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Azole derivatives of 1,4-benzothiazine as antifungal agents

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Abstract

A series of azole derivatives of 1,4-benzothiazine **7–14** was synthesized and evaluated for the in vitro and in vivo activity against *Candida albicans*. Secondary alcohol **10** and its ether derivative **13** showed very good efficacy against systemic candidiasis in a murine experimental model.

Key words: 1,4-Benzothiazine, antifungal activity, azole antifungals.

1. Introduction

Opportunistic fungal infections represent a significant cause of morbidity and mortality in the immunocompromised patients, such as those with AIDS, neoplasms, and transplants. *Candida albicans* infections occur in 41–79% of AIDS patients [1] and an increased rate of disseminated candidiasis has been observed. Furthermore, systemic mycoses are being recognized more frequently as serious infections in a diverse and emergent group of patients. In particular, mycotic infections have flourished in response to major medical and surgical achievements, such as potent antibacterial agents and intravascular catheters, which represent the major predisposing factors for opportunistic infections [2].

For the treatment of these diseases, orally active antifungal azoles have been developed and are currently used in antifungal chemotherapy.

However, the increasing incidence of fungal infection, associated with notoriously unsatisfactory therapeutic treatment in debilitated patients and the emergence of azole resistant strains, have increased the urgency of new alternative drugs [3–6].

Many of the orally active azole antifungal agents share common characteristic features [7–9] as shown in Fig. 1, the tertiary 1-phenylazolylethanol structure that seems to be the pharmacophore for this activity. Furthermore, numerous ethers of 1-(4-chlorophenyl)- or 1-(2,4-dichlorophenyl)-2-(1*H*-imidazolyl)ethanol have been developed as antifungal agents or show promise for clinical use [10–13]. With this in mind, our interest was directed to 1,4-benzothiazine derivatives in order to evaluate the effect of substituting the aromatic ring with 1,4-benzothiazine nucleus that, in itself, shows some antifungal activity [14], in fluconazole- and miconazole-like analogues. Herein, we report the synthesis and the in vitro and in vivo antifungal activity against *C. albicans* of a series of azole derivatives **7–14**.

2. Chemistry

Our synthetic pathway to the target compounds **7–14** is presented in Scheme 1. Starting from 7-acetyl-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (**1**), bromination

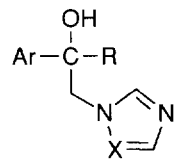

Fig. 1.

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to **2** and following condensation with 1*H*-imidazole or 1*H*-1,2,4-triazole led to key ketones **3** and **4**, respectively, which by reaction with dimethyloxosulfonium methylide afforded oxiranes **5** and **6**. Subsequent treatment of **5** with 1*H*-imidazole or 1*H*-1,2,4-triazole in the presence of NaH gave compounds **7** and **8**, while the treatment of **6** with 1*H*-imidazole under a variety of conditions, failed.

The reduction of the above-obtained ketone intermediates **3** and **4** with $NaBH_4$ gave secondary alcohols **9** and **10**, which were converted into **11–14** by *O*-alkylation with the appropriate arylchloromethyl compound.

3. Results and discussion

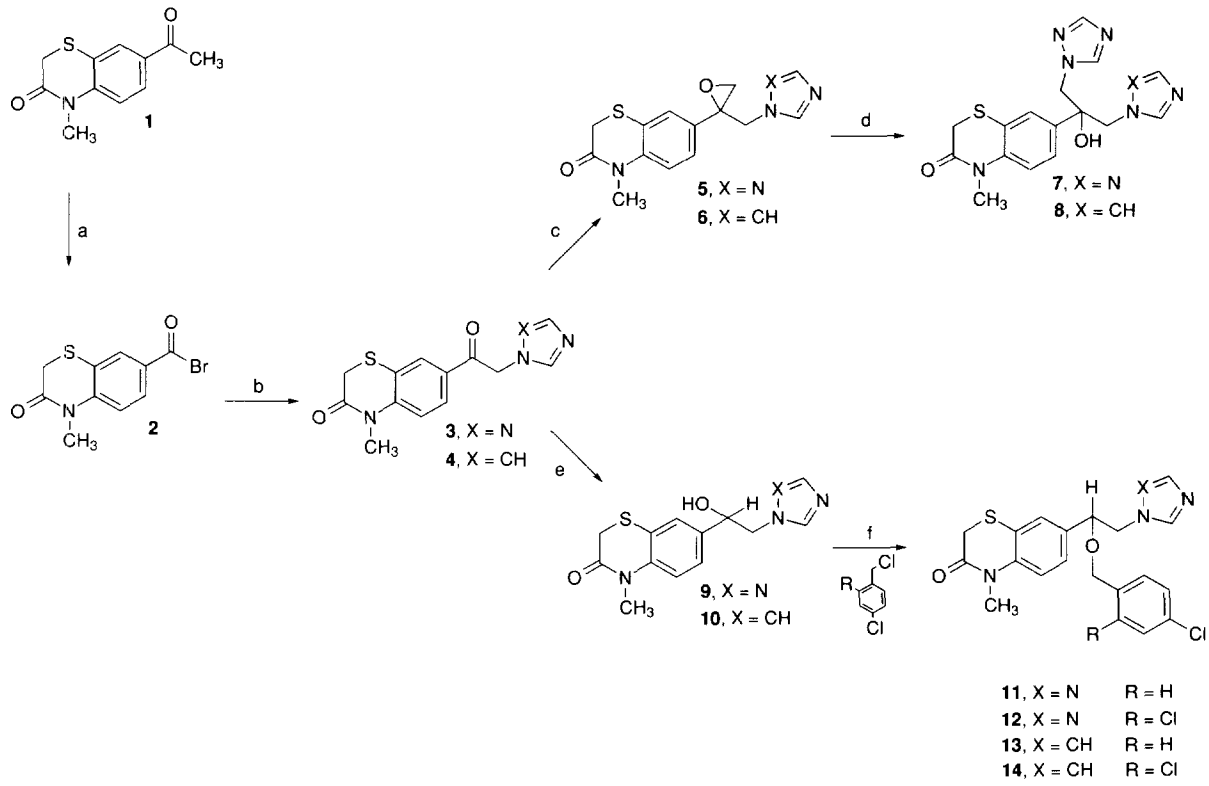
The in vitro antifungal activities of **7–14** derivatives, using the microdilution method and following National Committee for Clinical Laboratory Standards (NCCLS) guidelines [15], are presented in Table 1. The minimum inhibitory concentration (MIC) values (μg/ml) against *C. albicans* in comparison with fluconazole are given. No activity was exhibited in any of the compounds synthesized except a mild one for compound **13**. Nevertheless, knowing that in vitro activity among azoles is sometimes unreliable in predicting in vivo activity [16], compounds **7–14** were also subjected to studies in animal models of fungal infection. The results of the in vivo studies in mice with systemic candidiasis are summarized in Table 1.

Almost all control mice died within 12 days after infection, whereas a considerable number of mice treated intraperitoneally (ip) with these compounds at the dose of 10 mg/kg, survived appreciably longer. In fact, significant increases in median survival times (MST) were observed for compounds **7**, **10**, **11**, and **13**.

The tertiary alcohols **7** and **8**, fluconazole analogues, resulted inactive in spite of an increased MST (23 days) for compound **7**.

The secondary alcohol **10**, an imidazole derivative, was more active than the corresponding triazole analogue **9**, exhibiting a doubled MST with three mice surviving to 60 days.

Among ether derivatives **11–14**, 4-chlorobenzyl derivatives **11** and **13** were more active than the corresponding 2,4-dichlorobenzyl analogues **12** and **14**, with compound **13** showing a good activity that was comparable to fluconazole (MST > 60, D/T = 2/10).



Scheme 1. Reagents: (a) Br_2, AcOH; (b) amino-1,2,4-triazole, *i*-PrOH, reflux or 1*H*-imidazole, $CHCl_3$; (c) $(CH_3)_3S^+OI^-$, NaH, DMSO; (d) 1*H*-1,2,4-triazole, NaH, DMF, 80°C; (e) $NaBH_4$, MeOH; (f) NaH, DMF.

Table 1
In vitro and in vivo[a] antifungal activities of compounds **7–14** against *C. albicans* CA6

Compounds	MIC (μg/ml)	CFU ($\times 10^3$)[c]	MST[d]	D/T[e]
Diluent[b]	>250	22.1 ± 3.4	12	10/10
7	>250	49.6 ± 12.5	23[f]	9/10
8	>250	77.8 ± 15.7	13	10/10
9	>250	3.7 ± 0.8[f]	12	10/10
10	>250	1.1 ± 0.5[f]	26[f]	7/10
11	>250	3.7 ± 2.8[f]	16[f]	8/10
12	>250	13.7 ± 3.3	11	10/10
13	46	7.6 ± 3.5[f]	>60[f]	2/10
14	>250	18.3 ± 4.2	10	9/10
Fluconazole	<1	0	>60[f]	2/10

[a]Groups of 10 mice were inoculated iv with 2×10^5 cells of *C. albicans* CA6. Diluent, azoles, and fluconazole were given ip at the dose of 10 mg/kg, 2 h before and once daily for 7 consecutive days after infection. Data are from a representative experiment out of 3 performed. The MST, D/T, and CFU results in *C. albicans*-treated mice were similar in all determinations performed.
[b]Diluent = DMSO:H_2O, 1:4.
[c]Data represent the mean ± SD of colony forming units (CFU) recovered from the kidneys of 3 mice sacrificed 8 days after infection.
[d]Median survival time (days).
[e]Dead mice at 60 days over total number of animals tested.
[f]$p < 0.01$ (compounds-treated versus diluent-treated).

The increase of MST and reduction of CFU was evident for both **10** and **13**. The lack of correlation between CFU recovery and MST shown for some compounds could be ascribed to growth of *C. albicans* as mycelian form in the kidneys that could generate some inconstancies in CFU recovery.

Preliminary toxicity data showed excellent tolerance upon exposure to the new drugs. Thus, compounds **7–14** and fluconazole were injected ip into mice at the dose of 10 mg/kg, once daily for 7 consecutive days. A control group was treated with diluent only (DMSO:H_2O, 1:4). All mice in each group survived treatment.

4. Conclusion

In conclusion, we synthesized a series of azole derivatives linked to 1,4-benzothiazine moiety and found that secondary alcohol **10** and its ether derivative **13** showed appreciable in vivo antifungal activity. These results are encouraging to better define and optimize the antifungal effect of these compounds. Further investigations are currently in progress to verify the susceptibility of other fungi to these compounds and to outline their pharmacokinetic profile.

5. Experimental

Melting points were determined in capillary tubes (Electrothermal, Model 9100 melting point apparatus) and are uncorrected. Elemental analyses were performed on a Carlo Erba elemental analyzer 1106, and the data for C, H and N are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 200 MHz (Bruker AC-200 spectrometer) with Me_4Si as internal standard. Chemical shifts are given in ppm (δ) and the spectral data are consistent with the assigned structures. Reagents and solvents were purchased from common commercial suppliers and used as received. Column chromatography separations were carried out on Merck silica gel 40 (mesh 70–230) and Merck aluminium oxid 90, neutral, activity III (mesh 70–230). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure. Yields are of purified product and were not optimized. All starting materials were commercially available unless otherwise indicated.

5.1 7-Acetyl-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (1)

To a solution of 7-acetyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [17] (3 g, 14.4 mmol) in dry DMF (20 ml) under a nitrogen atmosphere at room temperature potassium *tert*-butoxide (1.8 g, 15.9 mmol) was added in one portion. The resulting homogeneous mixture was stirred at room temperature for 15 min followed by the dropwise addition of MeI (2.1 g, 14.7 mmol) in dry DMF (5 ml). The solution was stirred at room temperature for 4 h and then poured into ice water and neutralized with 2N HCl. The precipitate was filtered and crystallized from cyclohexane:EtOAc 7:3 to give **1** (2.8 g, 89%), mp 113–115°C. ^{1}H NMR ($CDCl_3$) δ 2.60 (3H, s, $COCH_3$), 3.40 (2H, s, SCH_2), 3.45 (3H, s, NCH_3), 7.15 (1H, d, $J = 8.6$ Hz, H-5), 7.85 (1H, dd, $J = 8.6$ and 2.1 Hz, H-6) and 7.95 (1H, d, $J = 2.1$ Hz, H-8). Anal. ($C_{11}H_{11}NO_2S$) C, H, N.

5.2 7-(2-Bromoacetyl)-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (2)

Bromine (2.5 g, 15.6 mmol) in AcOH (10 ml) was added slowly to a solution of acetyl derivative **1** (3 g, 13.5 mmol) in AcOH (50 ml) and stirred at room temperature for 30 min. The solvent was evaporated to dryness giving a solid that was recrystallized from EtOH to furnish **2** (2.8 g, 68.7%), mp 122–124°C. ^{1}H NMR ($CDCl_3$) δ 3.45 (2H, s, SCH_2), 3.50 (3H, s, NCH_3), 4.40 (2H, s, $COCH_2$), 7.15 (1H, d, $J = 8.6$ Hz, H-5), 7.90 (1H, dd, $J = 8.6$ and 2.1 Hz, H-6) and 8.00 (1H, d, $J = 2.1$ Hz, H-8). Anal. ($C_{11}H_{10}BrNO_2S$) C, H, N.

5.3 4-Methyl-7-[2-(1H-1,2,4-triazol-1-yl)acetyl]-3,4-dihydro-2H-1,4-benzothiazin-3-one (3)

4-Amino-1,2,4-triazole (0.6 g, 6.9 mmol), bromo derivative **2** (2 g, 6.6 mmol) and isopropyl alcohol (30 ml) were stirred and refluxed for 3 h. The product in this case crystallized at reflux temperature. The reaction mixture was cooled, filtered, and dried to give a product (2.5 g) that was solubilized with water (30 ml) and HCl (1.7 g, 17 mmol, 11.6 M) was added. After cooling to 0–5°C, a saturated aqueous solution of $NaNO_2$ (0.5 g, 7.2 mmol) was added dropwise. Gas evolution was observed. The reaction mixture was allowed to warm to room temperature and neutralized with 28% w/w NH_4OH solution to pH 7. The precipitate was filtered, washed with water, dried, and recrystallized from EtOH to yield **3** (1.65 g, 86%), mp 166–168°C. ^1H NMR ($CDCl_3$) δ 3.45 (2H, s, SCH_2), 3.50 (3H, s, NCH_3), 5.65 (2H, s, $COCH_2$), 7.20 (1H, d, $J = 8.6$ Hz, H-5), 7.90 (1H, dd, $J = 8.6$ and 2.1 Hz, H-6), 8.00 (2H, m, H-8 and H triazole) and 8.35 (1H, s, H triazole). Anal. ($C_{13}H_{12}N_4O_2S$) C, H, N.

5.4 7-[2-(1H-1-Imidazolyl)acetyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (4)

To a solution of bromo derivative **2** (1 g, 3.33 mmol) in $CHCl_3$ (25 ml) 1*H*-imidazole (0.5 g, 7.35 mmol) was added and the solution was stirred at room temperature for 2 h. The mixture was evaporated to dryness and chromatographed on silica gel eluting with $CHCl_3$:MeOH 97:3, to give **4** (0.82 g, 86%), mp 154–156°C. ^1H NMR ($CDCl_3$) δ 3.40 (2H, s, SCH_2), 3.43 (3H, s, NCH_3), 5.35 (2H, s, CH_2N), 6.90, 7.10 and 7.48 (each 1H, bs, H imidazole), 7.15 (1H, d, $J = 8.6$ Hz, H-5), 7.80 (1H, dd, $J = 8.6$ and 2.1 Hz, H-6), 7.95 (1H, d, $J = 2.1$ Hz, H-8). Anal. ($C_{14}H_{13}N_3O_2S$) C, H, N.

5.5 4-Methyl-7-[2-(1H-1,2,4-triazol-1-ylmethyl)-2-oxiranyl]-3,4-dihydro-2H-1,4-benzothiazin-3-one (5)

Trimethylsulfoxonium iodide (0.46 g, 2.1 mmol) was added portionwise to a stirred mixture of NaH (60% mineral oil dispersion, 0.07 g, 1.75 mmol) and DMSO (10 ml) under ice cooling over a period of 20 min and stirred for 30 min at room temperature. A solution of **3** (0.5 g, 1.74 mmol) in DMSO (8 ml) was added and the resulting mixture was stirred at room temperature for 8 h, then poured into water and extracted with $CHCl_3$. The combined organic layers were dried and evaporated to dryness. The residue was chromatographed on silica gel eluting with cyclohexane:EtOAc 1:1 to give **5** (0.31 g, 59%) as an oil. ^1H NMR ($CDCl_3$) δ 2.83 and 2.90 (each 1H, d, $J = 4.5$ Hz, OCH_2), 3.38 (2H, s, SCH_2), 3.42 (3H, s, NCH_3), 4.62 and 4.85 (each 1H, d, $J = 14.9$ Hz, CH_2N), 7.05 (1H, d, $J = 8.5$ Hz, H-5), 7.22 (1H, dd, $J = 8.5$ and 2.1 Hz, H-6), 7.35 (1H, d, $J = 2.1$ Hz, H-8), 7.90 and 8.12 (each 1H, s, H triazole). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N.

5.6 7-[2-(1H-1-Imidazolylmethyl)-2-oxiranyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (6)

In the same manner **6** was obtained as an oil, 83.7% yield. ^1H NMR (DMSO-d_6) δ 2.82 and 2.95 (each 1H, d, $J = 4.7$ Hz, CH_2O), 3.32 (3H, s, NCH_3), 3.50 (2H, s, SCH_2), 4.35 and 4.90 (each 1H, d, $J = 14.2$ Hz, CH_2N), 6.83, 7.08 and 7.55 (each 1H, s, H imidazole), 7.20 (1H, d, $J = 8.5$ Hz, H-5), 7.32 (1H, dd, $J = 8.5$ and 2.1 Hz, H-6) and 7.45 (1H, d, $J = 2.1$ Hz, H-8). Anal. ($C_{15}H_{15}N_3O_2S$) C, H, N.

5.7 7-[1-Hydroxy-2(1H-1,2,4-triazol-1-yl)-1-(1H-1,2,4-triazol-1-ylmethyl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (7)

1*H*-1,2,4-Triazole (0.08 g, 1.16 mmol) was added portionwise to a mixture of NaH (60% mineral oil dispersion, 0.05 g, 1.25 mmol) and DMF (6 ml) over a period of 15 min under a nitrogen atmosphere at 0°C. The mixture was stirred for 15 min at room temperature and a solution of **5** (0.3 g, 0.99 mmol) in DMF (6 ml) was added. The resulting mixture was heated at 80°C for 1.5 h. After cooling, it was poured into water (80 ml) and extracted with EtOAc. The combined organic layers were dried and evaporated to dryness to give a residue which was purified by chromatography on silica gel eluting with $CHCl_3$:MeOH 9:1 to yield **7** (0.22 g, 60%) as an amorphous solid. ^1H NMR ($CDCl_3$) δ 3.40 (5H, s, NCH_3, SCH_2), 4.45 and 4.55 (each 2H, d, $J = 14.9$ Hz, CH_2N), 5.15 (1H, bs, OH), 6.95 (1H, d, $J = 8.5$ Hz, H-5), 7.03 (1H, dd, $J = 8.5$ and 2.1 Hz, H-6), 7.38 (1H, d, $J = 2.1$ Hz, H-8), 7.90 and 8.05 (each 1H, s, H triazole). Anal. ($C_{16}H_{17}N_7O_2S$) C, H, N.

5.8 7-[1-Hydroxy-2-(1H-1-imidazolyl)-1-(1H-1,2,4-triazol-1-ylmethyl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (8)

By using a similar procedure, employing 1*H*-imidazole instead of 1*H*-1,2,4-triazole, **8** was obtained as an amorphous solid, 50% yield. ^1H NMR ($CDCl_3$) δ 3.40 (5H, s, NCH_3, SCH_2), 3.45 (1H, s, OH), 4.25 and 4.28 (each 1H, d, $J = 12.7$ Hz, CH_2N), 4.38 and 4.48 (each 1H, d, $J = 14.1$ Hz, CH_2N), 6.68, 6.78 and 7.30 (each 1H, s, H imidazole), 6.98 (1H, d, $J = 8.5$ Hz, H-5), 7.07 (1H, dd, $J = 8.5$ and 2.1 Hz, H-6), 7.40 (1H, d, $J = 2.1$ Hz, H-8), 7.82 and 7.92 (each 1H, s, H triazole). Anal. ($C_{17}H_{18}N_6O_2S$) C, H, N.

*5.9 7-[1-Hydroxy-2-(1H-1,2,4-triazol-1-yl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (**9**)*

To a solution of **3** (0.5 g, 1.7 mmol) in MeOH (20 ml) $NaBH_4$ (0.065 g, 1.7 mmol) was added in small fractions over 1 h. The mixture was then evaporated to dryness and the residue suspended in water and extracted with $CHCl_3$. The combined organic layers were dried and evaporated to dryness to yield **9**, which was recrystallized from EtOH (0.4 g, 79%), mp 199–200°C. ^1H NMR (DMSO-d_6) δ 3.35 (3H, s, NCH_3), 3.50 (2H, s, SCH_2), 4.30 (2H, d, $J = 6.3$ Hz, CH_2N), 4.93 (2H, dt, $J = 4.8$ and 6.3 Hz, C*H*OH), 5.78 (1H, d, $J = 4.8$ Hz, CHO*H*), 7.20-7.28 (2H, m, H-5 and H-6), 7.40 (1H, bs, H-8), 7.95 and 8.40 (each 1H, s, H triazole). Anal. ($C_{13}H_{14}N_4O_2S$) C, H, N.

*5.10 7-[1-Hydroxy-2-(1H-imidazolyl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (**10**)*

By a similar procedure, using 1*H*-imidazole instead of 1*H*-1,2,4-triazole, **10** was obtained and purified by chromatography on aluminium oxide eluting with $CHCl_3$:MeOH 97:3, 45% yield, mp 187–189°C. ^1H NMR ($CDCl_3$) δ 3.35 (3H, s, NCH_3), 3.50 (2H, s, SCH_2), 4.00 (1H, dd, $J = 13.9$ and 7.9 Hz, CHC*H*$_2$), 4.15 (1H, dd, $J = 13.9$ and 4.0 Hz, CHC*H*$_2$), 4.80 (1H, ddd, $J = 4.8$, 4.0 and 3.8 Hz, C*H*OHCH$_2$), 5.78 (1H, d, $J = 4.8$ Hz, CHO*H*CH$_2$), 6.85, 7.15 and 7.52 (each 1H, s, H imidazole), 7.28–7.20 (2H, m, H-5 and H-6), 7.40 (1H, bs, H-8). Anal. ($C_{14}H_{15}N_3O_2S$) C, H, N.

*5.11 General procedure for preparing **11-14** derivatives. 7-[1-[(4-Chlorobenzyl)oxy]-2-(1H-1,2,4-triazol-1-yl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (**11**)*

To a solution of **9** (0.5 g, 1.73 mmol) in DMF (6 ml), NaH (60% mineral oil dispersion, 0.1 g, 2.5 mmol) was added in small fractions to prevent any heating. 2,4-Dichlorobenzyl chloride (0.55 g, 3.44 mmol) in DMF (4 ml) was then added dropwise. The mixture was stirred at room temperature for 2 h and the excess of hydride was decomposed with a small amount of MeOH. After evaporation to dryness, the crude residue was suspended with water and extracted with $CHCl_3$. The combined organic layers were dried and evaporated to dryness to afford a crude residue that was purified by chromatography on aluminium oxide eluting with $CHCl_3$ to give **11** (0.28 g, 40%). ^1H NMR ($CDCl_3$) δ 3.35 (2H, s, SCH_2), 3.40 (3H, s, NCH_3), 4.15 and 4.40 (each 1H, d, $J = 11.8$ Hz, OCH_2), 4.28 (1H, dd, $J = 4.8$ and 14.0 Hz, CHC*H*$_2$), 4.35 (1H, dd, $J = 7.8$ and 14.0 Hz, CHC*H*$_2$), 4.65 (1H, dd, $J = 4.8$ and 7.8 Hz, C*H*CH$_2$), 6.90 (2H, d, $J = 8.5$ Hz, H-2 and H-6 benzyle), 7.03 (1H, d, $J = 8.4$ Hz, H-5 benzothiazine), 7.15 (1H, dd, $J = 8.4$ and 2.0 Hz, H-6 benzothiazine), 7.20 (2H, d, $J = 8.5$ Hz, H-3 and H-5 benzyle), 7.30 (1H, d, $J = 2.0$ Hz, H-8 benzothiazine), 7.85 and 8.01 (each 1H, s, H triazole). Anal. ($C_{20}H_{19}ClN_4O_2S$) C, H, N.

In the same manner, derivatives **12–14** were prepared by using the appropriate arylchloromethyl compound.

*5.11.1 7-[1-[(2,4-Dichlorobenzyl)oxy]-2-(1H-1,2,4-triazol-1-yl)ethyl]-4-methyl-3,4- dihydro-2H-1,4-benzothiazin-3-one (**12**)*

Obtained in a 42% yield, ^1H NMR ($CDCl_3$) δ 3.42 (2H, s, SCH_2), 3.45 (3H, s, NCH_3), 4.28 and 4.46 (each 1H, d, $J = 12.5$ Hz, OCH_2), 4.29 (1H, dd, $J = 12.5$ and 4.7 Hz, C*H*$_2$CH), 4.38 (1H, dd, $J = 12.5$ and 7.8 Hz, C*H*$_2$CH), 4.75 (1H, dd, $J = 7.8$ and 4.7 Hz, CH$_2$C*H*), 7.05–7.40 (6H, m, aromatic H), 7.92 and 8.08 (each 1H, s, H triazole). Anal. ($C_{20}H_{18}Cl_2N_4O_2S$) C, H, N.

*5.11.2 7-[1-[(4-Chlorobenzyl)oxy]-2-(1H-1-imidazolyl)ethyl]-4-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one (**13**)*

Obtained in a 38% yield, ^1H NMR ($CDCl_3$) δ 3.43 (2H, s, SCH_2), 3.46 (3H, s, NCH_3), 4.08 (1H, dd, $J = 14.2$ and 4.6 Hz, C*H*$_2$CH), 4.16 (1H, dd, $J = 14.2$ and 7.4 Hz, C*H*$_2$CH), 4.22 and 4.43 (each 1H, d, $J = 11.9$ Hz, OCH_2), 6.88–7.45 (10H, m, aromatic H and H triazole). Anal. ($C_{21}H_{20}ClN_3O_2S$) C, H, N.

*5.11.3 7-[1-[(2,4-Dichlorobenzyl)oxy]-2-(1H-1-imidazolyl)ethyl]-4-methyl-3,4-dihydro-2H-benzothiazin-3-one (**14**)*

Obtained in a 45% yield, ^1H NMR ($CDCl_3$) δ 3.43 (2H, s, SCH_2), 3.46 (3H, s, NCH_3), 4.13 (1H, dd, $J = 14.4$ and 4.8 Hz, C*H*$_2$CH), 4.19 (1H, dd, $J = 14.4$ and 6.8 Hz, C*H*$_2$CH), 4.36 and 4.48 (each 1H, d, $J = 12.7$ Hz, OCH_2), 4.55 (1H, dd, $J = 6.8$ and 4.8 Hz, CH$_2$C*H*), 6.92, 7.05 and 7.45 (each 1H, bs, H imidazole), 7.18–7.35 (6H, m, aromatic H). Anal. ($C_{21}H_{19}Cl_2N_3O_2S$) C, H, N.

5.12 Micology

Candida albicans strain CA6, used throughout this study, was isolated from a clinical specimen and identified by the taxonomic criteria of van Uden and Buckley [18]. The yeast was grown at 28°C in Saboraud dextrose agar. Under these conditions, the organism grew as an essential pure yeast-phase population. Before use, yeast cells were harvested from a 24 h culture, resuspended in pyrogen-free saline, washed twice and counted in a hemacytometer and adjusted to the desired concentration.

5.13 Drug stock solutions

Fluconazole and **7–14** derivatives were solubilized in DMSO and diluted with distilled water at a DMSO:H_2O ratio of 1:4. The stock solutions, sterilized by filtration and protected from light, were stored at 4°C until used.

5.14 In vitro assays

The yeast cell suspension (10^5 CFU/ml) was diluted with 1 ml of synthetic aminoacid medium (RPMI-1640, Gibco) and dispensed in multiwell microdilution plates (96 U-shaped wells, 100 μl/well), containing serially diluted azole compounds (0.49 to 250 μg/ml) or diluent (growth control) and incubated at 37°C for 48 h. The MIC was determined as the lowest concentration of compound preventing visible fungal growth.

5.15 In vivo assays

Pathogen-free female CD-1 mice, 8–10 weeks old, obtained from Charles River Breeding Laboratories (Calco, Milan, Italy), were injected intravenously (iv, tail vein) in a volume of 0.5 ml per mouse with 2×10^5 live cells of *C. albicans*. Compounds **7–14** and fluconazole were administered intraperitoneally (ip) at the dose of 10 mg/kg 2 h before and once daily for 7 consecutive days after infection. The animals were then examined for mortality parameters or yeast burden in the kidneys at 8 days post-infection by means of a standard colony forming unit (CFU) assay in Sabouraud dextrose agar [19].

5.16 Statistical analysis

Differences in survival times were assessed by the Mann-Whitney U test. Differences in the number of CFU were assessed by means of Student's t test. Each experiment was repeated three times.

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