of the 6-dimethylaminomethylenaminopyrimidine intermediate (formed *in-situ* by a condensation between aminopyrimidine **1** and *N*,*N*-dimethylformamide dimethylacetal), with subsequent cyclization of the previously formed adduct and water elimination to give **5**. To verify that, we realized the reaction, irradiated by microwave for

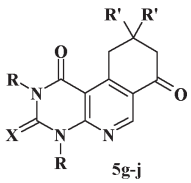
Table 1

Minimal Inhibitory Concentration (MIC) Values (μg/ml) of Pyrimido[4,5-*c*]isoquinolines Against a Panel of Yeasts, Hialohyphomycetes and Dermatophytes.

Type **A**



Type **B**



Comp	Type	R'	R	X	*C.a.*	*C.t.*	*S.c.*	*C.n.*	*A.fl.*	*A.n.*	*A.f.*	*M.c.*	*M.g.*	*T.m*	*T.r.*	*E.f*
5a	**A**	CH_3	CH_3	S	>250	>250	>250	>250	>250	>250	>250	100	100	50	50	20
5b	**A**	CH_3	H	S	>250	>250	>250	>250	>250	>250	>250	250	250	50	50	50
5d	**A**	CH_3	H	O	>250	>250	>250	>250	>250	>250	>250	250	250	50	50	50
5e	**A**	H	H	S	>250	>250	>250	>250	>250	>250	>250	250	200	100	200	100
5f	**A**	H	H	O	>250	>250	>250	>250	>250	>250	>250	250	200	200	200	100
5g	**B**	CH_3	H	S	>250	>250	>250	>250	>250	>250	>250	250	250	250	50	250
5h	**B**	CH_3	H	O	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
5i	**B**	CH_3	CH_3	O	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	250
5j	**B**	H	CH_3	O	200	100	100	100	100	200	100	>250	>250	>250	>250	>250
Keto					6.25	6.25	3.12	1.56	6.25	12.5	12.5	15	12.5	12.5	12.5	25
Amp					0.78	1.56	0.78	0.78	0.78	1.56	3.12	25	6.25	6.25	6.25	0.3
Terbin.					1.56	0.78	3.12	0.39	0.78	0.78	0.78	0.04	0.04	0.04	0.01	0.001

C.a.: *Candida albicans* ATCC 10231; *C.t.*: *Candida tropicalis* C131; *S.c.*: *Saccharomyces cerevisiae* ATCC 9763; *C.n.*: *Cryptococcus neoformans* ATCC 32264; *A.fl.*: *Aspergillus flavus* ATCC 9170, *A.n.*: *Aspergillus niger* ATCC 9029; *A.f.*: *Aspergillus fumigatus* ATCC 26934; *M.c:Microsporum canis* C 112; *M.g:· Microsporum gypseum* C 115. *T.m: Trichophyton mentagrophytes* ATCC 9972. *T.r.: Trichophyton rubrum* C113; *E.f.*: *Epidermophyton floccosum* C 114. Keto= Ketoconazole; Amp.= Amphotericin B. Terbin. = Terbinafine.

3 min, of 6-dimethylaminomethylenaminopyrimidin-4-one **7** with dimedone, which carried out the product **5** (see experimental) (compound **7** was synthesized by us [19]). The latter result discards the formation of linear structures **6** (see scheme 2). It is evident from these results that the preparation of compound **5** by multicomponent reaction between aminopyrimidines, *N*,*N*-dimethylformamide dimethylacetal and methylene-active compound (dimedone) by microwave irradiation for a short time has important advantages over recent reported procedures [19], which required of two-step reactions with previous preparation of either methylene derivative of aminopyrimidine **1** [19a] or in the some cases, previous condensation of *N*,*N* dimethylformamide dimethylacetal with methylene-actived component [19b].

Pyrimidoisoquinolines **5** were tested against a panel of yeasts, hialohyphomycetes and dermatophytes using the microbroth dilution method. Concentrations of compounds up to 250 μg/mL were incorporated to growth media according to the guideliness of the NCCLS [20].

Results showed that structures of type A (Table 1, compounds **5a-f**) displayed activity only against dermatophytes with MICs = 20-250 μg/ml. Since dermatophytes are a group of fungi, which characteristically infect the keratinized areas of the body and causes dermatomycoses that are very difficult to eradicate, it is important to note that these type of compounds showed selectivity against dermatophytes and not against another type of fungi. Among them, compound **5a** was the most active pyrimidoisoquinoline derivative showing a MIC = 20 μg/ml against *Epidermophyton floccosum* better than the activity showed by ketoconazole against the same fungus (MIC= 25 μg/ml).

Regarding compounds with a more oxidized substituent (=O or =S on position 3), (compounds **5g-j** structures type **B**), it is clear that in contrast with compounds type **A**, they are almost inactive against dermatophytes. Interesting enough, compound **5j**, with no substituent on position 9, showed activity against the yeasts *Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* and *Cryptococcus neoformans* and the filamentous fungi *Aspergillus niger*, *A. flavus* and *A. fumigatus* at concentrations below 250 μg/ml (MICs 100-200 μg/ml) in contrast with non-substituted structures type **A** in position 9. The activity showed by **5j**, although low, could be interesting for future research.

Conclusion.

This work contributes to the knowledge of the antifungal properties of pyrimido[4,5-*c*]isoquinolines which, although moderate, open new avenues for the design of new antifungal structures.

Since one of the strategies for avoiding the emergence of antifungal resistance, is the treatment of fungal infections with the appropriate antifungal agent in the case that the ethiological agent is known [6], our findings could be helpful in developing new antifungal agents, particularly analogues of structure **5a**, which selectively inhibit *E. floccosum*, a fungal species that is an environmental cause of contagion and is very difficult to eradicate.

The multicomponent regiospecific syntheses in solvent-free conditions under microwave, used for obtaining these pyrimido[4,5-*c*]isoquinolines, provide rapid access to them with great ease of execution and high yields, with the advantage of the simplicity, less pollution, and minimum reaction time brought in by the microwave heating.

EXPERIMENTAL

Melting points were taken on a Büchi melting point apparatus and are uncorrected. The ^{1}H- and ^{13}C nmr spectra were run on a Bruker AVANCE DRX 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 recorded on a Fisons-Platform interface APCI in methanol and recorded 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 elemental analyzer.

General Procedure for the Preparation of the 9,9-Dimethyl-9,10-dihydropyrimido[4,5-*c*]isoquinoline-1,7(2*H*,8*H*)-diones **5a-g**.

Equimolar amounts of amine **1** (0.2 mmol), dimedone **2** (0.2 mmol) and *N*,*N*-dimethylformamide dimethylacetal (0.24 mmol) were placed into pyrex-glass open vessels and irradiated in a domestic microwave oven for 2-6 min (at 600 watts). The products **5** were recrystallized from absolute ethanol.

2,9,9-Trimethyl-3-methylsulfanyl-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5a**).

This compound was obtained according to general procedure as crystals, yield 70 %, mp 253-254 °C. MS: EI m/z (relative abundance) = 304 (10), 303 (39, M^+), 288 (10, M^+-CH_3), 259 (23), 258 (100), 257 (14), 229 (15), 202 (10), 173 (13), 117 (10), 103 (10), 91 (12), 90 (11), 89 (13), 88 (36), 77 (17), 76 (10), 73 (12), 63 (23), 56 (24), 55 (28), 53 (20), 52 (12), 51 (18), 47 (38), 46 (29), 45 (31), 43 (21), 42 (50), 41 (74), 40 (17), 39 (55). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.05 (s, 6H, $C(CH_3)_2$), 2.62 (s, 2H, CH_2), 2.68 (s, 3H, CH_3S), 3.07 (s, 2H, CH_2), 3.51 (s, 3H, CH_3N), 8.71 (s, 1H, 6-H); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 15.0 (CH_3S), 27.8 ($C(CH_3)_2$), 30.3 (CH_3N), 32.4 (C-9), 46.0 (C-10), 51.1 (C-8), 112.4 (C-10b), 124.4 (C-6a), 134.9 (C-6), 157.9 (C-4a), 160.8 (C-1 (C=O)), 165.2 (C-3), 168.4 (C-10a), 196.0 (C-7 (C=O)).

Anal. Calcd. for $C_{15}H_{17}N_3O_2S$: C, 59.39; H, 5.65; N, 13.85. Found: C, 59.45; H, 5.72; N, 13.79.

9,9-Dimethyl-3-methylsulfanyl-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5b**).

This compound was obtained according to general procedure as crystals, yield 55 %, mp 198-200 °C. MS: EI m/z (relative abundance) = 289 (15, M^+), 274 (21), 259 (24), 258 (100), 229 (15), 88 (21), 42 (13), 41 (21). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.06 (s, 6H, $C(CH_3)_2$), 2.61 (s, 2H, CH_2), 2.69 (s, 3H, CH_3S), 3.01 (s, 2H, CH_2), 8.71 (s, 1H, 6-H), 11.70 (s, 1H, NH); ^{13}C nmr

(dimethyl sulfoxide-d_6) δ: 14.9 (CH_3S), 24.7 ($C(CH_3)_2$), 32.3 (C-9), 46.1 (C-10), 51.1 (C-8), 112.4 (C-10b), 124.5 (C-6a), 134.9 (C-6), 157.9 (C-4a), 160.7 (C-1 (C=O)), 165.1 (C-3), 168.3 (C-10a), 195.8 (C-7 (C=O)).

Anal. Calcd. for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52. Found: C, 58.06; H, 5.31; N, 14.57.

3-Methoxy-2,9,9-trimethyl-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5c**).

This compound was obtained according to general procedure as crystals, yield 75 %, mp 199-200 °C. MS: EI m/z (relative abundance) = 287 (100, M^+), 272 (7), 259 (9), 231 (96), 217 (11), 175 (7), 146 (11), 118 (13), 91 (8), 77 (7), 66 (7), 41 (9). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.05 (s, 6H, $C(CH_3)_2$), 2.60 (s, 2H, CH_2), 3.06 (s, 2H, CH_2), 3.23 (s, 3H, CH_3N), 3.59 (s, 3H, CH_3O), 8.60 (s, 1H, 6-H); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 27.6 ($C(CH_3)_2$), 28.0 (CH_3N), 32.4 (C-9), 45.8 (C-10), 50.8 (C-8), 54.9 (CH_3O), 119.3 (C-10b), 122.3 (C-6a), 134.9 (C-6), 150.8 (C-4a), 152.5 (C-1 (C=O)), 160.3 (C-3), 167.5 (C-10a), 195.6 (C-7 (C=O)).

Anal. Calcd. for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.62. Found: C, 62.63; H, 5.92; N, 14.68.

3-Methoxy-9,9-dimethyl-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5d**).

This compound was obtained according to general procedure as crystals, yield 75 %, mp 225-227 °C. MS: EI m/z (relative abundance) = 273 (100, M^+), 258 (13), 245 (38), 230 (10), 217 (99), 203 (10), 187 (5), 174 (10), 146 (8), 131 (9), 118 (12), 104 (7), 91 (6), 77 (9), 58 (12), 39 (16). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.05 (s, 6H, $C(CH_3)_2$), 2.57 (s, 2H, CH_2), 3.12 (s, 2H, CH_2), 3.80 (s, 3H, CH_3O), 8.50 (s, 1H, 6-H), 11.73 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 27.6 ($C(CH_3)_2$), 32.3 (C-9), 45.5 (C-10), 50.8 (C-8), 55.0 (CH_3O), 109.0 (C-10b), 122.5 (C-6a), 134.5 (C-6), 150.2 (C-4a), 154.5 (C-1 (C=O)), 161.8 (C-3), 168.1 (C-10a), 195.6 (C-7 (C=O)).

Anal. Calcd. for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 61.42; H, 5.43; N, 15.30.

3-Methylsulfanyl-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5e**).

This compound was obtained according to general procedure as crystals, yield 56 %, mp 282-284 °C. MS: EI m/z (relative abundance) = 261 (100, M^+), 233 (10), 215 (10), 188 (16), 187 (34), 186 (11), 177 (7), 160 (10), 159 (22), 132 (11), 131 (12), 130 (8), 104 (24), 103 (13), 91 (9), 90 (9), 89 (14), 78 (12), 77 (30), 76 (13), 75 (7), 74 (30), 66 (8), 65 (12), 64 (14), 63 (30), 62 (13), 55 (14), 53 (20), 52 (17), 51 (23), 50 (10), 47 (26), 46 (24), 44 (23), 42 (13), 41 (21), 39 (29). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 2.13 (s, 2H, CH_2), 2.60 (s, 3H, CH_3S), 2.67 (s, 2H, CH_2), 3.13 (s, 2H, CH_2), 8.67 (s, 1H, 6-H), 13.04 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 14.4 (CH_3S), 20.7 (C-9), 32.4 (C-10), 37.7 (C-8), 110.1 (C-10b), 125.3 (C-6a), 134.6 (C-6), 157.7 (C-4a), 160.0 (C-1 (C=O)), 167.0 (C-3), 169.4 (C-10a), 195.7 (C-7 (C=O)).

Anal. Calcd. for $C_{12}H_{11}N_3O_2S$: C, 55.16; H, 4.24; N, 16.08. Found: C, 55.22; H, 4.16; N, 16.19.

3-Methoxy-9,10-dihydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5f**).

This compound was obtained according to general procedure as crystals, yield 72 %, mp >300 °C. MS: EI m/z (relative abundance) = 245 (100, M^+), 217 (91), 216 (14), 215 (12), 174 (12), 131 (16), 118 (18), 104 (15), 78 (9), 77 (21), 68 (8), 63 (25), 58 (37), 55 (31), 53 (23), 52 (21), 51 (20), 44 (20), 43 (14), 42 (35), 41 (41) 39 (56). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 2.07 (s, 2H, CH_2), 2.60 (s, 2H, CH_2), 2.68, 3.01 (s, 2H, CH_2), 3.84 (s, 3H, CH_3O), 8.59 (s, 1H, 6-H), 13.04 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 21.3 (C-9), 32.6 (C-10), 38.0 (C-8), 53.9 (CH_3O), 110.0 (C-10b), 123.0 (C-6a), 134.6 (C-6), 157.9 (C-4a), 160.0 (C-1 (C=O)), 167.8 (C-3), 169.7 (C-10a), 196.2 (C-7 (C=O)).

Anal. Calcd. for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found: C, 58.72; H, 4.47; N, 17.18.

9,9-Dimethyl-3-thioxo-3,4,9,10-tetrahydro-2*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,7-dione (**5g**).

This compound was obtained according to general procedure as crystals, yield 70 %, mp 268-270 °C. MS: EI m/z (relative abundance) = 275 (100, M^+), 219 (57), 161 (10), 160 (7), 132 (5), 104 (7), 77 (9), 39 (9). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.08 (s, 6H, $C(CH_3)_2$), 2.59 (s, 2H, CH_2), 3.01 (s, 2H, CH_2), 8.50 (s, 1H, 6-H), 12.73 (s, 1H, NH); 13.34 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 27.6 ($C(CH_3)_2$), 32.3 (C-9), 45.6 (C-10), 50.8 (C-8), 110.9 (C-10b), 123.5 (C-6a), 134.3 (C-6), 153.3 (C-4a), 159.2 (C-1 (C=O)), 168.4 (C-3), 176.4 (C-10a), 195.4 (C-7 (C=O)).

Anal. Calcd. for $C_{13}H_{13}N_3O_2S$: C, 56.71; H, 4.76; N, 15.26. Found: C, 56.76; H, 4.64; N, 15.18.

9,9-Dimethyl-9,10-dihydro-4*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,3,7-trione (**5h**).

This compound was obtained according to general procedure as crystals, yield 70 %, mp 209-210 °C (dec). MS: EI m/z (relative abundance) = 259 (26, M^+), 203 (100), 132 (12), 104 (20), 77 (24), 41 (42), 39 (39). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.05 (s, 6H, $C(CH_3)_2$), 2.57 (s, 2H, CH_2), 2.98 (s, 2H, CH_2), 8.51 (s, 1H, 6-H), 11.60 (s, 1H, NH), 12.01 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 27.6 ($C(CH_3)_2$), 32.3 (C-9), 45.5 (C-10), 50.8 (C-8), 108.9 (C-10b), 122.5 (C-6a), 134.5 (C-6), 150.2 (C-4a), 154.5 (C-1 (C=O)), 161.8 (C-3), 168.1 (C-10a), 195.5 (C-7 (C=O)).

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.23; H, 5.05; N, 16.21. Found: C, 60.14; H, 5.12; N, 16.10.

2,4,9,9-Tetramethyl-9,10-dihydro-4*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,3,7-trione (**5i**).

This compound was obtained according to general procedure as crystals, yield 55 %, mp 185-187 °C. MS: EI m/z (relative abundance) = 287 (96, M^+), 259 (10), 244 (4), 231 (100), 205 (6), 175 (9), 146 (11), 118 (14), 91 (8), 77 (6), 66 (7), 41 (10). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.06 (s, 6H, $C(CH_3)_2$), 2.59 (s, 2H, CH_2), 3.06 (s, 2H, CH_2), 3.17 (s, 3H, CH_3N), 3.59 (s, 3H, CH_3N), 8.61 (s, 1H, 6-H); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 27.7 ($C(CH_3)_2$), 28.0 (CH_3N), 29.4 (CH_3N), 32.4 (C-9), 46.1 (C-10), 50.9 (C-8), 109.3 (C-10b), 122.5 (C-6a), 135.1 (C-6), 150.8 (C-4a), 152.6 (C-1 (C=O)), 160.3 (C-3), 167.6 (C-10a), 195.4 (C-7 (C=O)).

Anal. Calcd. for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.62. Found: C, 62.62; H, 5.85; N, 14.51.

2,4-Dimethyl-9,10-dihydro-4*H*,8*H*-pyrimido[4,5-*c*]isoquinoline-1,3,7-trione (**5j**).

This compound was obtained according to general procedure as crystals, yield 68 %, mp 198-200 °C. MS: EI m/z (relative

abundance) = 259 (100, M^+), 231 (78), 174 (5), 147 (12), 118 (12), 91 (8). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 2.08 (s, 2H, CH_2), 2.65 (s, 2H, CH_2), 3.09 (s, 2H, CH_2), 3.32 (s, 3H, CH_3N), 3.56 (s, 3H, CH_3N), 8.56 (s, 1H, 6-H); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 20.9 (C-9), 28.1 (CH_3N), 29.5 (CH_3N), 32.5 (C-10), 37.6 (C-8), 109.3 (C-10b), 123.4 (C-6a), 135.4 (C-6), 150.9 (C-4a), 152.1 (C-1 (C=O)), 160.3 (C-3), 169.0 (C-10a), 195.6 (C-7 (C=O)).

Anal. Calcd. for $C_{13}H_{13}N_3O_3$: C, 60.23; H, 5.05; N, 16.21. Found: C, 60.16; H, 5.13; N, 16.29.

5,5-Dimethyl-2-[(1-methyl-2-methylsulfanyl-6-oxo-1,6-dihydropyrimidin-4-ylamino)-methylene]-cyclohexane-1,3-dione (**5'**).

This compound was obtained according to general procedure by microwave irradiation for 2 min. as crystals, yield 75 %, mp 198-200 °C. MS: EI m/z (relative abundance) = 322 (21), 321 (100, M^+), 237 (36), 222 (10), 157 (18), 156 (83), 141 (18), 88 (88), 83 (16), 68 (12), 56 (11), 55 (29), 53 (19), 42 (18), 41 (22), 39 (15). ^{1}H nmr (dimethyl sulfoxide-d_6) δ: 1.00 (s, 6H, $(C(CH_3)_2)$, 2.39 (s, 2H, CH_2), 2.46 (s, 2H, CH_2), 2.62 (s, 3H, CH_3S), 3.38 (s, 3H, CH_3N), 6.24 (s, 1H, 5-H), 8.89 (d, 1H, CH=, ^3J = 12.82 Hz), 12.02 (d, 1H, NH, ^3J = 12.82 Hz); ^{13}C nmr (dimethyl sulfoxide-d_6) δ: 14.6 (CH_3S), 28.9 ($C(CH_3)_2$), 29.9 (CH_3N), 30.4 ($C(CH_3)_2$), 50.9 (CH_2), 51.3 (CH_2), 92.7 (C-5), 110.0 (=C(C=O)), 146.5 (NH-C=), 152.7 (C-6), 161.4 (C-4 (C=O)), 164.5 (C-2), 195.4 and 199.3 (C=O groups).

Anal. Calcd. for $C_{15}H_{19}N_3O_3S$: C, 56.06; H, 5.96; N, 13.07. Found: C, 56.15; H, 5.84; N, 13.13.

Reaction of 6-Dimethylaminomethylenaminopyrimidin-4-one **7** with Dimedone. Obtention of Compound **5a**.

A solution of 6-dimethylaminomethylenaminopyrimidin-4-one (**7**) (1 mmole) (compound **7** was obtained from aminopyrimidine **1** (R = CH_3, X = S) and *N*,*N*-dimethylformamide dimethylacetal by methodology described in our work [19a]) and dimedone (1 mmole) was irradiated in a domestic microwave oven for 3 min (at 600 watts). The solid obtained was treated with ethanol and recrystallized from absolute ethanol, obtaining compound **5a** with yield 50 % (from aminopyrimidine **1**), mp 252-254 °C. This sample presents the same characteristics that compound **5a** obtained by multicomponent reaction between aminopyrimidine, *N*,*N*-dimethylformamide dimethylacetal and dimedone.

Microorganisms and Media.

For the antifungal evaluation, strains from the American Type Culture Collection (ATCC, Rockville, MD, USA) and the Centro de Referencia Micológica Facultad de Ciencias Bioquímicas y Farmacéuticas (C, CEREMIC), Suipacha 531-(2000)-Rosario, Argentina, were used: *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, *Microsporum canis* C 112, *Trichophyton rubrum* C 113, *Epidermophyton floccosum* C 114 and *Microsporum gypseum* C 115. 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 [21].

Antifungal Susceptibility Testing.

The Minimal Inhibitory Concentration (MIC) of each extract was determined by using broth microdilution techniques following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [20]. 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 $5x10^3$ CFU/mL. Microtiter trays were incubated at 35 °C for yeasts and hialohyphomycetes 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. Ketoconazole, Terbinafine and Amphotericin B were used as standard drugs.

For the assay, stock solutions of compounds were diluted with RPMI from 250-0.98 μg/mL (final volume = 100 μL) and a final dimethyl sulfoxide concentration ≤ 2%. 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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REFERENCES AND NOTES

[1] B. Wong, B. Klei, T. Kozel, Inmunologic Approaches and Metabolite Detection. The Second NIAID Workshop in Medical Micology, University of Arizona, Northern Arizona University, Flagstaff, AZ, June 8-11, 1994.

[2] A. Groll, T. De Lucca, T. Walsh, *Trends in Microbiology*, **6**, 117 (1998).

[3] W. Timberlake, C. D'Enfert, W. Fonzi, Targets for Inhibiting Proliferaition of Virulence. The Second NIAID Workshop in Medical Micology, University of Arizona, Northern Arizona University, Flagstaff, AZ, June 8-11, 1994.

[4] M. Ghannoum, L. Rice, *Clin. Microbiol. Rev.*, **12**, 501 (1992).

[5] T. White, K. Marr, R. Bowden, R. *Clin. Microbiol. Rev.*, **11**, 382 (1998)

[6] F. Marco, M. A. Pfaller, S. Messer, R. Jones *Antimicrob. Agents Chemother.*, **42**, 161 (1998)

[7] A. Rashid, E. M. Scott, M. D. Richardson, *J. Am. Acad. Dermatol*., 718 (1995)

[8] P. H. Chandrasekar, E. K. Manavathu, *Drugs of Today***, 38**, 829 (2002).

[9a] C. C. Cheng, *Prog. Med. Chem.*, **6**, 67 (1969); [b] J. Scherkenbeck, T. Himmler, S. Beohm, H. Hagemann, K. Stroecl, S. Dutzmann, H. W. Dehne, Haenssler, G. Ger. Often DE 4,208,050, 23rd Sep. 1993; *Chem. Abstr.* **120**, 107033m (1994); [c] K. A. M. Walker, A. C. Braemer, S. Hitt, R. E. Jones, T. R. Mathews, *J. Med. Chem.*, **21**, 480 (1978); [d] T. Obata, K. Fujii, K. Tsutsumiuchi, Y. Nakamoto, Eur. Pat. Appl. EP 543,402, 26 May 1993; *Chem. Abstr.* **120**, 107043q (1994); [e]

P. Wachtler, W. Paulus, Ludwig, Georg-Wilhelm, Eur. Pat. Appl. EP 559, 002; 8 September 1993; *Chem. Abstr.* **120**, 107036q (1994); [f] S. Sakai, H. Hase, I. Narita, K. Hatano, H. Takunaga, JP05,294,945/93,294,945; *Chem. Abstr.* **120**, 217725w (1994); [g] S. Rollas, N. Kalyoncuoglu, D. Sur-Altiner, Yegenoglu, Y. *Pharmazie* **48**, 308 (1993); [h] A. Kreutzberger, A. Burger, *Arch. Pharm. (Weinheim)* **328**, 473 (1993).

[10] B. K. Shoichet, R. M. Stroud, D. U. Santi, I. D. Kuntz, K. M. Perry, *Science*, **259**, 1445 (1993).

[11] L. Pagano, L. Mele, L. Fianchi, L. Melillo B. Martino, D. D'Antonio, M. Tosti, B. Posteraro, M. Sanguinetti, G. Trape, F. Equitani, F. Nobile, M. Carotenuto, G. Leone, *Haematologica*, **87**, 535 (2002).

[12] J. Quiroga, A. Hormaza, B. Insuasty, A. J. Ortiz, A. Sánchez, M. Nogueras, *J. Heterocyclic Chem.*, **35**, 231 (1998).

[13a] A. Gangjee, J. K. Ohemeng, J. J. Tulachka, F.-T. Lin, A. A. Katoh, *J. Heterocyclic Chem.*, **22**, 1149 (1985); [b] A. Gangjee, J. K. O'Donnell, T. J. Bardos, T. I. Kalman, *J. Heterocyclic Chem.,* **21**, 873 (1984); [c] S. R. Stone, J. A. Montgomery, J. F. Morrison, *Biochem. Pharmacol.*, **33**, 175 (1984).

[14a] R. S. Varma, D. Kumar, *Tetrahedron Lett.*, **40**, 7665 (1999); [b] K. Groebke, L. Weber, F. Mehlin, *Synlett*, 661 (1998); [c] C. O. Kappe, D. Kumar, R. S. Varma, *Synthesis*, 1799 (1999); [d] L. Weber, K. Illegen, M. Almstetter, *Synlett*, 366 (1999); [e] R. W. Armstrong, A. P. Combs, P. A. Tempest, S. D. Brown, T. A. Keating, *Acc. Chem. Res.*, **29**, 123 (1996).

[15a] D. Dallinger, N. Y. Gorobets, C. O. Kappe, *Org. Lett.*, **5**, 1205 (2003); [b] U. Bora, A. Saikia, R. C. Boruah, *Org. Lett.*, **5**, 435 (2003); [c] J. F. Cheng, M. Chen, T. Arthenius, A. Nadzen, *Tetrahedron Lett.*, **43**, 6293 (2002); [d] M. C. Bagley, J. W. Cale, J. Bower, *Chem. Commun.*, 1682 (2002); [e] V. Nair, A. U. Vinod, C. Rajesh, *J. Org. Chem.*, **66**, 4427 (2001); [f] R.V.A. Orru, M. de Greef, *Synthesis*, 1471 (2003).

[16a] A. Loupy, A. Petit, J. Hamelin, F. Texier-Boullet, P. Jacquault, D. Mathe, *Synthesis*, 1213 (1998); [b] R. S. Varma, *Green Chemistry*, 43 (1999); [c] S. Deshayes, M. Liagre, A. Loupy, J.-L. Luche, A. Petit, *Tetrahedron*, **55**, 10951 (1999); [d] R. S. Varma, *Clean Products and Processes*, **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. Singh, *Arkivok*, **xiii**, 68; [h] M. Nüchter, U. Müller, B. Ondruschka, A. Tied, W. Lautenschläger, *Chem. Eng. Technol.,* **26**, 1207 (2003); [i] A. de la Hoz, A. Díaz-Ortiz, A. Moreno, *Chem. Soc. Rev.,* **34**, 164 (2005).

[17] A. Bax and S. Subramanian*, J. Magn. Reson*., **65**, 565 (1986).

[18] A. Bax and M. F. Summers, *J. Am. Chem. Soc*., **108**, 2093 (1986).

[19a] J. Quiroga, G. Viveros, B. Insuasty, M. Nogueras, A. Sánchez, J. Cobo, *J. Heterocyclic Chem.*, **36**, 501 (1999); [b] E. I. Al-Afaleq, *Synthetic Commun*., **30**, 1985 (2000).

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

[21] L. R. Wright, E. M. Scott, S. P. Gorman, *J. Antimicrob. Chemother*., **12**, 317 (1983).