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N-haloacetyl phenothiazines and derivatives: Preparation, characterization and structure-activity relationship for antifungal activity

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N-acetyl phenothiazines;
Microwave assisted synthesis;
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Conformational isomers;
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Abstract A serie of *N*-acetyl phenothiazines and related compounds was synthesized by means of the acetylation reaction of the corresponding phenothiazine with the appropriate reagent using microwave irradiation. Structural elucidation of these heterocyclic derivatives was done using ^{1}H, ^{13}C NMR spectra. The equilibra between conformationals enantiomers were studied as a possible atropisomerism case. The single crystal X-ray diffraction of some derivatives was also recorded and the presence of enantiomers could be confirmed. The antifungal activity evaluation of all *N*-acetyl phenothiazines prepared by us in this and in a previous work, was performed *in vitro* against 163 isolated human pathogenic yeasts and filamentous fungi, including: *Cryptococcus neoformans, Candida albicans, Candida non-albicans, Aspergillus* and *Acremonium-Fusarium*. The most promising compounds were those bearing one chlorine or bromine atom attached to the *N*-acetyl group. These

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compounds were as active as 5-fluorocitosine (5-FC) or fluconazole (FCZ), currently in clinical use. A structure-activity relationship (SAR) and a quantitative structure-activity relationship (QSAR) for antifungal activity of each genus were established.

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1. Introduction

In recent years, the incidence and severity of fungal diseases has increased, particularly in population with a broad list of inmunocompromised medical conditions, such as cancer, AIDS, solid-organ and hematopoietic stem cell transplantation and in aging population (Castón-Osorio et al., 2008; Shao et al., 2007). Due to the small number of available antifungal agents and infections caused, specially by unusual fungal species, and having into account the different mechanism of action of them, searching for new and more effective and less toxic drugs is mandatory (Pfaller, 2012; Kathiravan et al., 2012). Moreover, the growing of antifungal resistance to the commercial drugs causes serious problems to the public health (Shao et al., 2007; Tobudic et al., 2012; Nucci and Marr, 2005).

Phenothiazine, an aromatic tricyclic compound, is the parent molecule of a multitude of drugs that have enjoyed varied and extensive use throughout medical and veterinary practice. These compounds are non-antibiotics with insecticidal, antifungal, antibacterial and anthelmintic properties (Mitchell, 2006; Aaron et al., 2009). Following its extensive usage over many years, unwanted reactions including neuromuscular incoordination, photosensitization and hemolytic anemia have been reported and these have limited its use (Pluta et al., 2011).

In the last decade, the antifungal properties of some antipsychotic phenothiazine derivatives, such as trifluoroperazine, chlorpromazine and fluphenazine, have been described (Fig. 1) (Afeltra and Verweij, 2003). The active phenothiazine derivatives had more potent inhibitory activity against fungi, including phytopathogen filamentous, human pathogen filamentous fungi and yeasts, than against gram-positive and negative bacteria.

Some years ago our research program has been directed to synthesis of phenothiazines and their *N*-acyl derivatives (Sarmiento et al., 2009; Moltrasio et al., 2006) using conventional and non-conventional methodologies, as microwave irradiation using a household MW oven, and to investigate their biological properties. The antifungal activity of several synthesized phenothiazines, related compound as well as yet known pharmacological available ones such as pipotiazine (PIP), a neuroleptic phenothiazine, and promethazine (PMZ), an antihistaminic phenothiazine (Fig. 1), were reported by us showing interesting results as antimicotic agents (Sarmiento et al., 2011). In that opportunity we found that *N*-chloroacetyl phenothiazine (**1**), the simplest of the compounds tested, showed to be as active as antifungal agents currently used in clinical (Sarmiento et al., 2011). Due to the important biological properties of this heterocyclic class, the aim of this work was to improve the synthesis of *N*-acyl substituted phenothiazines and related compounds, as well as to synthesize some others not yet described in the literature, and evaluate their antifungal activity. Then, with the battery of compounds available, we attempted to establish the potential structure activity relationship (SAR and QSAR).

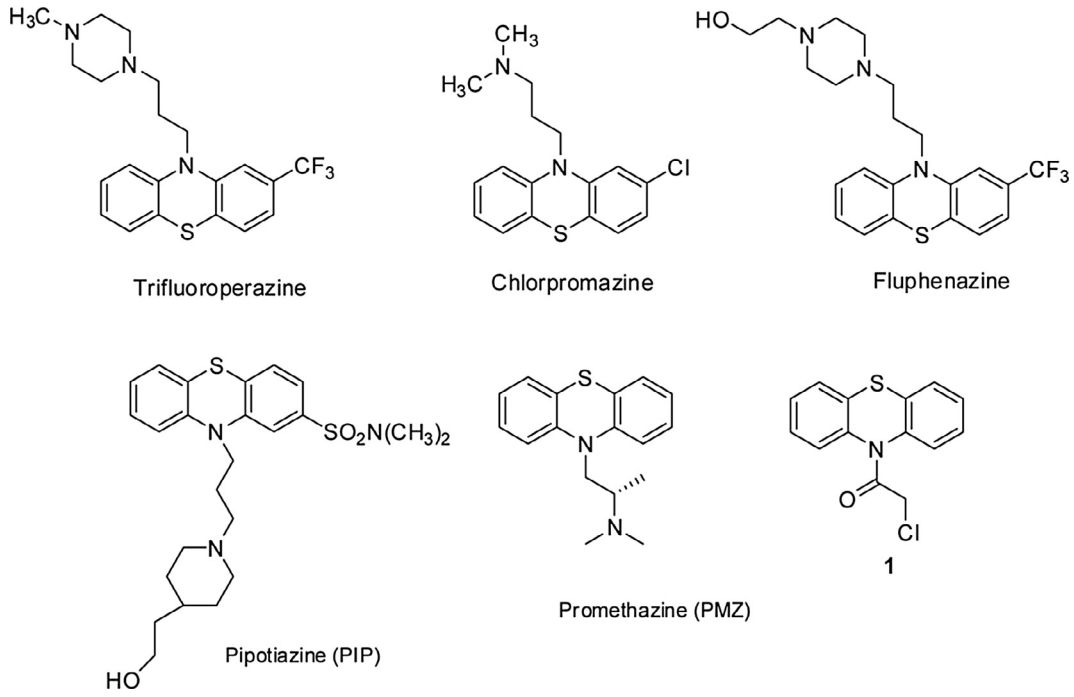

Fig. 1 Some phenothiazine derivatives with antifungal activity.

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2. Experimental section

2.1. Chemistry

All the reagents and solvents were analytical or chemical pure class. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC 300 or on Bruker Avance 500 or on Varian 200 spectrometers. Shifts reported are relative to the signal of the solvent used in each case, and coupling constants (J) are reported in Hertz (Hz). Splitting patters are designated as follows: s: singlet, bs: broad singlet, d: doublet, bd: broad doublet; t: triplet, dd: double doublet, td: triple doublet, q: quartet, m: multiplet. IR spectra were recorded using a Perkin Elmer Spectrum One FT-IR spectrophotometer and are expressed in cm^{-1}. High resolution mass spectra were obtained on Bruker micrOTOF-Q II spectrometer. Microwave-assisted reactions were carried out into using an Anton Paar Monowave 300 as microwave reactor, heating at 100 °C and stirring at 500 rpm. Melting points were obtained by using a Thomas Hoover melting point apparatus. Thin layer chromatography was performed using aluminum plates precoated with Silicagel $60F_{254}$ (E-Merck). Spots were visualized in the ultraviolet light chamber (254 nm). Preparative thin layer chromatography (p-TLC) was done on Silicagel 60 GF_{254}, Merck. Column chromatography was performed by using Silicagel (200–400 mesh, Merck) with the indicated solvent system. THF was distilled from sodium/benzophenone.

2.2. Preparation of N-acylphenothiazines and phenoxazine **2-7**

2.2.1. 10-Chloroacetyl-2-chlorophenothiazine (**2**)

To a solution of 2-chlorophenothiazine (234 mg, 1 mmol) and triethylamine (0.2 mL; 1.5 mmol) in ethyl acetate (2 mL) was added drop by drop chloroacetyl chloride (0.2 mL; 2.4 mmol). The reaction mixture was irradiated with MW during 15 min (100 °C), then it was allowed to reach room temperature. After that, it was washed with NaOH 5% (2 × 3 mL), and finally with brine. The organic phase was dried with anhydrous Na_2SO_4. Finally the solvent was removed *in vacuo*. The residue was purified by p-TLC using *n*-hexane/dichloromethane (1:1) as eluent. Compound **2** was obtained as a green solid (280 mg, 90%); m.p. 105–107 °C (dichloromethane); m.p. lit. (Bansode et al., 2009) 115–118 °C (diethyl ether). IR (Film): 1694 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.15 (d, ^{2}J = 12.5 Hz, 1H, —C*H*HCl), 0.25 (d, ^{2}J = 12.5 Hz, 1H, —CH*H*Cl), 7.28 (dd, ^{3}J = 8.2 Hz, ^{4}J = 2.1 Hz, 1H, Ar), 7.33 (td, ^{3}J = 7.5 Hz, ^{4}J = 1.4 Hz, 1H, Ar), 7.40 (m, 2H, Ar), 7.50 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.3 Hz, 1H, Ar), 7.57 (d, ^{3}J = 8 Hz, 1H, Ar), 7.67 (d, ^{4}J = 2 Hz, 1H, Ar) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 41.6 (CH_2), 126.3, 127.1, 127.6, 127.7, 127.8, 128.4, 128.6, 133.1, 137.5, 138.9, 165.4 (C═O) ppm.

2.2.2. 10-Chloroacetyl-2-methylthiophenothiazine (**3**)

To a solution of 2-methylthiophenothiazine (245 mg, 1 mmol) and triethylamine (0.2 mL; 1.5 mmol) in ethyl acetate (2 mL) was added drop by drop chloroacetyl chloride (0.2 mL; 2.4 mmol). The reaction mixture was irradiated with MW during 8 min (100 °C), then it was allowed to reach room temperature. After that, it was washed with NaOH 5% (2 × 3 mL), and finally with brine. The organic phase was dried with anhydrous Na_2SO_4. Finally the solvent was removed *in vacuo*. The residue was purified by p-TLC using *n*-hexane/dichloromethane (1:1) as eluent. Compound **3** was obtained as a white solid (270 mg, 85%); m.p. 117–120 °C. IR (Film): 1693 (CO). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.55 (s, 3H, CH_3), 4.19 (d, ^{2}J = 12.5 Hz, 1H, —C*H*HCl), 4.22 (d, ^{2}J = 12.5 Hz, 1H, —CH*H*Cl), 7.17 (dd, ^{3}J = 8.5 Hz, ^{4}J = 1.8 Hz, 1H, Ar), 7.29 (td, ^{3}J = 7.5 Hz, ^{4}J = 1.3 Hz, 1H, Ar), 7.38 (m, 2H, Ar), 7.48 (dd, ^{3}J = 7.8 Hz, ^{4}J = 1.3 Hz, 1H, Ar), 7.51 (bs, 1H, Ar), 7.59 (d, ^{3}J = 8.1 Hz, 1H, Ar) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.0 (CH_3), 41.8 (CH_2), 124.2, 125.6, 126.6, 127.4, 127.5, 128.1, 128.2, 137.7, 138.5, 138.7, 165.4 (C═O) ppm. HRMS (ESI): calc. for $C_{15}H_{12}ClNNaOS_2$, 343.9941, found 343.9944. Anal. Calcd for $C_{15}H_{12}ClNOS_2$: C, 55.98; H, 3.76; N, 4.35, S, 19.93. Found: C, 55.95; H, 3.80; N, 4.25, S, 19.80.

2.2.3. 10-Bromoacetylphenothiazine (**4**) (Diehl and Himbert, 1986)

To a solution of phenothiazine (520 mg, 2.6 mmol) and triethylamine (0.6 mL; 4 mmol) in ethyl acetate (3 mL) was added drop by drop bromoacetyl bromide (0.5 mL; 6.2 mmol). The reaction mixture was irradiated with MW during 6 min (100 °C), then it was allowed to reach room temperature. After that, it was washed with NaOH 5% (2 × 3 mL), and finally with brine. The organic phase was dried with anhydrous Na_2SO_4. Finally the solvent was removed *in vacuo*. The reaction mixture obtained was purified p-TLC using *n*-hexane/dichloromethane (1:3) as eluent. Compound **4** was obtained as a grey solid (815 mg, 98%); m.p. 113–115 °C. IR (Film): 1677 (CO). ^{1}H NMR (300 MHz, $CDCl_3$): δ4.00 (s, 2H, CH_2), 7.27 (td, ^{3}J = 7.6 Hz, ^{4}J = 1.4 Hz, 2H, Ar), 7.36 (td, ^{3}J = 7.4 Hz, ^{4}J = 1.5 Hz, 2H, Ar), 7.47 (dd, ^{3}J = 7.6 Hz, ^{4}J = 1.5 Hz, 2H, Ar), 7.62 (dd, ^{3}J = 7.6 Hz, ^{4}J = 1.2 Hz, 2H, Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 29.7 (CH_2), 126.5, 127.3, 127.4, 128.1, 138.0, 165.7 (C═O) ppm.

2.2.4. 10-dichloroacetylphenothiazine (**5**) (Kanzawa et al., 1972)

To a solution of phenothiazine (200 mg, 1 mmol) and triethylamine (0.2 mL; 1.5 mmol) in ethyl acetate (2 mL) was added drop by drop dichloroacetyl chloride (0.22 mL; 2.3 mmol). The reaction mixture was irradiated with MW during 20 min (100 °C), then it was allowed to reach room temperature. After that, it was washed with NaOH 5% (2 × 3 mL), and finally with brine. The organic phase was dried with anhydrous Na_2SO_4. Finally the solvent was removed *in vacuo*. The residue was purified by p-TLC using *n*-hexane/dichloromethane (1:2) as eluent. Compound **5** was obtained as a green solid (190 mg, 60%); m.p. 152–154 °C. IR (Film): 1702 (CO).^{1}H NMR (300 MHz, $CDCl_3$): δ6.37 (s, 1H, CH), 7.35 (m, 4H, Ar), 7.50 (d, ^{3}J = 7.7 Hz, 2H, Ar), 7.61 (bd, ^{3}J = 7.7, 2H, Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 63.7 (CH), 126.5, 127.4, 127.8, 128.3, 137.2, 163.1 (C═O) ppm.

2.2.5. 10-Trifluoroacetylphenothiazine (**6**) (Mais et al., 1999; De Meester and Chu, 1986)

To a solution of phenothiazine (1.00 g, 5 mmol) in ethyl acetate (2 mL) was added drop by drop trifluoroacetic acid (0.8 mL; 13 mmol). The reaction mixture was irradiated with MW during 5 min. and then it was washed with NaOH 5% (2 × 3 mL). The organic phase was dried with anhydrous

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Na_2SO_4. Finally the solvent was removed *in vacuo*. The residue was purified by crystallization in ethanol to give **6** as a white solid (1.00 g, 70%); m.p. 104–105 °C. IR (Film): 1698 (CO).^{1}H NMR (300 MHz, $CDCl_3$): δ7.30 (td, ^3J = 7.6 Hz, ^4J = 1.7 Hz, 2H, Ar), 7.38 (td, ^3J = 7.8 Hz, ^4J = 1.7 Hz, 2H, Ar), 7.47 (dd, ^3J = 7.5 Hz, ^4J = 1.8 Hz, 2H, Ar), 7.60 (da, ^3J = 7.7 Hz, 2H, Ar) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 116.2 (q, ^1J = 288.2 Hz, CF_3), 126.5, 127.4, 127.9, 128.2, 133.4, 136.8, 156.0 (q, ^2J = 37.9 Hz, C═O) ppm.

2.2.6. 10-Acetylphenoxazine (7) (Ragg at al., 1983)

To a solution of phenoxazine (480 mg, 2.62 mmol) in ethyl acetate:pyridine (1:1, 3 mL) was added drop by drop acetyl chloride (0.5 mL; 6.2 mmol). The reaction mixture was irradiated with MW during 15 min, then it was washed with NaOH 5% (2 × 3 mL) and the organic phase was dried with anhydrous Na_2SO_4. Finally the solvent was removed *in vacuo*. The reaction mixture obtained was purified p-TLC using *n*-hexane/dichloromethane (1:4) as eluent. Compound **7** was obtained as a green solid (526 mg, 90%); m.p. 138–140 °C. IR (KBr): 1668 (CO). ^{1}H NMR (200 MHz, $CDCl_3$): δ2.35 (s, 3H, CH_3), 7.05–7.25 (m, 6H, Ar), 7.50 (m, 2H, Ar) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.3 (CH_3), 117.1, 123.6, 125.4, 127.1, 129.7, 151.3, 169.3 (C═O) ppm.

2.3. Crystallographic data of compounds *1*, *3*, *9* and *18*

Data for suitable single crystals were collected at room temperature, using a Gemini A diffractometer, Oxford Diffraction, Eos CCD detector with graphite-mono chromate Mo Kα (λ = 0.71073 Å) radiation. Data-collection strategy (ω-scan) and data reduction (integrated and scaled intensities) followed standard procedures implemented in the CrystAlisProsuite of programs (CrysAlisPro, 2012). Data were corrected empirically for absorption employing the multi-scan method implemented in CrysAlisPro. The structure was solved using program SHELXS-97 (Sheldrick, 1990) and refined using the full-matrix LS procedure with SHELXL-2014/7 (Sheldrick, 2008). We provide supplementary crystallographic information containing crystal data and structure refinement results for the compounds (Table S1). CIF files with further details of the crystal structures reported in the paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC), under deposition numbers CCDC 1550502, 882149, 882148, 1550503, for compound **1**, **3**, **9** and **18** respectively. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.

2.3.1. X-ray single crystal structure for compound *1*

$C_{14}H_{10}ClNOS$, M_r = 275.74 g mol^{-1}, crystal size = 0.60 × 0.26 × 0.14 mm^3, triclinic, space group P-1, a = 8.6648(5) Å, b = 10.5621(6) Å, c = 14.3541(9) Å, α = 76.201(5)°, β = 85.160(5)°, γ = 84.746(5)°, V = 1267.74(14) Å^3, Z = 4. 5687 independent reflections were collected (from 10,246 total reflections), 3792 with I > 2σ(I), R_{int}: 0.0193. Anisotropic displacement parameters were employed for non-hydrogen atoms. All H atoms were located at the stereo-chemically expected positions and they were refined using a riding model. LS weights of the form $w = 1/[\sigma^2(F_o^2) + (0.0489P)^2]$ where P = (Fo^2 + 2Fc^2)/3, were employed. $R[F^2 > 2\sigma(F^2)]$ = 0.0366, $wR(F^2)$ = 0.0882.

A bond lengths and angles table is provided as supplementary crystallographic information for the compound (Table S2).

2.3.2. X-ray single crystal structure for compound *3*

$C_{15}H_{12}ClNOS_2$, M_r = 321.83 g mol^{-1}, crystal size = 0.40 × 0.30 × 0.15 mm^3, triclinic, space group P-1, a = 9.8991(3) Å, b = 12.0627(5) Å, c = 13.0691(4) Å, α = 81.548(3)°, β = 72.306 (3)°, γ = 82.987(3)°, V = 1465.65(8) Å^3, Z = 4. 6798 independent reflections were collected (from 14,763 total reflections), 4069 with I > 2σ(I), R_{int}: 0.0254. Anisotropic displacement parameters were employed for non-hydrogen atoms. All H atoms were located at the expected positions and they were refined using a riding model. LS weights of the form w = 1/[σ2(Fo2) + (0.0557P)^2] where P = (Fo^2 + 2Fc^2)/3, were employed. $R[F^2 > 2\sigma(F^2)]$ = 0.0424, $wR(F^2)$ = 0.1001.

A bond lengths and angles table is provided as supplementary crystallographic information for the compound (Table S3).

2.3.3. X-ray single crystal structure for compound *9*

$C_{16}H_{15}ClN_2O_3S_2$, M_r = 382.87 g mol^{-1}, crystal size = 0.25 × 0.22 × 0.16 mm^3, monoclinic, space group P21/c, a = 8.9238(15) Å, b = 14.987(3) Å, c = 12.730(3) Å, β = 89.883 (10)°, V = 1702.6(5) Å^3, Z = 4. 3338 independent reflections were collected (from 5448 total reflections), 2102 with I > 2σ (I), R_{int}: 0.0454. Anisotropic displacement parameters were employed for non-hydrogen atoms. All H atoms were located at the expected positions and they were refined using a riding model. LS weights of the form w = 1/[σ2(Fo2) + (0.1126P)^2] where P = (Fo^2 + 2Fc^2)/3, were employed. $R[F^2 > 2\sigma(F^2)]$ = 0.067, $wR(F^2)$ = 0.1728. The crystal structure was refined as two component merohedric twin. The twin law was defined by the matrix 1 0 0, 0 −1 0, 0 0 −1. The fractional contribution for each twin domain was obtained from refinement (0.47/0.53). A bond lengths and angles table is provided as supplementary crystallographic information for the compound (Table S4).

2.3.4. X-ray single crystal structure for compound *18*

$C_{14}H_{14}N_2O_2S_2$, M_r = 306.39 g mol^{-1}, crystal size = 0.35 × 0.25 × 0.19 mm^3, monoclinic, space group P21/n, a = 10.0358 (5) Å, b = 11.3657(6) Å, c = 13.0516(9) Å, β = 101.758(6)°, V = 1457.48(15) Å^3, Z = 4. 3248 independent reflections were collected (from 5132 total reflections), 2068 with I > 2σ(I), R_{int}: 0.0342. Anisotropic displacement parameters were employed for non-hydrogen atoms. All H atoms were located at the expected positions and they were refined using a riding model. LS weights of the form w = 1/[σ2(Fo2) + (0.0580P)^2] where P = (Fo^2 + 2Fc^2)/3, were employed. $R[F^2 > 2\sigma(F^2)]$ = 0.0424, $wR(F^2)$ = 0.1024.

A bond lengths and angles table is provided as supplementary crystallographic information for the compound (Table S5).

2.4. Evaluation of the antifungal activity

The compounds **1**–**17** and conventional antifungal agents against yeasts and filamentous fungi: amphotericina B (AMB), 5-fluorocitosine (5-FC), fluconazole (FCZ), itracona-

zole (ITZ), voriconazole (VCZ) and terbinafine (TBF), were evaluated following the CLSI guidelines (formerly NCCLS), document M27A3 and M38A2 (Clinical and Laboratory Standards Institute reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2008, CLSI Document M38-A2; Wayne, PA and Clinical and Laboratory Standards Institute, reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard, 2008, CLSI Document M27-A3; Wayne, PA). The assayed yeast were: a) *Candida albicans* (n = 40) and *Candida non-albicans* (n = 26) that included: *C. parapsilosis, C. glabrata, C. krusei, C. tropicalis* and b) *Cryptococcus neoformans* (n = 60). The filamentous fungi tested were: a) *Aspergillus* (n = 25) that included: *Aspergillus fumigatus, A. flavus, A. niger, A. nidulans, A. terreus* and b) *Fusarium* and *Acremonium* species (n = 12). Briefly, fungi were grown onto Saboureaud glucose agar during 2 days for yeasts and up to 7 days for the filamentous fungi. The media used was RPMI-1640 buffered with MOPS at pH 7. For each drug, stock solutions were prepared in the appropriate solvent and the final concentrations were ranged from 256 to 0.25 μg/mL for AMB, 5-FC, FCZ, ITZ, VCZ, TBF and compound **1**, and from 512 to 0.50 μg/mL for the remaining compounds. After, the stock solutions were diluted in the media to be inoculated into 96-well-flat-bottom micro titer plates. Final inoculums for yeasts were 0.5–5 × 10^3 CFU/mL, and for filamentous fungi 0.5–5 × 10^4 CFU/mL. After incubation at 30 °C, spectrophotometer and visual reading were determined in order to obtain the minimal inhibitory concentration (MIC) for each strain and drug tested. The mathematical treatment of the values found for the Gmean and MIC_{90}, was carried out using Microsoft Office Excel 2007 program.

2.5. QSAR study

The ground-state geometry of compounds **1**–**17** was optimized within the Density Functional Theory (DFT) using the B3LYP functional and 6-31G* basis set. The quantum calculations included full optimization of the geometric structures and evaluations of the atomic charges according to the Merz-Kollman scheme.

We use Gaussian, ChemAxon and HyperChem software in order to obtain the different descriptors used to calculate the potential QSAR models.

We analyzed uniparameter models in all the cases and biparameter models when the number of compounds was statistically enough to validate this model ($n \geq 5$ for each descriptor used). In all biparameter models, we checked the orthogonality of the descriptors.

3. Results and discussion

3.1. Chemistry

A serie of *N*-acetyl phenothiazines (**2**–**6**) and one *N*-acetylphenoxazine (**7**) were synthesized, in excellent yields and very short reaction time, using microwave irradiation (MW) (Table 1). The use of MW allowed reduce the reflux time and use of friendly solvents, compared with that previously reported in the literature (Bansode et al., 2009). Compounds **2** and **3** were obtained by treatment of 2-chlorophenothiazine (**12**) and2-methylthiophenothiazine (**13**), both commercially available, with chloroacetyl chloride. Compounds **4**, **5** and **6** were synthesized from phenothiazine (**14**), also commercially available, and bromoacetyl bromide, dichloroacetyl chloride and trifluoroacetic acid, respectively. Compound **7** was obtained by treatment of phenoxazine with acetyl chloride.

Compounds **1**, **8**–**11** (Table 1) and compounds N-chloroacetylcarbazol (**15**), 2-chloro-N,N-diphenylacetamide (**16**) and 2-chloro-N-[2-(phenylthio)phenyl]acetamide (**17**) were obtained as it was previously described (Sarmiento et al., 2011).

3.1.1. ^1H NMR analysis

^{1}H NMR spectra of *N*-chloroacetyl substituted phenothiazines, at room temperature, such as compounds **2**, **3** and, **9** showed asymmetry in the molecules, which manifested itself through the display of methylene hydrogen atoms as an AB system with a geminal coupling constant between 10 and 14 Hz; in contrast to that observed in the ^{1}H NMR spectrum of compounds **1**, **4**–**8** and **10** where these hydrogen atoms appeared as a singlet. Furthermore, other authors described the multiplicity of these hydrogen atoms, for example for compound **2**, as a singlet (Bansode et al., 2009). The correlation spectrum (HMQC) of compounds **2** and **9** helped us to affirm that both hydrogen atoms were joined to the same carbon atom, which confirmed their diastereotopicity. The non-equivalence of a pair of hydrogen atoms joined to a single carbon atom, in a molecule of such characteristics, indicates the presence of asymmetry in it (Silverstein et al., 2005). The acquisition of ^{1}H NMR spectra in deuteron chloroform of compound **2**, when it was heating from 290 K to 322 K, showed that both signals coalesce near 313.0 K (show in Supplementary Material: Fig. S1). A similar experiment conducted on compound **9** enabled the observation of the coalescence of both doublets into one singlet at a temperature near 323.0 K. When the temperature was lowered until 270 K in both compounds, **2** and **9**, could be observed that AB system corresponding to the methylene group was conserved. Similar results were observed for compound **3**.

Considering the dynamic process involved in the conversion of **2**, **3** and **9** and based on the conducted ^{1}H NMR experiments, the energetic barriers ($\Delta G_c^{\neq}$) related to the interconversion of compound **2** and **9** were calculated using Eq. (1):

$$\Delta G_c^{\neq} = 4.5710^{-3} T_c(\log T_c - \log k_{coal} + 10.32)\frac{kcal}{mol} \quad (1)$$

where:

T_c: coalescence temperature,

k_{coal}: coalescence constant.

Coalescence constant was calculated using the Eq. (2) that takes into account the coupling constant of the exchangeable hydrogen atoms (Jackman and Cotton, 1975).

$$k_{coal} = \frac{1}{2}\pi\sqrt{2}\sqrt{\left[(\nu_1 - \nu_2)^2 + 6J^2\right]} \quad (2)$$

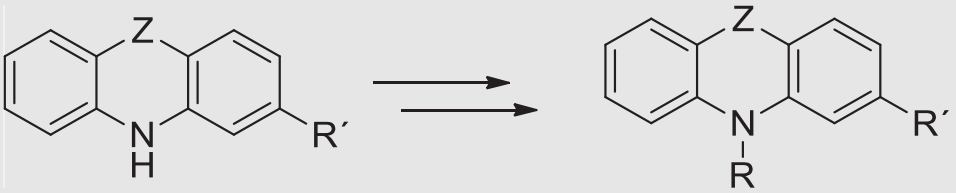
where:

J: coupling constant of the exchangeable hydrogen atoms,

ν: frequency,

ν and *J:* both values are measured at a lower temperature than the T_c, where it is possible to determine the maximum spacing between ν_1 and ν_2.

Table 1 Synthesis of phenothiazines **2–6**, **9–11** and phenoxazines **7**, **8**.



Compound	Z	R	R'	Reagent	%
1	S	$COCH_2Cl$	H	Chloroacetyl chloride	Sarmiento et al. (2011)
2	S	$COCH_2Cl$	Cl	Chloroacetyl chloride	90.0
3	S	$COCH_2Cl$	SCH_3	Chloroacetyl chloride	85.0
4	S	$COCH_2Br$	H	Bromoacetyl bromide	98.0
5	S	$COCHCl_2$	H	Dichloroacetyl chloride	60.0
6	S	$COCF_3$	H	Trifluoroacetic acid	70.0
7	O	$COCH_3$	H	Acetyl chloride	90.0
8	O	$COCH_2Cl$	H	Chloroacetyl chloride	Sarmiento et al. (2011)
9	S	$COCH_2Cl$	$SO_2N(CH_3)_2$	Chloroacetyl chloride	Sarmiento et al. (2011)
10	S	$COCH_3$	H	Acetyl chloride	Sarmiento et al. (2011)
11	S	CH_2CH_2Cl	H	**1** + B_2H_6	Sarmiento et al. (2011)

For compound **2** the Δν was 37 Hz at 280.0 K and *J* was 13.06 Hz so the k_{coal} calculated was 108.66 s^{-1}.

For compound **9** the Δν was 86 Hz at 280.0 K and *J* was 13.33 Hz so the k_{coal} calculated was 204.35 s^{-1}.

Using the values of k_{coal} found, $\Delta G_c^{\neq}$ calculated was:

For compound **2**, where T_c is 313 K, $\Delta G_c^{\neq}$ = 15.42 kcal/mol,

For compound **9**, where T_c is 323 K, $\Delta G_c^{\neq}$ = 15.53 kcal/mol.

3.1.2. ^{13}C NMR analysis

^{13}C NMR spectra of *N*-chloroacetyl substituted phenothiazines, recorded at room temperature, show coincidence of the observed ^{13}C NMR shift values (Table 2) for *N*-acetylphenothiazine (**10**) and *N*-acetylphenoxazine (**7**) (Sarmiento et al., 2011) with those reported in the literature for these compounds (Ragg et al., 1983). The chemical shifts are in agreement with the values presented in this work for related structures: **1**, **4–6**, **8** and confirm a certain degree of nitrogen-pyramidalization (*sp3*) of the atom of nitrogen of the *N*-acylderivatives of the phenothiazines, even if they are nitrogen atoms which simultaneously aniline-amide characteristic. The displacement values at the carbon atoms, especially those at the *ortho* and *para* positions at the *N*-10 atom, indicate a clear deshielding effect, from the expected values for carbon atoms in the presence of the electron donor effect (by resonance) of a nitrogen atom. This fact can be attributed to the folding of the molecules which leads to the pyramidalization of the nitrogen atom and determines shift values in the ^{13}C NMR spectrum close to those of an unsubstituted benzene ring.

3.2. X-ray crystallographic analysis

In an initial approach, a non-common NMR spectrum may be associated with the atropisomerism phenomenon. Then, the crystallographic analysis allows the proper characterization of the phenomena. Taking this into account, the single crystal X-ray diffraction (SCXRD) studies were performed from those compounds whose crystals were suitable. One molecule of compound **1**, in the asymmetric unit by SCXRD, shows a "butterfly like" folding of the tricycle system and the *extra*position of the substituent on the *N*-10, larger than a hydrogen atom (Supplementary Material Fig. S2), as was described by Malrieu and Pullman (1964).

Fig. 2 shows the molecule obtained by SCXRD in the asymmetric unit of compound **9** (R = $SO_2N(CH_3)_2$) and its precursor **18**, previously described (Sarmiento el al., 2011). It is possible to observe that in compound **9**, the substituent group in the *N*-8 occupies the *extra* position, whereas in its precursor **18**, the hydrogen atom on the *N*-7 is in the *endo* position.

In the same figure, the packing diagram in the unit cell for compounds **9** and **18** are shown (bottom panel). In the unit cell of compound **9**, four molecules can be observed, two of which are the enantiomeric forms of the other two. This confirms the inherent chirality of the tricycle system asymmetrically substituted.

As calculated, taking into account NMR dynamic experiments, the energy barrier of interconversion between these conformational enantiomers would be 15.53 kcal/mol. The calculated energetic barrier would correspond to the ring movement ("ring flipping") and the consequent conversion of one enantiomer into another (Fig. 3) and would be the cause of observed spectra.

According to the classification proposed by LaPlante, (LaPlante et al., 2011) the energetic barrier calculated for compounds **2** and **9** would position them as Class 1 of the conformational isomers, which leads us to assert that their development as an only active species is a correct approach.

It could be also obtained the crystallographic data for compound **3** (R = SCH_3), in whose crystals two type of conform-

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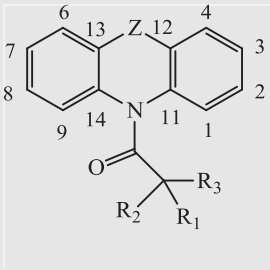
Table 2 ^{13}C NMR shift values (ppm) for *N*-acetylphenothiazine and *N*-acetylphenoxazine derivatives.



1 Z=S; R_1=R_2=H; R_3=Cl
4 Z=S; R_1=R_2=H; R_3=Br
5 Z=S; R_1=R_2=Cl; R_3=H
6 Z=S; R_1=R_2=R_3=F
7 Z=O; R_1=R_2=R_3=H
8 Z=O; R_1=R_2=H; R_3=Cl
10 Z=S; R_1=R_2=R_3=H

Compound number	C1 (C9)	C2 (C8)	C3 (C7)	C4 (C6)	C11 (C14)	C12 (C13)	CO	CH_3 (CH_2) (CH)
1	126.5	127.4	127.3	128.1	137.9	133.2	165.5	41.8
4	126.5	127.3	127.3	127.4	138.0	128.1	165.7	29.7
5	126.5	127.4	127.4	127.8	137.2	128.3	163.1	63.7
6	126.5	127.4	127.9	128.2	136.8	133.4	156.0	116.2
7	125.4	123.6	127.1	117.1	129.7	151.3	169.3	23.3
8	124.3	123.7	127.6	117.1	128.5	150.9	165.3	41.5
10	126.7	126.9	127.1	127.9	138.9	132.9	169.3	23.0

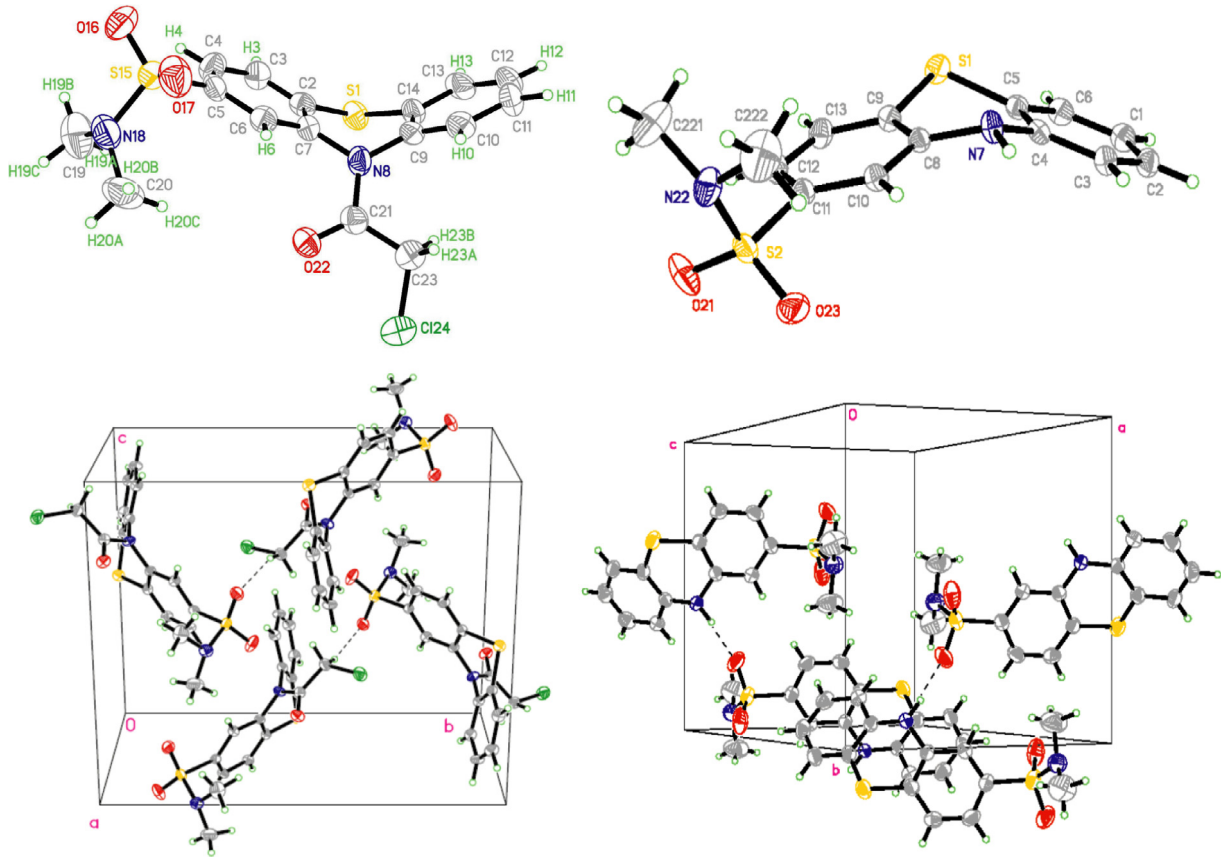


Fig. 2 Molecule of compounds **9** (left top panel) and **18** (right top panel) showing the numbering scheme used and displacement ellipsoids drawn at the 50% probability level. Unit cell diagram in the crystal packing of compounds **9** (left bottom panel) and **18** (right bottom panel).

ers (forms A and B) were observed. They arise from the different spatial orientation adopted by the substituent chain on the nitrogen atom. A disorder manifests itself to level of the methylthio group (such conformers are shown in Supplementary Material Fig. S3). The crystal of this compound also shows the existence of both enantiomers for each of the two

conformers, containing the unit cell four molecules, two corresponding to the enantiomers of form A and two to the enantiomers of form B.

3.3. Antifungal evaluation

In a previous study, the antifungal activity of PIP and PMZ, as well as some synthetic precursors of them has been described (Sarmiento et al., 2011). Given the fact that the length of the carbon chain joined to the *N*-10 atom is very important in the physiological activity of phenothiazine compounds (antipsychotic *vs*. antihistaminic activities) and having into account that *N*-chloroacetyl phenothiazine (**1**) was found to be the most active one against different strains of yeast and filamentous fungi, related compounds **2**–**11** and **15**–**17**, have been evaluated as antifungal agents against: *Cryptococcus neoformans, Candida albicans, Candida non-albicans* (*C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*), *Aspergillus* (*A. fumigatus, A. flavus, A. terreus, A. niger, A. nidulans*) *and Acremonium-Fusarium*. In this study three phenothiazine precursors (**12**–**14**), as well as six antifungal drugs clinically used have been included: amphotericina B (AMB), 5-fluorocitosine (5-FC), fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ) and terbinafine (TBF). The results obtained (Gmean and MIC_{90}) against different strains corresponding to 163 (n = 163) clinical isolates are showed in Table 3.

Among phenothiazine derivatives, compounds **1** and **4** were active against all the strains tested. Compound **4** was much more active against yeasts than filamentous fungi, and compound **1** was more active against filamentous fungi than yeasts. Compounds **15**–**17**, which not conserved the phenothiazine motif in their structure, but they are derivatives of *N*-α-haloacylaniline, showed interesting activity against all the strains assayed. For the other compounds different activities were observed. Compounds **2** and **3** were much more active against yeast than filamentous fungi. However, compound **3** was inactive against *Candida non-albicans*. Compound **8**, a phenoxazine compound, showed good activity against yeast and filamentous fungi with the exception of *Aspergillus* species. Compounds **5**–**7** and **9**, **11** as well as **15** have poor activity or are inactive against almost all strains, with the exception of *Cryptococcus neoformans*. Compounds **10**, **16** and **17** are practically inactive with all the strains assayed.

3.4. QSAR studies

For the QSAR study, 20 molecular descriptors were considered. They include **electronic descriptors**: HOMO, LUMO, Dipole Moment, Hydration Energy, Polarizability; **steric descriptors**: Surface Area, Volume, Molar Mass, Minimum projection area (mPA), Maximum projection Area (MPA), Minimum projection radius (mPR), Maximum projection radius (MPR), Length perpendicular to minimum area, Length perpendicular to maximum area, Topological polar surface area, van der Waals Surface Area, Solvent accessible surface area; **lipophilic descriptors**: logP, as well as some **hybrid descriptors**: Molar Refractivity, logS. As biological descriptor, the pGmean expressed in millimolar^{-1} was used. In Table 4, the pGmean values for each strain and the values of the descriptors which resulted in a suitable correlation with the biological descriptor are included.

In order to construct equations which relate the antifungal activity with the descriptors, the lineal correlation coefficients for each one were estimated. When the R^2 was higher than 0.60, it was checked the possibility of biparameter equation, according with the number of active compounds for each strain and the orthogonality of the descriptors. A quadratic correlation was also attempted when it enhanced the lineal correlation previously obtained. Having into account the selective activity of some compounds on particular strain, the calculation were carried out independently for each strain assayed (see Table 4).

Analyzing the possible mathematical functions that explain the activity on *Aspergillus* strains, for which 8 of the 17 compounds tested were moderately active, no acceptable linear relationship could be found with any descriptor tested. The best of them, the MPR, resulted in an R^2 of 0.45 with a standard deviation (sd) of 0.48, which although unacceptable, allowed to estimate from the shape of the corresponding graph (Fig. 4A), a quadratic relation, expressed by the Eq. (3).

$$pGmean = 241.05 - 77.76MPR + 6.28MPR^2 \quad (3)$$

$$n = 8 \quad R^2 = 0.82 \quad sd = 0.20$$

When a similar analysis was performed on *Acremonium-Fusarium* strains where only 7 of the 17 compounds studied showed activity, an acceptable linear relationship was found, also with the MPR descriptor, resulting in an R^2 of 0.82 with

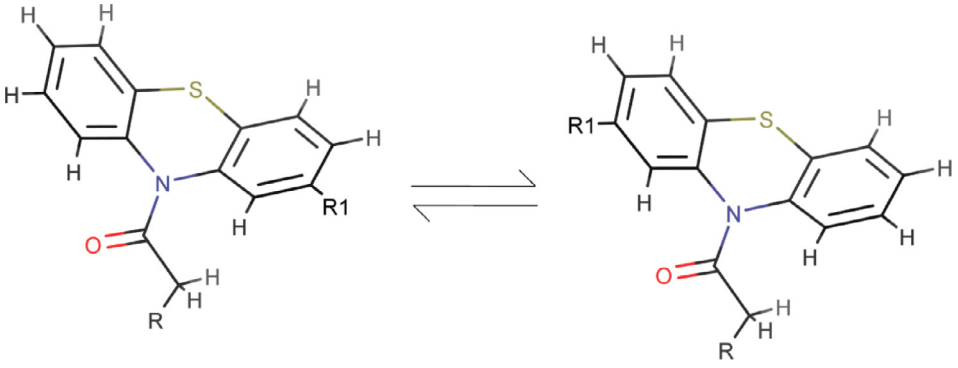


Fig. 3 Equilibrium between conformational enantiomers.

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Table 3 Antifungal activity of the compounds assayed. The concentrations are expressed in μg/mL.

Assayed compounds	*Aspergillus*[a]	*Acremonium-Fusarium*	*Cryptococcus neoformans*	*Candida albicans*	*Candida non albicans*[b]
	(n = 25)[c] Gmean[d]/ MIC_{90}^{e}	(n = 12)[c] Gmean[d]/ MIC_{90}^{e}	(n = 60)[c] Gmean[d]/ MIC_{90}^{e}	(n = 40)[c] Gmean[d]/ MIC_{90}^{e}	(n = 26)[c] Gmean[d]/ MIC_{90}^{e}
AMB	1.15/4.00	1.00/2.40	0.18/0.5	0.20/1.00	0.25/1.00
5-FC	66.50/256.00	211.90/256.00	7.60/32.00	0.10/0.925	0.11/1.50
FCZ	160.21/256.00	256.00/256.00	8.40/32.00	0.13/2.80	0.37/13.60
ITZ	0.06/0.25	11.27/32.00	0.02/0.06	0.01/0.25	0.04/0.25
VCZ	0.04/0.29	3.03/8.00	0.03/0.25	0.01/0.50	0.02/0.50
TBF	0.09/0.50	10.08/32.00	1.10/12.00	1.98/4.40	0.34/4.00
1	**5.15**/64.00	**2.27**/32.00	**9.05**/32.00	**11.62**/256.00	**15.72**/256.00
2	135.01/ > 512	i	**1.80**/4.00	**3.86**/32.00	**25.40**/32.00
3	77.71/ > 512	120.18/ > 512	**2.67**/32.0	**2.50**/16.00	i
4	**3.78**/12.80	**3.31**/4.00	**0.83**/4.00	**1.22**/4.00	**2.38**/6.00
5	i	i	**21.02**/ > 512	84.03/ > 512	181.02/ > 512
6	i	i	**11.41**/ > 512	i	i
7	i	i	**13.22** /512	i	i
8	i	**3.76**/ > 512	**7.53**/ > 512	**3.39**/ > 512	**19.17**/ > 512
9	i	i	**20.46**/ > 512	i	92.18/614.40
10	i	i	108.74/ > 512	i	i
11	i	i	**20.49**/ > 512	i	i
12	39.95/ > 512	i	92.63/ > 512	i	i
13	i	i	103.75/ > 512	i	i
14	i	i	**30.68**/ > 512	172.28/ > 512	181.02/ > 512
15	58.59/ > 512	**32.00**/ > 512	**12.39**/ > 512	**8.28**/64.00	**7.13**/128.00
16	39.95/ > 512	**9.07**/64.00	**14.47**/64.00	54.03/ > 512	87.32/ > 512
17	98.71/ > 512	38.66/ > 512	**7.71**/ > 512	150.20/ > 512	113.46/ > 512

(a) Include: *A. fumigatus, A. flavus, A. terreus, A. niger, A. nidulans*. (b) Include: *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*. (c) Where n: represents the number of isolates tested for the indicated strain; (d) Gmean is the resulting concentration of calculating the n^{th} root of the product of the CIM n determined experimentally; (e) MIC_{90} is the concentration that inhibits growth of 90% of the isolates tested for each case. For example for 40 isolates of *Candida albicans*, the compound **1** need a concentration of 256 μg/mL to inhibit the growth of 36 isolates and i: inactive at the concentrations tested.
Note: There were highlighted in bold the studied compounds' Gmean values $\leq$ 32 μg/mL.

Table 4 Biological and molecular descriptors used in the best models found.

	pGmean[c] (mM^{-1})								
Assayed compound	*Aspergillus*[a]	*Acremonium-Fusarium*	*Cryptococcus neoformans*	*Candida albicans*	*Candida non-albicans*[b]	HOMO (eV)	LUMO (eV)	mPR (Å)	MPR (Å)
1	1.7287	2.0845	1.4839	1.3753	1.2441	−8.4553	−0.3974	5.06	5.72
2	0.3613	–	2.2364	1.9051	1.0868	−8.5823	−0.5327	4.99	6.31
3	0.6172	0.4278	2.0811	2.1097	–	−8.5713	−0.5466	5.19	6.5
4	1.9279	1.9856	2.5863	2.4191	2.1288	−8.4477	−0.3881	5.16	5.71
5	–	–	1.1690	0.5672	0.2339	−8.5243	−0.4762	5.09	5.71
6	–	–	1.4129	–	–	−8.5745	−0.4470	4.81	5.68
7	–	–	1.2314	–	–	−8.6471	−0.1348	4.55	5.77
8	–	1.8393	1.5377	1.8843	1.1318	−8.8304	−0.3065	5.11	5.72
9	–	–	1.2722	–	0.6184	−8.7248	−1.3572	5.06	7.13
10	–	–	0.3462	–	–	−8.3028	−0.2304	4.46	5.75
11	–	–	1.1064	–	–	−7.8886	−0.0470	4.72	5.79
12	0.7672	–	0.4019	–	–	−7.9747	−0.3458	4.11	6.47
13	–	–	0.3738	–	–	−7.9025	−0.3936	4.11	6.87
14	–	–	0.8126	0.0632	0.0417	−7.8137	−0.0976	4.18	5.9
15	0.6190	0.8817	1.2938	1.4688	1.5337	−8.9684	−0.5542	5.48	5.97
16	0.7889	1.4328	1.2300	0.6578	0.4493	−9.0728	−0.1004	4.57	5.77
17	0.4269	0.8340	1.5342	0.2446	0.3664	−8.4212	−0.0841	5.07	6.08

(a). Include: *A. fumigatus, A. flavus, A. terreus, A. niger, A. nidulans*. (b) Include: *C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*. (c) Gmean is the resulting concentration of calculating the n^{th} root of the product of the CIM n determined experimentally, express in mM.

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an sd of 0.30. This relationship could be optimized by studying the quadratic relation with the same descriptor, in a similar way of the *Aspergillus* behavior, resulting in the Eq. (4) (Fig. 4A):

$$pGmean = 162.41 - 51.15MPR + 4.04MPR^2 \quad (4)$$

$$n = 7 \quad R^2 = 0.95 \quad sd = 0.07$$

The MPR is the radius for the maximal projection area (MPA) of the studied compound, which is read at a length perpendicular to maximum area (LpMA), from the center of mass of the molecule. mPR is the radius for the minimum projection area (mPA) of the studied compound, which is read at a length perpendicular to minimum area (LpmA), from the center of mass of the molecule (for a better comprehension see Supplementary Material Fig. S4).

These results would indicate the dependence of the activity observed for the genus *Aspergillus*, *Acremonium-Fusarium* of a parameter of steric nature that would find an improvement in its activity, in the absence of substituent on the tricycle system. The mathematical function shed light a similar dependence of the activity for both filamentous fungi (Fig. 4A).

A similar study was carried out for the strains of *Candida albicans* and *non-albicans*, which were sensitive to 10 of the 17 compounds tested, in most cases with very near Gmean values to each other for the same compound assayed. The search for linear relationships was not optimal, with none of the 21 descriptors. In both cases, the mPR and LUMO descriptors showed an incipient linear correlation, although statistically unacceptable. Both parameters were studied, both in a biparametric relation, as in a quadratic relation, obtaining no improvement with respect to the initial situation. Discarding compound **5**, which has low activity for both strains, was achieved in both cases an important improvement, Eqs. (5) and (6):

Candida albicans

$$pGmean = -0.74 - 12.80\ LUMO - 14.92\ LUMO^2 \quad (5)$$

$$n = 9 \quad R^2 = 0.84 \quad sd = 0.38$$

Candida non-albicans

$$pGmean = -0.07 - 4.79\ LUMO - 3.16\ LUMO^2 \quad (6)$$

$$n = 9 \quad R^2 = 0.69 \quad sd = 0.43$$

In the case of Eq. (6), the statistics values are border for the cut-off values, so it was decided to analyzed a new selection, discarding compound **9**, in which the sulphonamide group lead to a very different distribution of electronic density in the molecule. Therefore, an optimization of the model could be reached, Eq. (7) (Fig. 4B):

$$pGmean = -0.53 - 9.78\ LUMO - 11.57\ LUMO^2 \quad (7)$$

$$n = 9 \quad R^2 = 0.76 \quad sd = 0.34$$

In Fig. 4B, it can be seen that the shape corresponding to Eqs. (5) and (7) has the same pattern for all studied yeasts. In this case, it could be concluded that for the genus *Candida*, the dependence of the activity would be linked to an electronic type factor, such as LUMO, finding the optimal value close to −0.4 eV.

When *Cryptococcus neoformans* strains were studied, having into account that they were sensitive to all compounds tested (Table 4) no linear, multiparametric, or quadratic acceptable correlation was found with any of the descriptors, or combinations thereof. So, it was decided to choose a set of compounds that did not include that ones whose pGmean was less than 0.5 (values with a Gmean > 90 μg/ml) –therefore compounds **10**, **12** and **13** were discarded - leaving a population of 14 compounds. This selection also did not show acceptable linear, multiparametric or quadratic correlation. Taking into account the values of pGmean and comparing these with the values obtained for the strains previously studied, it was decided to separate two sets of compounds. One of them consists of 6 inactive compounds or with a pGmean less than 0.5 (**5**, **6**, **7**, **9**, **11** and **14**), for all other strains studied. These ones are only active on *Cryptococcus neoformans* strains. The best mathematical relation obtained for this group was the quadratic expression dependent on mPR, Eq. (8):

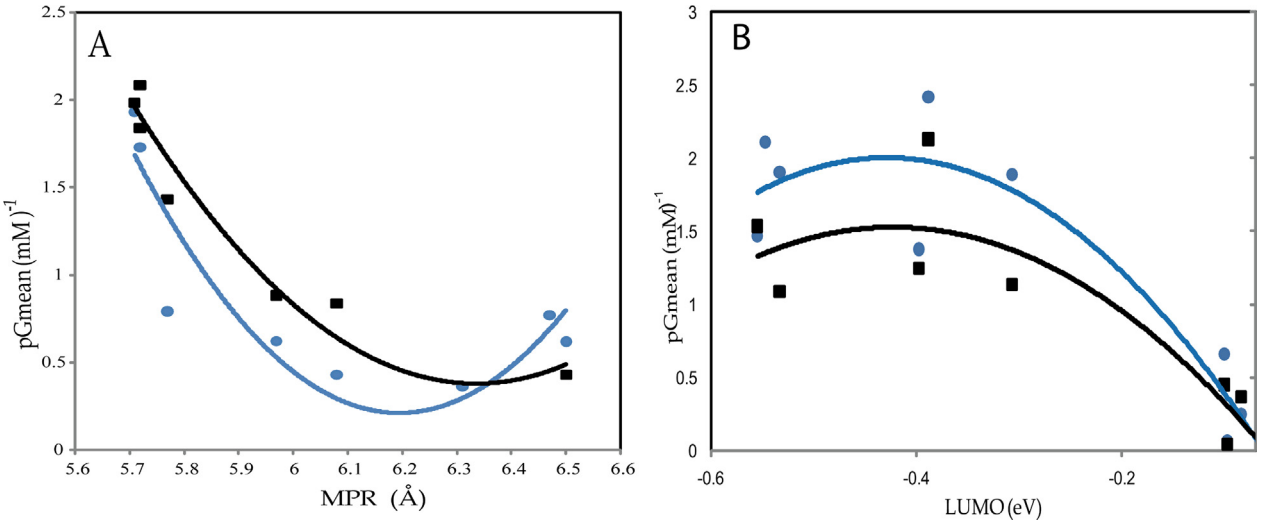

Fig. 4 Estimated QSAR models of: A. Eqs. (3) (blue line) and (4) (black line) and B. Eqs. (5) (blue line) and (7) (black line).

$$pGmean = -22.72 + 9.90mPR - 1.02mPR^2 \quad (8)$$

$$n = 6 \quad R^2 = 0.76 \quad sd = 0.03$$

The other group of compounds, **1**–**4**, **8** and **15**–**17**, comprises 8 derivatives which are also active with most of the other fungal genera studied, independently of the morphology of the fungi strain assayed. In studying the mathematical function between pGmean values and the 21 molecular descriptors, no acceptable linear or quadratic correlation could be established in any case.

4. Conclusion

In this work, it was developed a simple and successful method of synthesis of *N*-acylphenotiazines and derivative compounds using MW. The synthesized compounds were tested as antifungal agents and the obtained results were compared with the activity showed for six antifungal drugs clinically used: AMB, 5-FC, FCZ, ITZ, VCZ and TBF. Compounds **4** and **8**, resulting of the isosteric replacement in compound **1**, select as a lead compound, of the atom of chlorine by bromine in compound **4** and sulphur by oxygen in compound **8** represent an optimized structure for **4**, and equivalent one for **8**. A possible phenomena of atropisomerism, in mono-substituted phenothiazines in one of their benzene ring, and observed through the diastereotopic signals of the methylene protons, was studied using dynamic NMR. Through these experiments it was possible to calculate an energetic barrier, near to16kcal/mol at room temperature, for the conversion of the conformers. Thus, it was possible to conclude that such compounds do not exhibit atropisomerism, since atropisomers present rotational barriers of above 20 kcal/mol at room temperature.

Surprisingly, compounds **1**, **2** and **15** previously synthesized by other authors, as precursors of more complex phenothiazines or diphenylamine *N*-acyl substituted, had not been tested as antifungal agents (Bansode et al., 2009; Kumar and Mishra, 2015).

As a first sight, a qualitative relationship between molecular structure and biological activities (SAR) of the compounds synthesized and evaluated, would conclude that:

- The presence of the residue chloroacetyl on the nitrogen atom increases the activity. Precursors were less active or inactive with respect to the corresponding *N*-chloroacetyl derivatives for most of the strains tested.
- The replacement of the chlorine atom (compound **1**) by the bromine one (compound **4**) led to the most active compound in this series, being more active that some antifungal agents currently in clinical use, such as 5-FC or FCZ.
- Introducing another chlorine atom in the chloroacetyl group (**5**) or replacement of the chloromethyl group by trifluoromethyl (**6**) leads to less active compounds than those monohalogenated derivatives.
- The presence of phenothiazine system seems not to be essential for the antifungal activity.

In order to shed light in the structural requirements for the antifungal activity, a QSAR study was carried out. The antimicotic activity of the studied compounds over the strains with filamentous morphology: *Acremonium-Fusarium, Aspergillus* shows a quadratic dependence with MPR. It is higher as MPR decrease, in the studied compounds. This descriptor is involved with both MA and mA, so the activity is enhanced in the absence of substituent in the aromatic rings, as well as the presence of *N*-monohaloacetyl chain on the aniline nitrogen atom.

In the case of genus *Candida*, the activity is enhanced in the case of electron acceptor molecules, with an optimal value of LUMO near to −0.4 eV, as well as the presence of *N*-monohaloacetyl chain on the aniline nitrogen atom. This last requirement is the same as in the cases of *Acremonium-Fusarium, Aspergillus*.

Finally, in the case of *Cryptococcus neoformans*, where all the compounds tested resulted active, this activity seems to be explain by different ways. For that compounds that are active only on *Cryptococcus neoformans*, the dependence with mPR with an optimal value of 4.85 Å, would indicate that the presence of a substituent on the aniline nitrogen atom, enhanced the activity. However, for the more active derivatives it could not be possible found an adequate mathematical correlation. As *Cryptococcus neoformans* is an unconventional dimorphic fungus that can grow either as yeast or in a filamentous form (Zhai et al., 2013), and taking into account that we worked with clinical isolations, the dependence of their activity could be related with more than one molecular descriptor, related with one of filamentous or yeast fungi.

All these results together, might lead to propose that the *N*-haloacylaniline could be part of the pharmacophoric group, which should be included in further structural optimization, for the development of prototype molecules as potential antifungal agents.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.arabjc.2017.11.019.

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