2*H*-Benzo[1,4]thiazin-3-one Derivatives Endowed with Antifungal Activity

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Resumo

Os testes de susceptibilidade in vitro com terbinafina e fluconazol, utilizando a metodologia CLSI, foram descritos para avaliar os derivados de 2H-benzo [1,4] tiazin-3-ona. Todos os compostos sintetizados foram investigados *in vitro* contra Candida spp. Os pontos finais da concentração inibitória mínima (CIM) foram lidos visualmente após 48 horas de incubação e as susceptibilidades foram medidas.
Palavras-chave: 1,4-2H-benzotiazinonas, atividade antifúngica, cândida.

Abstract
Derivados de 2H-benzo [1,4] tiazin-3-ona dotados de actividad antifúngica

The in vitro susceptibility tests with terbinafine and fluconazole, using the CLSI methodology were described to evaluate the 2H-benzo[1,4]thiazin-3-one derivatives. All compounds synthesized were investigated in vitro against Candida spp. Minimum inhibitory concentration (MIC) endpoints were read visually after 48 hours of incubation and the susceptibilities were measured.
Key words: 1,4-2H-benzothiazinones, antifungal activity, candida.

Resumen
Derivados 2H-Benzo [1,4] tiazin-3-ona dotados de atividade antifúngica

Las pruebas de susceptibilidad in vitro con terbinafina y fluconazol, utilizando la metodología CLSI, se describieron para evaluar los derivados de 2H-benzo [1,4] tiazin-3-ona. Todos los compuestos sintetizados fueron investigados in vitro contra Candida spp. Los puntos finales de la concentración inhibitoria mínima (CIM) se leyeron visualmente después de 48 horas de incubación y se midieron las susceptibilidades.
Palabras clave: 1,4-2H-benzotiazinonas, actividad antifúngica, candida.

INTRODUCTION

Notwithstanding the good antifungal activities of the agents used to treat Candida infections, especially the azole and non-azole antifungal, candidemia is increase of the prevalence and still cause of death in population immunocompromised (PERES-BOTA, D., et al, 2004; IMWIDTHAYA, P.; POUNGVARIN, N., 2000; IMWIDTHAYA, P.; POUNGVARIN, N., 2000. Therefore, new effective anti-Candida agents are needed to

combat the drug-resistant strains of Candida spp. Phenothiazines and 1,4- benzothiazines are structural analogs and have been widely studied since a long time due chemical and pharmacological properties, (GUPTA, R.; OJHA, K., 1988). The Benzothiazines possess a wide spectrum of biological and therapeutics activities generally justified by spatial parameters of the privileged core moiety (SAVCHUK, N. P.; TKACHENKO, S. E.; BALAKIN, K. V., 2005). Antifungal properties were reported as part of the spectrum of activities (FRINGUELLI, R. et al, 1998; FRINGUELLI, R., SCHIAFELLA, F., VECCHIARELLI, A., 2001). The alkylation of 4-N position of 2*H*-benzo[1,4]thiazin-3-ones affords bactericidal and antifungal derivatives (ZIMMERMANN, M., 1958; LOWRIE, H. 1961). Guarda et al., (2000) synthesized three series of 6-alkylacylamino-2*H*-benzo[1,4]thiazin-3-ones substituted on position N-4. The 6-alkylacylamino-4-methyl-2*H*-benzo[l,4]thiazin-3-ones and 6- alkylacylamino-4-buthyl-2*H*-benzo[1 ,4]thiazin-3-ones has been previously reported (GUARDA, V. et al, 2001; GUARDA, V., 1988).

EXPERIMENTAL

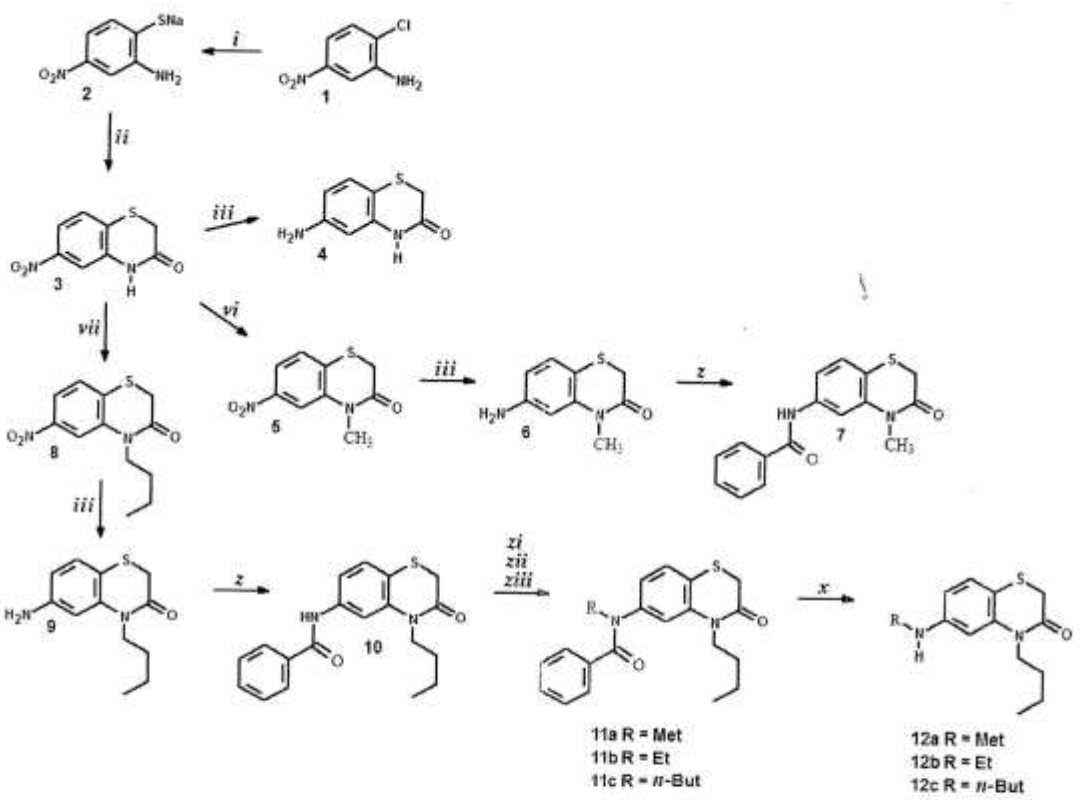
Chemistry

Melting points were determined with a capillary Büchi apparatus and are uncorrected. The purity of the compounds is controlled by thin layer chromatography on silica pre-coated plates Merck $60F_{254}$. The spots are revealed under UV light or iodine vapor and the Rf values are measured.

IR spectra were recorded in KBr tablets (2%) with a Perkin-Elmer 1310 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Brucker AC 200 spectrometer in DMSO-d6. Chemicals shifts (δ) are expressed in ppm and coupling (J) in Hertz (Hz).

Several steps are necessary to synthesize the 4-methyl-2*H*-benzo[1,4]-thiazin-3-ones (SAVCHUK, N. P.; TKACHENKO, S. E.; BALAKIN, K. V., 2005; FRINGUELLI, R. et al, 1998; FRINGUELLI, R., SCHIAFELLA, F., VECCHIARELLI, A., 2001)) and 4-butyl-2*H*-benzo[1,4]-thiazin-3-ones (ZIMMERMANN, M., 1958; LOWRIE, H. 1961, GUARDA et al., 2001; GUARDA et al., 2000 and GUARDA, 1998. Synthetic pathway is portrayed in Fig. 1. All compounds has been previously reported, (GUARDA et al., 2000 and GUARDA, 1998), an exception to the 6-benzoilethylamino and 6-ethylamino derivatives, **11b** and **12b**.

Figure 1 - Synthesis of the 2*H*-benzo- [1,4]- thiazin-3-ones derivatives



Reagents and conditions: (i) Ethanol, $Na_2S.9H_20$, S, reflux; (ii) $ClCH_2COOH$, H_20, NaOH, reflux; (iii) $SnCl_2.2H_20$, HCl, reflux; (vi) DMSO, Methanol, KOH, CH_3I, 50°C; (vii) DMSO, Methanol, KOH, $CH_3CH_2CH_2CH_2Br$, 50°C; (z) NaOH 5%, Benzoyl chloride; (zi, zii, ziii) Toluene, NaOH, $CaCO_3$, tetrabutylammonium, [CH_3I, CH_3CH_2I, $CH_3CH_2CH_2CH_2Br$] reflux; (x) H_2SO_4 70%, 150°C.

6-Benzoylethylamino-4-butyl-2H-benzo[1,4]thiazin-3-one - **11b**

To a suspension of 1.18 g (5 mmol) of compound 10 in 20 mL of 5% NaOH, 2 mL of benzoyl chloride were added dropwise. The mixture is vigorously stirred for 10 min.The precipitate is separated, washed with water and purified by recrystallised from 95% ethanol.

$C_{21}H_{24}N_2O_2S$. Yield 49%. TLC Hexane: ethyl acetate (6 : 4) Rf 0,74. IR (KBr): —v 2930, 2860, 1670, 1640 cm^{-1}. ^1H NMR ($CHCI_3$-d) δ: 0.93 (t. 3H. CH_3-6. J=6.1 Hz). 1.20 (t. 3H. CH_3-4, J=7.1 Hz) 1.31- 1.42 (m. 2H. CH_2). 1.55- 1.70 (m. 2H. CH_2). 2.85 (s. 2H. CH_2-2). 3.32-3.49 (m. 2H. CH_2-6). 3.96 (t. 2H. CH_2-4). 6.37 (dd. 1H. aromatic H. J=8.2 and 1.7 Hz). 6.45 (d. 1H. aromatic H. J=1.7 Hz). 7.14-7.35 (m. 6H. aromatic H). ^{13}C NMR ($CHCl_3$;-d, DEPT)δ:13.72 (CH_3), 13.82(CH_3), 19.99(CH_2), 29.59 (CH_2), 31,28 (CH_2), 38.30 (CH_2), 44.47 (CH_2), 119.15 (C), 122.26 (CH), 125.00 (2CH), 126.40 (2CH), 128.11 (CH) 128.61 (CH), 129.19 (C), 137.02 (C), 140.50 (C), 147.98 (C), 165.70 (CO), 176.18 (CO).

6-Ethylamino-4-butyl-2H-benzo[1,4]thiazin-3-one - **12b**

A mixture of 5 mmol of acylated compounds dissolved in toluene (100mL), 1,4 g of potassium carbonate, 7,0 g of sodium hydroxide and 0,16 g of tetrabutylammonium bromide is refluxed under vigorous stirring. The ethyl iodide (7,5 mmol) in toluene (10 mL) is added dropwise. Stirring is continued for 4 hours at the refluxing temperature. After cooling, the mixture is filtered and the filtrate added with water (50 ml; the organic phase is separated, washed with water, dried over anhydrous magnesium sulfate and evaporated.

$C_{14}H_{19}N_2OS$. Yield 73%. TLC Hexane : ethyl acetate (6 : 4) Rf 0,57. IR (KBr): —v. 3360, 2930, 2860, 1670, 1640 cm^{-1}. ^1H NMR ($CHCl_3$-d)δ: 0.89 (t. 3H. CH_3-6. *J*=6.95 Hz). 0.98 (t. 3H. CH_3-4, *J*=7.26 Hz); 1.32 -1.57 (m. 2H. CH_2-4); 1.65 - 1.83 (m. 2H. CH_2-4). 3.31 (s. 2H. CH_2-2); 3.57 - 3.70 (m. 2H. CH_2-6). 3.90 (t. 2H. CH_2-4). 4.33 (t. 1H. NH-6. *J*=6.56 Hz). 6.84 (dd. 1H. aromatic H. *J*=8.59 and 1.01 Hz). 6.58 (d. 1H. aromatic H. *J*=1.02 Hz). 7.14 - 7.34 (m. 6H. aromatic H). ^{13}C NMR ($CHCl_3$-d, DEPT) δ:13.77 (CH_3), 13.85, (CH_3). 19,29 (CH_2), 20.22 (CH_2), 29,92 (CH_2), 30.79(CH_2), 64.84 (CH_2), 128.31 (CH), 129.53 (CH) 132.79 (CH), 136.12 (C), 139.55 (C), 142.72 (C), 166.72 (CO).

Micology Samples

The MICs of compounds synthesized (3, 4, S, 6, 7, 8, 9, 10, 11a, 11b, 11c,12a, 12b and 12c) were determined by the broth microdilution technique following COMMITTEE CLINICAL LABORATORY STANDARDS – CLSI, (2003), formely NCCLS, document M27-A2. The compounds were tested against four different strains of Candida. Four reference isolates were included in this study: *Candida albicans* (ATCC 18804), *Candida krusei* (ATCC 6258), *Candida parapsilosis* (ATCC 90018) and *Candida tropicalis* (ATCC 750).

Inoculum

The inocula were performed according to CLSI guideline M27-A2. The strains of *Candida* cultivated in Potato Dextrose Agar were suspended in saline and turbidity standardized to 0.5 McFarland scale, i.e. 10^6 $CFU.mL^{-1}$. This suspension is diluted using RPMI-1640 medium (SIGMA — ALDRICH LOT.:125K83551) supplemented with 2% glucose (MERK 3053029) and buffered to a pH of 7.0 with 0.165 mol/L MOPS (3-[N-

morpholino] propanesulfonic acid) (VETEC LOT.: 0501076), for constituting the final inoculum (10^3 CFU mL^{-1}).

Compounds and Reference antibiotic

Mother solutions of each compound synthesized, fluconazole and terbinafine were prepared in DMSO (Synth, Brazil) at the concentration of 1024 $mg.mL^{-1}$, 256 $mg.mL^{-1}$ and 32 $mg.\ mL^{-1}$, respectively. Fluconazole (Pfizer, USA) and Terbinafine (Novartis-Pharma, AG, Swiss) were used as reference antifungal agents. A serial dilution were made in RPMI-1640 medium according to a geometric progression of ratio 2, with the aim to obtain ten (10) different concentrations disposed on Flat-bottom microdilution plates.

Interpretive breakpoint

All tests were done in duplicate and the endpoints were read visually after 48 hours of incubation at 28°C. For the compounds synthesized and Terbinafine the MICs considered is the lowest concentration at which fungal growth is completely inhibited, as evidenced by an optically clear well. MIC for fluconazole was read with prominent reduction in growth as 80%.

RESULTS AND DISCUSSION

Studies for antifungal agent determination against Candida spp are very important in last decade. *Candida parapsilosis* emerge like an important pathogen for finger infections, (FIGUEIREDO, V. et al., 2007), and found in blood and catheter in children from a hospital in Brazil (MATSUMOTO, F. et al., 2001). Fluconazole is the main drug against Candida species and terbinafine is used against dermatophytes but it is very effective against C. parapsilosis.

The antifungal activity for the 6-alkylacylamino-4-methyl-2*H*-benzo[1,4]thiazin-3-ones and 6- alkylacylamino-4-buthyl-2*H*-benzo[1,4]thiazin-3-ones; against strains of *Candida* was assessed. The results for all compounds synthesized (**3, 4, 5, 6, 7, 8, 9, 10, 11a, 11b, 11c, 12a, 12b** and **12c**) (**scheme 1**) are collected in the Table 1. Their activity of all compounds is inferior to that of fluconazole and terbinafine, the reference antifungal.

The increase of substituting of N-4 (H, -CH_3, -C_4H_9) in the nitro derivatives (**3**, **5** and **8**), give an increase on the antifungal activity with the augmentation of the group, but the 6-amino derivatives, only the radical butyl give a good one, compounds **4**, **6** and **9**.

The presence of a benzoyl group in the structure, like in compounds **7** and **10**, exhibit the poorest antifungal susceptibility, (MIC > 512 μg/mL). The substitution of the hydrogen of the acylamino group in the position 6 by the radicals' methyl, ethyl or n-butyl obtaining acylalkylamino derivatives **11a**, **11b**, **11c** exhibit an increase of the activity (MIC = 259 for **11b** and **11c** and MIC = 128 for 11a against *Candida tropicalis*). But the desacylation, giving the alkylamino derivatives, **12a**, **12b**, **12c**, didn't promote de exchange on the activity.

Table 1 - MIC values for 2H-Benzo[1,4]thiazin-3-one and derivatives against Candida spp

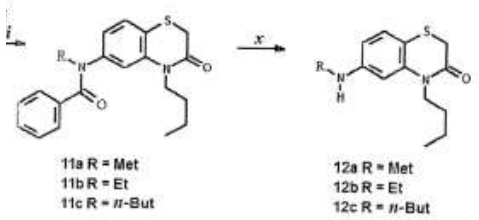


Table 1: MIC values for 2*H*-Benzo[1,4]thiazin-3-one and derivatives against *Candida* spp

Compounds	MIC (μg/ml)			
	Candida albicans	*Candida krusei*	*Candida parapsilosis*	*Candida tropicalis*
3	32	32	32	256
4	256	256	256	256
5	128	128	128	256
6	256	256	256	256
7	>512	>512	>512	>512
8	256	256	256	256
9	128	128	128	256
10	>512	>512	>512	>512
11a	259	259	259	128
11b	256	256	256	256
11c	256	256	256	256
12a	259	259	259	256
12b	256	256	256	256
12c	256	256	256	256
Fluconazole	0,5	0,5	0,5	8
Terbinafine	1,0	1,0	1,0	16

Barufini, A., Pagani, G. Amoretti, L. (1967) demonstrated that the 1,4-benzothiazine nucleus shows some antifungal activity. Fringuelli, F. et al., (1998) were synthesized a series of azole derivatives of 1,4-benzothiazin-3-one and evaluated for the in vitro and in vivo activity against *Candida albicans*. Only a secondary alcohol and its ether derivative showed very good efficacy against systemic candidiasis in a murine experimental model. No activity in vitro was exhibited in any compounds synthesized in comparison with fluconazole, except a mil one for the compound {1[(4-chorobenzyl)oxy]-2-(1*H*-1- imidazolyl)ethyl]-4-methyl-3,4-dihydro-2*H*-benzothiazin-3-one (MIC = 46μg/mL). The compounds essayed presented the semelhant activity.

CONCLUSIONS

Our compounds showed some activity against *Candida spp*, but do not have a breakpoint for these drugs. We can observe only susceptibility profile of them and we can make many tests to determine these breakpoints. It is still not possible declare the resistance or susceptibility for any compounds differing those enumerated in CLSI document. [t is necessary many additional studies to establish the correct criteria to evaluate this susceptibility.

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