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Growth inhibition of *Cryptococcus neoformans* by 2-(1-piperazinyl)-5-(1,2-diarylethyl)-4,6-dichloropyrimidines: synthesis and *in vitro* studies

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Summary — 2-(1-Piperazinyl)-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine and 2-(1-piperazinyl)-5-[1-(4-chlorophenyl)-2-(3-chlorophenyl)ethyl]-4,6-dichloropyrimidine were synthesized *via* organozinc reagents. These new pyrimidine derivatives were tested against human mycoflora. Biological tests showed that these compounds are selective growth inhibitors of *Cryptococcus neoformans*.

pyrimidine derivative / *Cryptococcus neoformans*

Introduction

Infection with *Cryptococcus neoformans* has been diagnosed in an increasing number of immunocompromised patients, such as those with the acquired immunodeficiency syndrome (AIDS). This has led to the development of new antifungal agents for the inhibition of the growth of this yeast. Some of these agents are used for chemotherapy of cryptococcis in human immunodeficiency virus (HIV)-infected persons. Among the compounds tested against *C neoformans*, there is a great diversity of chemical structures such as trifluoroperazine and chlorpromazine [1], sodium diethyldithiocarbamate [2], a copyrine alkaloid, 3-methoxysampangine [3], α-difluoromethylornithine and cyclohexylamine [4], pradimicin derivative [5], azole derivatives [6-8], 1,2,4-triazino[5,6-*b*]-indole compounds [9], a cyclic depsipeptide, aureobasidin A [10] and amphotericin-B [11].

In this paper, we show that other chemical series such as pyrimidine derivatives can inhibit growth of *C neoformans*. Moreover, we have observed that certain 2-(1-piperazinyl)-5-(1,2-diarylethyl)-4,6-dichloropyrimidines have a high selectivity against this yeast. The synthesis and *in vitro* studies of these compounds are described.

Chemistry

Scheme 1 shows the synthesis of different pyrimidine derivatives from 5-(4-chlorobenzylidene) barbituric acid **1**. This precursor **1** was easily prepared by condensing barbituric acid with 4-chlorobenzaldehyde according to a previously described method [12]. The 5-[1-(4-chlorophenyl)-2-phenylethyl]barbituric acid **2a** and 5-[1-(4-chlorophenyl)-2-(3-chlorophenyl)-ethyl]barbituric acid **2b** were obtained following the 1,4-addition of benzylzinc bromide or 3-chlorobenzylzinc bromide respectively to the same 5-(4-chlorobenzylidene) barbituric acid **1**. In a previous paper [13] we proved that the benzylzinc bromide also undergoes hydrogen-metal exchange with both NH sites of the substrate **1**. The reaction led to substantial yields of products **2** only if three molecular equivalents of the organozinc reagent were used. The reaction of a mixture of phosphorus oxychloride and phosphorus pentachloride with 5-(1,2-diarylethyl) barbituric acid **2a** or **2b** yielded 5-[1-(4-chlorophenyl)-2-phenylethyl]-2,4,6-trichloropyrimidine **3a** or 5-[1-(4-chloro-

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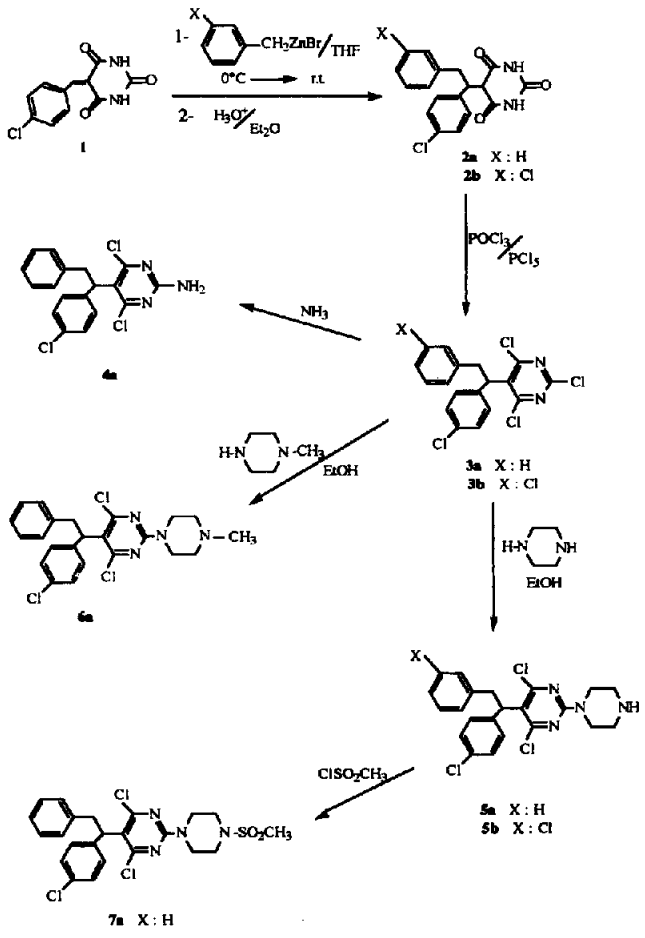


Scheme 1. Synthesis of 2-substituted-5-(1,2-diarylethyl)-4,6-dichloropyrimidine derivatives.

phenyl)-2-(3-chlorophenyl)ethyl]-2,4,6-trichloropyrimidine **3b**, respectively. Compound **3a** reacts at reflux of the solvent with an excess of concentrated ammonium hydroxide resulting in the corresponding 2-amino-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **4a**. The piperazinyl derivatives **5a** and **5b** were also prepared according to the procedure reported in scheme 1 by heating trichloropyrimidine **3a** or **3b** with an excess of piperazine at reflux of the ethanol. The same reaction of trichloropyrimidine **3a** with *N*-methylpiperazine yielded 2-[1-(4-methylpiperazinyl)]-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **6a**. The reaction of the compound **5a** with methanesulfonyl chloride gave the product **7a** resulting from methanesulfonation on the NH site of the substrate.

After purification, the purity of these products was checked on the basis of their elution profile in a capillary gas chromatography procedure. Yields of pyrimidine derivatives are reported in table I.

Pharmacology

In order to investigate the antifungal activity, the seven pyrimidine derivatives (**3a**, **3b**, **4a**, **5a**, **6a**, and **7a**), were tested *in vitro* against six species of human mycoflora: *Candida albicans*, *C neoformans*, *Aspergillus fumigatus*, *Scopulariopsis brevicaulis*, *Trichophyton rubrum*, and *T mentagrophytes*.

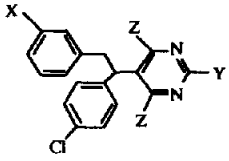
Results and discussion

Scheme 1 shows a convenient method for the synthesis of 5-(1,2-diarylethyl)-2,4,6-trichloropyrimidines **3a** and **3b** from their corresponding 5-(1,2-diarylethyl)barbituric acids **2a** and **2b**.

^{1}H-NMR analysis of 5-substituted barbituric acids at 200 MHz showed a doublet in the range of chemical shifts δ = 3.40–3.50 ppm for COCHCO proton. This doublet was well observed at δ = 3.41 ppm with a coupling constant ^3J = 3.2 Hz for 5-[1-(4-chlorophenyl)-2-(3-chlorophenyl)ethyl] barbituric acid **2b**. The barbituric ring exhibited a broad singlet for the two NH protons at δ = 9.85 ppm in acetone-d_6 as solvent for compound **2a** and δ = 7.75 ppm in $CDCl_3$ as solvent for compound **2b**. As expected, these characteristic signals of the barbituric ring disappeared in the NMR spectra of the corresponding trichloropyrimidines **3a** and **3b**. The experimental protocol demonstrated that ^{1}H-NMR spectrum of the ethyl chain was characteristic of the 5-(1,2-diarylethyl)-2,4,6-trichloropyrimidines. In particular, the tertiary proton exhibited a multiplet at 3.80 ppm for barbituric acid **2a** and 3.86 ppm for barbituric acid **2b**, whereas its signal was a triplet at 5.14 ppm for trichloropyrimidine **3a** and 5.13 ppm for trichloropyrimidine **3b** with a coupling constant about 8.5 Hz. Compared to their precursors **3a** and **3b**, the 2-substituted dichloropyrimidine derivatives **4a**, **5a**, **5b**, **6a** and **7a** retained the same NMR structure of the ethyl chain with small differences for the corresponding chemical shifts and coupling constants.

Mass spectra of dichloropyrimidines and trichloropyrimidines showed the expected fragments with isotopic peaks due to the presence of chlorine atoms 35 and 37 in their structure. For instance, 5-[1-(4-chlorophenyl)-2-phenylethyl]-2,4,6-trichloropyrimidine **3a** displayed molecular isotopic peaks at *m/z* = 396, 398, 400 and 402 and the fragment $C_{11}H_{15}N_2Cl_4^+$ at *m/z* = 305, 307 and 309. For the 2-(1-piperazinyl)-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **5a** the molecular isotopic peaks were at *m/z* =

Table I. Data for 5-(1,2-diarylethyl)pyrimidines.



Compound	*X*	*Y*	*Z*	*Yield (%)*	*Molecular formula*	*Analysis*[a]
2a	H	OH	OH	75	$C_{18}H_{15}ClN_2O_3$ (342.8)	
2b	Cl	OH	OH	86	$C_{18}H_{14}Cl_2N_2O_3$ (377.2)	
3a	H	Cl	Cl	50	$C_{18}H_{12}Cl_4N_2$ (398.1)	C, H, Cl, N
3b	Cl	Cl	Cl	53	$C_{18}H_{11}Cl_5N_2$ (432.6)	C, H, Cl, N
4a	H	NH_2	Cl	84	$C_{18}H_{14}Cl_3N_3$ (378.7)	C, H, Cl, N
5a	H		Cl	74	$C_{22}H_{21}Cl_3N_4$ (447.8)	C, H, Cl, N
5b	Cl		Cl	84	$C_{22}H_{20}Cl_4N_4$ (482.2)	C, H, Cl, N
6a	H		Cl	95	$C_{23}H_{23}Cl_3N_4$ (461.8)	C, H, Cl, N
7a	H		Cl	85	$C_{23}H_{23}Cl_3N_4O_2S$ (525.9)	C, H, Cl, N, S

[a]Analyses indicated by the symbols of the elements were within ±0.4% of chemical values.

446, 448 and 450 and the fragment $C_{15}H_{14}N_4Cl_3^+$ at m/z = 355, 357 and 359.

Among the seven pyrimidine derivatives used for biological tests, we observed an antifungal activity with 2-(1-piperazinyl)-5-(1,2-diarylethyl)-4,6-dichloropyrimidines **5a** and **5b**. Moreover, this activity was very specific to *C neoformans* colonies with a minimum inhibitory concentration of 150 µg/ml for the two growth inhibitors **5a** and **5b**. There was no antifungal activity with the trichloropyrimidines **3a** and **3b** used as precursors of the products **5a** and **5b**. No antifungal activity was observed with 2-amino-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **4a**, the *N*-methyl derivative **6a** and the *N*-methanesulfonyl derivative **7a**. This suggested that 2-(1-piperazinyl)-4,6-dichloropyrimidine group, with a free NH site, was the active part of the cryptococcis growth inhibitors. These preliminary results, which showed a high specific activity of pyrimidine derivatives **5a** and **5b** against *C neoformans*, encouraged us to continue this work in order to increase their inhibition and improve the understanding of the structure–activity relationships.

Experimental protocols

Chemistry

Infrared spectra were recorded on a Perkin-Elmer IR 1310 spectrometer and ultraviolet spectra on a Secomam S 1000 spectrometer. ^{1}H-NMR spectra were recorded on a Bruker AM 200 (200 MHz) spectrometer using $CDCl_3$, acetone-d_6 or DMSO-d_6 as solvent and tetramethylsilane as internal standard; mass spectra were obtained on a Hewlett Packard HP5989 A spectrometer (electronic impact at 70 eV). Thin layer chromatography analysis was conducted using silica gel $60F_{254}$. TLC plates were purchased from Merck; compounds were revealed by UV detection. Melting points were not corrected. The purity of the synthesized compounds was verified by gas chromatography (GC, HP 5890A, II) coupled with mass spectrometry.

A 25 m x 0.2 mm fused silica capillary column OVI (HP_1) Hewlett Packard was directly inserted into the ion source of the HP quadrupole mass spectrometer through a heated (250°C) interface box. Helium was used as carrier gas with a flow rate through the column of 0.7 ml/min. The temperature remained at 70°C for 1 min and was then programmed up to 300°C at 10°C/min. The final time was 60 min. The temperature of the ion source was 200°C and the energy of bombarding electrons was 70 eV. Elemental analyses were within ±0.4% of theoretical values and were determined in the Laboratory of the Service Central d'Analyse du CNRS (Vernaison, France).

5-(4-Chlorobenzylidene)barbituric acid 1

Compound **1** was obtained according to a described method [12] by condensing the 4-chlorobenzaldehyde from Janssen (7.0 g, 50 mmol) with barbituric acid from Janssen (6.4 g, 50 mmol) dissolved in hot water (60 ml). The precipitate was filtered off, washed with small quantities of hot water and dried. Compound **1** (11.5 g, 92% yield) was characterized; IR (KBr) 3270 (NH), 1755, 1700, 1645 (C=O), 1593, 1570, 1547 (aromatic ring) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ ppm: 7.29 (m, 2H arom, ^3J = 8.5 Hz); 7.84 (d, 2H arom, ^3J = 8,5 Hz); 8.00 (s, 1H, Ar-CH=); 11.02 (s, 1H, NH); 11.17 (s, 1H, NH).

Preparation of organozinc reagents

According to the procedure described by Gaudemar [14] benzylzinc bromide or 3-chlorobenzylzinc bromide was ob-

tained by reaction between benzylbromide from Aldrich (7.18 g, 42 mmol) or 3-chlorobenzyl bromide from Aldrich (8.63 g, 42 mmol) and zinc from Labosi (2.75 g, 42 mmol) in dry tetrahydrofuran (THF, 20 ml) at 25–30°C, blanketed under nitrogen gas. The organozinc reagents were used *in situ* for the synthesis of compounds **2a** and **2b**.

*5-(1,2-Diarylethyl)barbituric acids **2a** and **2b***
According to a described method [13] a solution of benzylzinc bromide or 3-chlorobenzylzinc bromide (42 mmol) in THF was cooled to 0°C and the 5-(4-chlorobenzylidene) barbituric acid **1** (2.50 g, 10 mmol) was added while stirring and cooling. The temperature of the mixture quickly rose to 30°C. When it began to fall the cooling bath was removed. After stirring at room temperature for 1 h, the mixture was hydrolysed with crushed ice (30 g) and concentrated hydrochloric acid (5 ml); ether (30 ml) was then added. The two phases were separated and the aqueous layer was extracted with ether (4 x 20 ml). The combined organic phase was washed with brine (50 ml), dried with anhydrous sodium sulfate and evaporated to give the crude solid product **2a** or **2b**. For purification, the crude product was dissolved in aqueous 2 N sodium hydroxide (40 ml). The aqueous layer was washed with ether (40 ml). Strong hydrochloric acid (10 ml) was then added to precipitate product **2a** or **2b**. This product was dissolved in ether (100 ml), washed with brine (4 x 20 ml) and dried with anhydrous sodium sulfate. The solvent was removed under vacuum.

*5-[1-(4-Chlorophenyl)-2-phenylethyl]barbituric acid **2a**.* The reaction of benzylzinc bromide with 5-(4-chlorobenzylidene) barbituric acid **1** yielded compound **2a** (2.57 g, 75% yield); mp: 124–126°C; IR (KBr) 3200 (NH), 3045 (CH arom), 2920, 2840 (CH, CH_2), 1755, 1705, 1695 (C=O), 1595, 1570, 1490 (aromatic rings) cm^{-1}; UV (EtOH) 209, 212, 216, 267 nm; ^{1}H-NMR (acetone-d_6) δ ppm: 3.07 (dd, 1H, ^2J = 13.8 Hz, ^3J = 6.7 Hz, Ar-CH); 3.42–3.55 (m, 2H, Ar-CH and COCHCO); 3.80 (m, 1H, Ar CH); 6.89–7.27 (m, 9H arom); 9.85 (broad s, 2H, 2NH).

*5-[1-(4-Chlorophenyl)-2-(3-chlorophenyl)ethyl] barbituric acid **2b**.* The reaction of 3-chlorobenzylzinc bromide with 5-(4-chlorobenzylidene) barbituric acid **1** yielded compound **2b** (3.24 g, 86% yield); mp: 118–122°C; IR ($CHCl_3$) 3210, 3100 (NH), 2910, 2860 (CH, CH_2), 1705 (C=O), 1595, 1570, 1490 (aromatic rings) cm^{-1}; UV (EtOH) 212, 216, 265 nm; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.08 (dd, 1H, ^2J = 13.8 Hz, ^3J = 6.0 Hz, Ar-CH); 3.41 (d, 1H, ^3J = 3.2 Hz, CO-CH-CO); 3.54 (dd, 1H, ^2J = 13.8 Hz, ^3J = 10.7 Hz, Ar-CH); 3.86 (m, 1H, Ar-CH); 7.08 (d, 2H arom, ^3J = 8.5 Hz); 7.17 (m, 4H arom); 7.32 (d, 2H arom, ^3J = 8.5 Hz); 7.75 (broad s, 2H, 2NH).

*5-(1,2-Diarylethyl)-2,4,6-trichloropyrimidines **3a** and **3b***
According to a described method [15] a mixture of barbituric acid **2a** or **2b** (5 mmol) and phosphorus oxychloride from Prolabo (1.53 g, 10 mmol) was heated at reflux (105°C) overnight. After cooling to room temperature, phosphorus pentachloride from Janssen (3.12 g, 15 mmol) was added. Refluxing was then continued overnight. After cooling, the reaction mixture was poured onto ice and allowed to stand 30 min. Product **3a** or **3b** was extracted with ether (3 x 20 ml), decolorized with charcoal and then filtered. The organic layer was treated by 2 N sodium hydroxide (10 ml) and washed with brine until neutrality. The organic phase was then dried with anhydrous sodium sulfate and the solvent was removed. The residue was purified by recrystallization.

*5-[1-(4-Chlorophenyl)-2-phenylethyl]-2,4,6-trichloropyrimidine **3a**.* The reaction with barbituric acid **2a** (1.71 g) yielded **3a** (1.0 g, 50% yield); mp: 142–143°C (EtOH); silica-gel preparative TLC (*n*-hexane/$CHCl_3$ 80:20; R_f = 0.57). GC/MS: R_t = 24.9 min; IR (KBr) 3030 (CH arom), 2930 (CH, CH_2), 1600, 1525, 1495 (aromatic rings) cm^{-1}; UV (EtOH) 270 nm; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.58 (d, 2H, ^3J = 8.6 Hz, Ar-CH_2); 5.14 (t, 1H, ^3J = 8.6 Hz, Ar-CH); 7.00–7.36 (m, 9H arom); MS *m/z* $M^{\bullet+}$: 396 (2.8%, $C_{18}H_{12}N_2{}^{35}Cl_4{}^{\bullet+}$); $M^{\bullet+}$ + 2: 398 (3.9%, $C_{18}H_{12}N_2{}^{35}Cl_3{}^{37}Cl^{\bullet+}$); $M^{\bullet+}$ + 4: 400 (1.8%, $C_{18}H_{12}N_2{}^{35}Cl_2{}^{37}Cl_2{}^{\bullet+}$); $M^{\bullet+}$ + 6: 402 (0.4%, $C_{18}H_{12}N_2{}^{35}Cl{}^{37}Cl_3{}^{\bullet+}$); 91 (100%, $C_7H_7{}^+$); 305 (6.9%, $C_{11}H_5N_2{}^{35}Cl_4{}^+$); 307 (8.5%, $C_{11}H_5N_2{}^{35}Cl_3{}^{37}Cl^+$); 309 (4%, $C_{11}H_5N_2{}^{35}Cl_2{}^{37}Cl_2{}^+$); 174 (4.8%, $C_{10}H_5N^{35}Cl^+$); 65 (12.2%, $C_5H_5{}^+$).

*5-[1-(4-Chlorophenyl)-2-(3-chlorophenyl)ethyl]-2,4,6-trichloropyrimidine **3 b**.* The reaction with barbituric acid **2b** (1.88 g) yielded **3b** (1.15 g, 53% yield); GC/MS: R_t = 26.1 min; IR (KBr) 3025 (CH arom), 2920 (CH, CH_2), 1600, 1550, 1495 (aromatic rings), cm^{-1}; UV (EtOH) 270 nm; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.53 (d, 2H, ^3J = 8.4 Hz, Ar-CH_2); 5.13 (t, 1H, ^3J = 8.4 Hz, Ar-CH); 6.86–7.29 (m, 8H arom); MS *m/z* $M^{\bullet+}$: 430 (8.5%, $C_{18}H_{11}N_2{}^{35}Cl_5{}^{\bullet+}$); $M^{\bullet+}$ + 2: 432 (13.6%, $C_{18}H_{11}N_2{}^{35}Cl_4{}^{37}Cl^{\bullet+}$); $M^{\bullet+}$ + 4: 434 (8.7%, $C_{18}H_{11}N_2{}^{35}Cl_3{}^{37}Cl_2{}^{\bullet+}$); $M^{\bullet+}$ + 6: 436 (2.7%, $C_{18}H_{11}N_2{}^{35}Cl_2{}^{37}Cl_3{}^{\bullet+}$); $M^{\bullet+}$ + 8: 438 (0.6%, $C_{18}H_{11}N_2{}^{35}Cl{}^{37}Cl_4{}^{\bullet+}$); 305 (76%, $C_{11}H_5N_2{}^{35}Cl_4{}^+$); 307 (100%, $C_{11}H_5N_2{}^{35}Cl_3{}^{37}Cl^+$); 309 (48.4%, $C_{11}H_5N_2{}^{35}Cl_2{}^{37}Cl_2{}^+$); 311 (10.4%, $C_{11}H_5N_2{}^{35}Cl{}^{37}Cl_3{}^+$); 269 (8.3%, $C_{11}H_4N_2{}^{35}Cl_3{}^+$); 271 (8.8%, $C_{11}H_4N_2{}^{35}Cl_2{}^{37}Cl^+$); 125 (16.9%, $C_7H_6{}^{35}Cl^+$); 127 (5.6%, $C_7H_6{}^{37}Cl^+$).

*2-Amino-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **4a***
Trichloropyrimidine **3a** (184 mg, 462 µmol) was stirred at reflux for 15 h with 28% ammonium hydroxide (10 ml) and ethanol (35 ml). After cooling, ethanol was removed under vacuum and the aqueous layer was then extracted with chloroform (6 x 20 ml). The combined organic layer was washed with brine (5 x 10 ml) and dried with Na_2SO_4. The solvent was evaporated and the residue recrystallized from acetone. Product **4a** (146 mg, 385 µmol) was obtained with 84% yield. GC/MS: R_t = 27.2 min; IR (KBr) 3317, 3190 (NH_2), 3032 (CH arom), 2968, 2937 (CH, CH_2), 1632, 1568, 1552, 1540, 1496 (aromatic rings) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm: 3.49 (d, 2H, ^3J = 8.1 Hz, Ar-CH_2); 4.98 (t, 1H, ^3J = 8.1 Hz, Ar-CH); 5.24 (s, 2H, NH_2); 6.95–7.65 (m, 9H arom); MS *m/z* $M^{\bullet+}$: 377 (3.8%, $C_{18}H_{14}N_3{}^{35}Cl_3{}^{\bullet+}$); $M^{\bullet+}$ + 2: 379 (3.7%, $C_{18}H_{14}N_3{}^{35}Cl_2{}^{37}Cl^{\bullet+}$); $M^{\bullet+}$ + 4: 381 (1.2%, $C_{18}H_{14}N_3{}^{35}Cl{}^{37}Cl_2{}^{\bullet+}$); $M^{\bullet+}$ + 6: 383 (0.1%, $C_{18}H_{14}N_3{}^{37}Cl_3{}^{\bullet+}$); 286 (100%, $C_{11}H_7N_3{}^{35}Cl_3{}^{\bullet+}$); 288 (94%, $C_{11}H_7N_3{}^{35}Cl_2{}^{37}Cl^+$); 290 (30.7%, $C_{11}H_7N_3{}^{35}Cl{}^{37}Cl_2{}^+$); 292 (3,4%, $C_{11}H_7N_3{}^{37}Cl_3{}^+$); 216 (12.8%, $C_{11}H_7N_3{}^{35}Cl^+$); 218 (12.6%, $C_{11}H_7N_3{}^{37}Cl^+$); 180 (6.7% $C_{11}H_6N_3{}^+$); 91 (16.5%, $C_7H_7{}^+$); 65 (9.8%, $C_5H_5{}^+$).

*2-(1-Piperazinyl)-5-(1,2-diarylethyl)-4,6-dichloropyrimidines **5a** and **5b***
Trichloropyrimidine **3a** (2.79 g, 7 mmol) or trichloropyrimidine **3b** (3.03 g, 7 mmol) and piperazine from Aldrich (1.20 g, 14 mmol) in absolute ethanol (30 ml) were heated at reflux for 15 h. The organic layer was then removed under vacuum. The residue was treated with 2 N sodium hydroxide (40 ml) and the solid dissolved in CH_2Cl_2 (6 x 20 ml). The combined organic layer was washed with brine (5 x 10 ml) and dried with Na_2SO_4. The solvent was evaporated to give product **5a** or **5b**, respectively.

*2-(1-Piperazinyl)-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **5a**.* The reaction yielded product **5a** (2.10 g; 67% yield); mp: 80–82°C; GC/MS: R_t = 30.7 min; UV

(EtOH) 261 nm; IR (KBr): 3330 (NH), 3090, 3020 (CH arom), 2830 (CH, CH_2), 1565, 1515, 1480 (aromatic rings) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm: 166 (s, 1H, NH); 2.80 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 3.49 (d, 1H, ^3J = 6.7 Hz, Ar-CH); 3.50 (d, 1H, ^3J = 9.5 Hz, Ar-CH); 3.63 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 4.98 (m, 1H, Ar-CH); 7.08–7.20 (m, 9H arom); MS *m/z* $M^{+\cdot}$: 446 (5.3%, $C_{22}H_{21}N_4{}^{35}Cl_3{}^{+\cdot}$); $M^{+\cdot}$ + 2: 448 (6%, $C_{22}H_{21}N_4{}^{35}Cl_2{}^{37}Cl^{+\cdot}$); $M^{+\cdot}$ + 4: 450 (0.9%, $C_{22}H_{21}N_4{}^{35}Cl^{37}Cl_2{}^{+\cdot}$); 355 (100%, $C_{15}H_{14}N_4{}^{35}Cl_3{}^+$); 357 (96.1%, $C_{15}H_{14}N_4{}^{35}Cl_2{}^{37}Cl^+$); 359 (30.3%, $C_{15}H_{14}N_4{}^{35}Cl^{37}Cl_2{}^+$); 313 (8.5%, $C_{13}H_{10}N_3{}^{35}Cl_3{}^{+\cdot}$); 91 (15.1%, $C_7H_7{}^+$); 84 (29.4%, $C_4H_8N_2{}^{+\cdot}$); 69 (47%, $C_4H_7N^{+\cdot}$); 56 (49%, $C_2H_4N_2{}^{+\cdot}$).

2-(1-Piperazinyl)-5-[1-(4-chlorophenyl)-2-(3-chlorophenyl)ethyl]4,6-dichloropyrimidine ***5b***. The reaction yielded product **5b** (2.85 g; 84% yield); mp: 110–114°C. Silica-gel preparative TLC (CH_3CN /28% NH_3: 90/10; R_f: 0.59); GC/MS: R_t = 32.9 min; UV (EtOH) 274 nm; IR (KBr): 3430 (NH), 3020 (CH arom), 2920, 2830 (CH, CH_2), 1565, 1515, 1480 (aromatic rings) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm: 1.55 (s, 1H, NH); 2.78 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 3.47 (d, 2H, ^3J = 7.8 Hz, Ar-CH_2), 3.61 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 4.95 (t, 1H, ^3J = 7.8 Hz, Ar-CH), 7.00–7.20 (m, 8H arom); MS *m/z* $M^{+\cdot}$: 480 (6.9%, $C_{22}H_{20}N_4{}^{35}Cl_4{}^{+\cdot}$); $M^{+\cdot}$ + 2: 482 (8.8%, $C_{22}H_{20}N_4{}^{35}Cl_3{}^{37}Cl^{+\cdot}$); $M^{+\cdot}$ + 4: 484 (4.2%, $C_{22}H_{20}N_4{}^{35}Cl_2{}^{37}Cl_2{}^{+\cdot}$); $M^{+\cdot}$ + 6: 486 (0.96%, $C_{22}H_{20}N_4{}^{35}Cl^{37}Cl_3{}^{+\cdot}$); 355 (100%, $C_{15}H_{14}N_4{}^{35}Cl_3{}^+$); 357 (97.8%, $C_{15}H_{14}N_4{}^{35}Cl_2{}^{37}Cl^+$); 359 (32.2%, $C_{15}H_{14}N_4{}^{35}Cl^{37}Cl_2{}^+$); 361 (4%, $C_{15}H_{14}N_4{}^{37}Cl_3{}^+$); 125 (13.6%, $C_7H_6{}^{35}Cl^+$).

2-[1-(4-Methylpiperazinyl)]-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine ***6a***
5-[1-(4-Chlorophenyl)-2-phenylethyl]-2,4,6-trichloropyrimidine **3a** (200 mg, 0.5 mmol) was stirred at reflux for 15 h with *N*-methylpiperazine from Aldrich (111 µl, 100 mg, 1 mmol) and absolute ethanol (6 ml). After cooling, the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (10 ml). The organic layer was treated with 2 N NaOH (2 x 10 ml), washed with brine (4 x 5 ml) and dried with Na_2SO_4. The solvent was evaporated to give product **6a**. The residue was purified by flash chromatography, silica-gel 230-400 Mesh from Merck; the eluent was CH_2Cl_2/MeOH: 9/1; R_f = 0.78. Purification gave compound **6a** (142 mg; 63% yield); GC/MS; R_t = 30.17 min; ^{1}H-NMR ($CDCl_3$) δ ppm: 2.22 (s, 3H, N-CH_3); 2.32 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 3.47 (d, 1H, ^3J = 6.5 Hz, Ar-CH); 3.48 (d, 1H, ^3J = 9.5 Hz, Ar-CH); 3.66 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 4.95 (dd, 1H, ^3J = 6.5 Hz, ^3J = 9.5 Hz, Ar-CH), 7.05–6.15 (m, 9H arom); MS *m/z* $M^{+\cdot}$: 460 (8.3%, $C_{23}H_{23}N_4{}^{35}Cl_3{}^{+\cdot}$); $M^{+\cdot}$ + 2: 462 (7.8%, $C_{23}H_{23}N_4{}^{35}Cl_2{}^{37}Cl^{+\cdot}$); $M^{+\cdot}$ + 4: 464 (2.7%, $C_{23}H_{23}N_4{}^{35}Cl^{37}Cl_2{}^{+\cdot}$); $M^{+\cdot}$ + 6: 466 (0.2%, $C_{23}H_{23}N_4{}^{37}Cl_3{}^{+\cdot}$); 369 (57.6%, $C_{16}H_{16}N_4{}^{35}Cl_3{}^+$); 371 (56.2%, $C_{16}H_{16}N_4{}^{35}Cl_2{}^{37}Cl^+$); 373 (18.1%, $C_{16}H_{16}N_4{}^{35}Cl^{37}Cl_2{}^+$); 375 (2%, $C_{16}H_{16}N_4{}^{37}Cl_3{}^+$); 312 (7.7%, $C_{13}H_9N_3{}^{35}Cl_3{}^+$); 314 (7.5%, $C_{13}H_9N_3{}^{35}Cl_2{}^{37}Cl^+$); 316 (2.8%, $C_{13}H_9N_3{}^{35}Cl^{37}Cl_2{}^+$); 318 (1%, $C_{13}H_9N_3{}^{37}Cl_3{}^+$); 98 (32.2%, $C_5H_{10}N_2{}^{+\cdot}$); 91 (13.9%, $C_7H_7{}^+$); 83 (22.7%, $C_4H_7N_2{}^+$); 70 (100%, $C_4H_8N^+$); 58 (47.7%, $C_3H_8N^{+\cdot}$); 56 (12.2%, $C_2H_4N_2{}^{+\cdot}$); 43 (50.7%, $C_2H_5N^{+\cdot}$); 42 (46.6%, $C_2H_4N^+$).

2-[1(4-Methanesulfonylpiperazinyl)]-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine ***7a***
2-(1-Piperazinyl)-5-[1-(4-chlorophenyl)-2-phenylethyl]-4,6-dichloropyrimidine **5a** (100 mg, 223 µmol) was stirred at room temperature for 30 min with CH_2Cl_2 (4 ml) and CH_3SO_2Cl (400 µl, 590 mg, 5.1 mmol). The mixture was then stirred at room temperature for 30 min with 10% aqueous Na_2CO_3 (4 ml). The two phases were separated and the aqueous layer extracted with CH_2Cl_2 (2 x 5 ml). The combined organic phase was washed with brine (3 x 5 ml) and dried with Na_2SO_4. The solvent was removed under vacuum and the residue was washed with ether and filtered to give compound **7a** (99.7 mg, 85% yield) which was purified by silica-gel TLC (the eluent was $CHCl_3$: R_f = 0.55); IR ($CHCl_3$): 3090, 3020 (CH arom), 2950 (CH, CH_2, CH_3), 1565, 1515, 1490 (aromatic rings), 1350, 1150 (SO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ ppm: 2.70 (s, 3H, SO_2CH_3); 3.18 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 3.50 (d, 1H, ^3J = 7.0 Hz, Ar-CH); 3.51 (d, 1H, ^3J = 9.5 Hz, Ar-CH); 3.80 (t, 4H, ^3J = 5.0 Hz, CH_2NCH_2); 5.00 (dd, 1H, ^3J = 7.0 Hz, ^3J = 9.5 Hz, Ar-CH); 7.10–7.20 (m, 9H arom).

Pharmacological evaluation

Test organisms were provided by the Laboratory of Microbiology, Besançon Hospital (CHR) (France) and the Laboratory of Microbiology, Faculty of Pharmacy (Professor Senet), University of Angers (France).

Fungi were grown on Sabouraud 1.5% dextrose Agar (bioMerieux, France) plates. The compound suspensions were added at increasing concentrations first to the sterilized culture broth, kept at 40°C, and then distributed into Petri plates before solidification. Fungi (yeasts and *Aspergillus*) were inoculated by spore dissemination and *Trichophyton* mycelium disposition in center plates. These methods were described previously [16]. Cultures were incubated for 24–36 h at 37°C (yeasts and *A fumigatus*) and for 10 d at 25°C (dermatophytes and *Scopulariopsis*). The lowest concentration of compounds that completely inhibited fungal growth was considered to be the minimum inhibitory concentration (MIC), expressed in µg/ml. The experiments were performed and compared to control tests.

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