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Oxime and Oxime Ether Derivatives of 1,4-Benzothiazine Related to Oxiconazole

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The synthesis, in vitro antifungal activity, and molecular docking experiments of some oxime and oxime ether derivatives of azole 1,4-benzothiazine are reported herein, with the aim of evaluating the influence of a partially constrained scaffold that is structurally related to Oxiconazole and bearing the 1,4-benzothiazine moiety, on the inhibition of Candida albicans CYP51.

Introduction

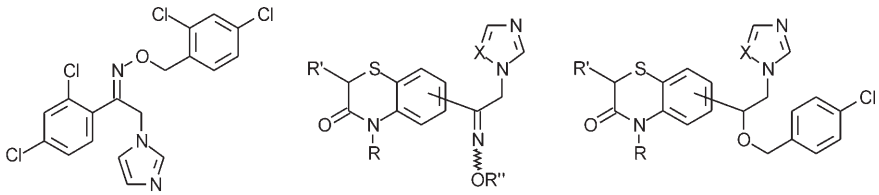
The opportunistic human pathogen *Candida albicans* and other non-*albicans* species have acquired considerable significance in recent years because of the enhanced susceptibility of immunocompromised patients.[1] These pathogenic species of *Candida* derive their importance not only from the severity of their infections but also from their ability to develop resistance against antifungal drugs.[2] Therefore, the need to develop new antifungal drugs is higher than ever.

Recently we reported the synthesis and antifungal activity of a series of *C. albicans* CYP51 inhibitors[3] related to Econazole and Ketoconazole based on the replacement of the 2,4-dichlorophenyl group with the 1,4-benzothiazine moiety that, in itself, shows some antifungal activity.[4]

Herein, we report the synthesis and in vitro antimycotic activity of some oxime and oxime ether derivatives of azole 1,4-benzothiazine **3a**–**g** and **4a**–**g** related to Oxiconazole and to our previously synthesized compounds. The aim was to extend the current structure–activity relationship (SAR) of *C. albicans* CYP51 inhibitors by investigating the effect of partially constraining the molecule through the insertion of oxime and oxime ether groups.

Oxiconazole (Oxistat®),[5] is a well-known antifungal agent with a broad spectrum of activity, used for the treatment of skin mycoses. It is structurally characterized by an oxime ether group in the *Z* configuration. Several compounds containing an oxime or an oxime ether function have been reported to exhibit antimicrobial activity.[6] (see Figure 1)

Initially our attention was focused on the modification of compound **5a** as it had shown an appreciable inhibitory activity on *C. albicans* growth, a very good in vivo antimycotic activity combined with an interesting capability to stimulate the immune response.[3a,b]



Oxiconazole **3a–g, 4a–g** **5a–g**

Figure 1. Structures of Oxiconazole, target compounds, and reference ether derivatives.

Results and Discussion

The synthesis of compound **4a** was planned and carried out starting from the known ketone **2a**[3a] that was first converted into the corresponding oxime derivative **3a** by reaction with hydroxylamine hydrochloride and then alkylated with 4-chlorobenzyl chloride (see Scheme 1).

The increased antifungal activity of **4a** (MIC = 15.6–7.8 μg mL^{-1} against *C. albicans* CA-6) with respect to **5a** (MIC = 46 μg mL^{-1}) motivated us to extend this structural modification to a series of compounds with a different lateral chain position, different substituents at the C2 position of the nucleus-base, a triazole instead of the imidazole, or longer alkyl substituents

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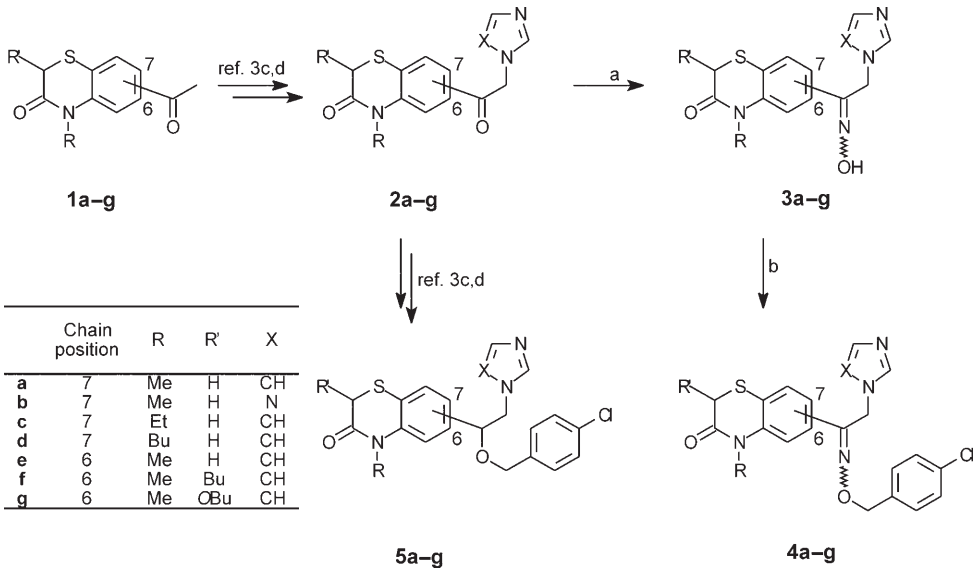
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author. Elemental analysis for target compounds **3a**–**g**, **4a**–**g** and **5d**. Synthetic pathway to obtain compound **5d**.



	Chain position	R	R'	X
a	7	Me	H	CH
b	7	Me	H	N
c	7	Et	H	CH
d	7	Bu	H	CH
e	6	Me	H	CH
f	6	Me	Bu	CH
g	6	Me	OBu	CH

Scheme 1. Synthesis of oxime and oxime ether derivatives and corresponding ether compounds: a) $NH_2OH{\cdot}HCl$; b) NaH, 4-chlorobenzyl chloride.

on the cyclic amide, thus obtaining compounds **3a**–**g** and **4b**–**g**.

Ketones **2a**–**g** were the key intermediates for the synthesis of the new compounds. Whereas derivatives **2a**–**c** and **2e**–**g** were recently synthesized,[3a,c,d] compound **2d** was obtained starting from 7-acetyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one,[7] following a synthetic pathway similar to the one previously reported.[3a] Moreover, to be able to have a direct comparison between the activities of ether and oxime ether compounds, the *N*-butyl derivative **5d**, to date unreported, was synthesized from ketone **2d** by reduction with $NaBH_4$ and subsequent alkylation with 4-chlorobenzyl chloride.[3a] The azole ketones **2a**–**g** were converted into the corresponding oxime derivatives **3a**–**g** by reaction with hydroxylamine hydrochloride in pyridine or in EtOH/NaOH. The choice of reaction conditions depended on the differences in solubility between the 6- and 7-substituted 1,4-benzothiazines.

Z/*E* configurations of the oximes thus obtained were established by comparing their ^{1}H NMR spectra and the literature data. The 7-substituted 1,4-benzothiazines **3a**–**d** gave the *Z* isomer as a single product; their ^{1}H NMR spectra showed only one signal for the methylene protons, and only one signal attributable to the oxime proton; the values of these signals are consistent with the *Z* configuration.[6a] Moreover, the *syn* relationship was confirmed by Overhauser correlations measured between the oxime proton and the methylene ones. Compounds **3e**–**g** were obtained as *Z*/*E* mixtures, as deduced from their ^{1}H NMR spectra. For compound **3g**, for instance, the ^{1}H NMR spectrum of the mixture showed two signals as singlets at $\delta = 5.10$ and 5.25 ppm (1.4 H and 0.6 H, respectively), corresponding to the protons in position 2′. The most unshielded signal was attributed to the *Z* isomer, because of the presence of the nearby hydroxyl function.[6e] Similar signals were observed for compounds **3e** and **3f**. Based on these findings, the configuration of each compound was assigned by repeating ^{1}H NMR experiments after the separation of the *Z*/*E* mixtures. With regard to the *Z*/*E* ratio, the *E* isomer was only predominant for compound **3g** (*Z*/*E* ratio 3:7), whereas the *Z* isomer was predominant for both oximes **3e** (*Z*/*E* ratio 9:1) and **3f** (*Z*/*E* ratio 7:3). The high ratio did not allow the *E* isomer to be isolated for compound **2e**. The oximes **3a**–**g** thus obtained and characterized were then converted into the corresponding 4-chlorobenzyl ether derivatives **4a**–**g** by reaction with 4-chlorobenzyl chloride in the presence of sodium hydride (see Scheme 1). ^{1}H NMR spectra of the final products confirmed the retention of the configuration, in accord with the literature data.[8]

The in vitro antifungal activity against *C. albicans* and *C. krusei* of compounds **3a**–**g** and **4a**–**g** was evaluated in comparison with their corresponding previously synthesized ether derivatives **4a**–**g**.[3a,c,d] Unfortunately, the microbiological data confirmed a good activity only for the oxime ether derivative (*Z*)-**4a**.

To gain some insight into the structure–activity relationships and binding mode of compounds **4a**, **5a**, **4c**, and **5c**, a number of docking experiments were performed in a 3D model of *C. albicans* CYP51 as detailed in the experimental section. The results partially clarified the different activities observed among the selected compounds. In particular, the main variation was in the different binding modes adopted by the compounds at the active site of the enzyme. Whereas the access channel to the catalytic site is filled with the 4-chlorophenyl side chain in the case of the *N*-methyl ether derivative **5a** and *N*-ethyl ether derivative **5c**,[9] the *N*-methyl 1,4-benzothiazine moiety occupies the channel in the binding mode of the oxime ether analogue **4a**. This latter orientation seems to favor the binding of compound **4a** over **5a** and **5c** at the enzyme (glide scores for **4a** = 6.97, **5a** = 5.57, **5c** = 6.07). In particular, the contribution made by the steric interaction term (van der Waals energy, Evdw) of compound **4a** to the binding energy was about twice that of **5c** and three times that of compound **5a**. These results indicate that the molecular shape of this compound has a good fit within the enzymatic binding site (see Figure 2).

Despite the good activity displayed by the ether derivative **5c** (MIC = 15.6 $\mu g\,mL^{-1}$), the *N*-ethyl oxime ether **4c** was inactive. Docking results of **4c** indicated that there were no possible binding modes present in the catalytic site of CYP51 for this molecule. The failure of the docking algorithm to identify a binding pose for compound **4c** can be ascribed to the presence of two structural features (Figure 2B): 1) the small size of the binding pocket (residues Gln 72, Arg 95, Leu 96, and

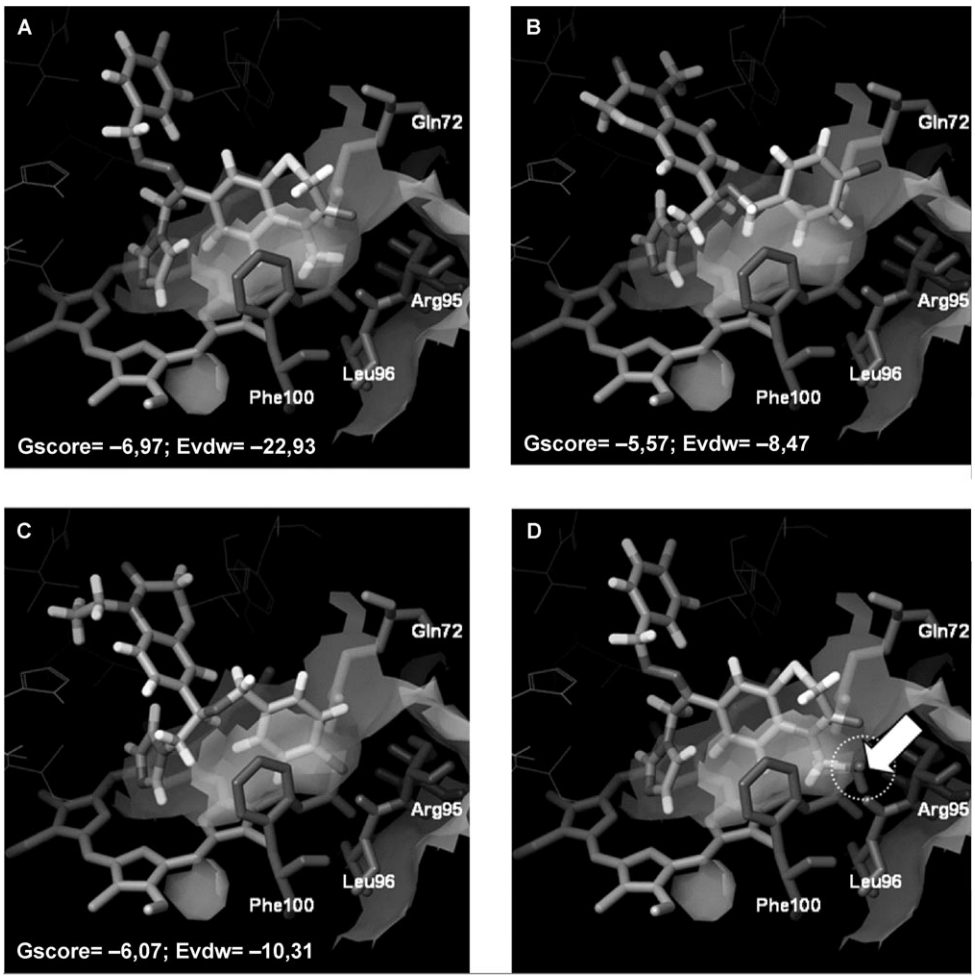


Figure 2. Docking results of studied compounds within the catalitic site of CYP51. The residues lining the pocket of the binding site are in stick style and their van der Waals molecular surface is highlighted. The images A, B, and C show the results of derivatives **4a**, **5a**, and **5c**, respectively. In D, the hypothetical pose of compound **4c** is shown to clarify the steric clashes of the *N*-ethyl group (highlighted with white point circle) in the small pocket constituted by residues Gln 72, Arg 95, Leu 96, and Phe 100.

Phe 100) that hosts the *N*-methyl 1,4-benzothiazine moiety of compound **4a** does not allow the enzyme to accept the *N*-ethyl 1,4-benzothiazine group of **4c** and 2) the structural stiffness of the oxime function of the molecule that hampers any conformational fitness within the above described enzymatic pocket.

Conclusions

As a continuation of an articulated program, the aim of this work was twofold: 1) to synthesize new azole derivatives containing a 1,4-benzothiazine nucleus, that, in itself, shows a moderate antifungal activity and 2) to improve the antifungal activity and extend the SAR for this class of compounds.

As a result of structural change, it was possible to establish that the side chain that bears the azole nucleus must be flexible, as its constraint in a more rigid moiety generally proved to be detrimental to the activity.

Experimental Section

Melting points determined in capillary tubes (Electrothermal, model 9100, melting point apparatus) were uncorrected. Elemental analysis was performed on a Carlo Erba element analyzer 1106, and the data for C, H, and N are within $\pm 0.4\%$ of the theoretical values. ^{1}H NMR spectra were recorded at 200 MHz (Bruker AC-200 spectrometer) or at 400 MHz (Bruker Avance-DRX 400 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 60 (mesh 230–400). Yields of purified products were not optimized. All starting materials were commercially available unless otherwise indicated.

7-Acetyl-4-butyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (1d). *t*BuOK (0.16 g, 1.45 mmol) was added to a solution of 7-acetyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one[7] (0.20 g, 0.97 mmol) in dry DMF (3 mL). The mixture was stirred at room temperature for 15 min, then BuI (0.27 g, 1.45 mmol) in dry DMF (2 mL) was added dropwise. After being stirred for 4 h, the mixture was poured into ice-chilled water and extracted with EtOAc. The residue was chromatographed, eluting with cyclohexane/EtOAc 85:15. **1d** was obtained as oil, (0.18 g, 70% yield). ^{1}H NMR ($CDCl_3$): $\delta = 0.92$ (t, $J = 6.9$ Hz, 3H; CH_2CH_3); 1.20–1.45 and 1.50–1.65 (m, each 2H; $CH_2CH_2CH_3$); 2.55 (s, 3H; $COCH_3$); 3.38 (s, 2H; SCH_2); 4.01 (t, $J = 7.4$ Hz, 2H; NCH_2); 7.17 (d, $J = 8.6$ Hz, 1H; H-5); 7.82 (dd, $J = 8.6$ and 2.1 Hz, 1H; H-6); 7.95 ppm (d, $J = 2.1$ Hz, 1H; H-8). Anal. ($C_{14}H_{17}NO_2S$) C, H, N.

4-Butyl-7-(1*H*-1-imidazolylacetyl)-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (2d). 1) A solution of **1d** (0.20 g, 0.76 mmol) in CCl_4 (5 mL) was cooled to 0 °C, then Br_2 (0.13 g, 0.84 mmol) in CCl_4 (5 mL) was added dropwise. After being stirred for 1 h at room temperature, the mixture was evaporated to dryness and the residue was chromatographed, eluting with cyclohexane/EtOAc 92:8 to give 7-(bromoacetyl)-4-butyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one as an oil (0.10 g, 40% yield). ^{1}H NMR ($CDCl_3$): $\delta = 0.96$ (t, $J = 7.2$ Hz, 3H; CH_2CH_3); 1.25–1.50 and 1.55–1.80 (m, each 2H; $CH_2CH_2CH_3$); 3.45 (s, 2H; SCH_2); 3.95–4.15 (m, 2H; NCH_2); 4.41 (s, 2H; CH_2Br); 7.15–7.25 (m, 1H; aromatic H); 7.75–8.05 ppm (m, 2H; aromatic H). Anal. ($C_{14}H_{16}BrNO_2S$) C, H, N. 2) Imidazole (0.06 g, 0.88 mmol) was added to a solution of 7-(bromoacetyl)-4-butyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (0.10 g, 0.29 mmol) in $CHCl_3$ (3 mL). The mixture was stirred at room temperature for 24 h, then

evaporated to dryness, and the residue chromatographed eluting with $CHCl_3$/MeOH 96:4 to give the **2d** (0.82 g, 87% yield) as an orange amorphous solid. ^{1}H NMR ($CDCl_3$): $\delta = 0.95$ (t, $J = 7.2$ Hz, 3H; CH_2CH_3); 1.20–1.65 and 1.65–1.80 (m, each 2H; $CH_2CH_2CH_3$); 3.43 (s, 2H; SCH_2); 3.95–4.20 (m, 2H; NCH_2CH_2); 5.40 (s, 2H; $COCH_2N$); 6.96, 7.14, and 7.53 (bs, each 1H; imidazolic H); 7.20–7.30 (m, 1H; aromatic H); 7.75–8.05 ppm (m, 2H; aromatic H). Anal. ($C_{17}H_{19}N_3O_2S$) C, H, N.

4-Butyl-7-{1-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethyl}-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (5d). 1) $NaBH_4$ (0.02 g, 0.60 mmol) was added portionwise to a solution of **2d** (0.20 g, 0.60 mmol) in dry MeOH (3 mL) over 1 h. The mixture was then evaporated to dryness, and the residue was chromatographed, eluting with $CHCl_3$/MeOH 92:8 to furnish, 4-butyl-7-[1-hydroxy-2-(1*H*-1-imidazolyl)ethyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-one as an oil, in quantitative yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.95$ (t, $J = 7.3$ Hz, 3H; CH_2CH_3); 1.30–1.50 and 1.50–1.75 (m, each 2H; $CH_2CH_2CH_3$); 3.37 (s, 2H; SCH_2); 3.80–4.20 (m, 5H; NCH_2CH_2, $COCH_2N$ and CHOH); 4.80–4.95 (m, 1H; CHOH); 6.89 (bs, 2H; imidazolic H); 7.10–7.50 ppm (m, 4H; imidazolic and aromatic H). Anal. ($C_{17}H_{21}N_3O_2S$) C, H, N. 2) NaH (0.023 g, 0.59 mmol) was added portionwise to a solution of 4-butyl-7-[1-hydroxy-2-(1*H*-1-imidazolyl)-ethyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-one (0.13 g, 0.39 mmol) in DMF dry (3 mL) and stirred for 30 min. 4-Chlorobenzyl chloride (0.095 g, 0.59 mmol) in DMF dry (2 mL) was added dropwise. After 90 min the mixture was poured into water and extracted with EtOAc. After evaporation the residue was chromatographed eluting with CH_2Cl_2/MeOH 98:2 to furnish **5d** as an oil (0.12 g, 70% yield). ^{1}H NMR ($CDCl_3$): $\delta = 0.95$ (t, $J = 7.4$ Hz, 3H; CH_2CH_3); 1.25–1.50 and 1.50–1.75 (m, each 2H; $CH_2CH_2CH_3$); 3.37 (s, 2H; SCH_2); 3.80–4.50 ppm (m, 7H; NCH_2CH_2, $CHOCH_2N$ and OCH_2Ph); 6.75–7.50 (m, 6H; imidazolic and aromatic H). Anal. ($C_{24}H_{26}ClN_3O_2S$) C, H, N.

General method for the preparation of oxime derivatives 3a–d. The procedure is illustrated by the synthesis of 7-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-**3a**]. $NH_2OH{\cdot}HCl$ (0.16 g, 2.30 mmol) was added to a solution of ketone **2a**[3a] (0.50 g, 1.70 mmol) in EtOH (12 mL), and the pH of the mixture was adjusted to 11 with 15 N NaOH. The solution was refluxed for 3 h, then evaporated to dryness, and the residue was solubilized in H_2O. The aqueous layer was acidified with 1 N HCl to pH 5 and then adjusted to pH 9 with $NaHCO_3$-saturated aqueous solution. Compound (*Z*)-**3a**, precipitated out of solution, was crystallized from EtOH (0.27 g, 52%) as a white solid, mp 201–202 °C. ^{1}H NMR ([D_6]DMSO): $\delta = 3.38$ (s, 3H; NCH_3); 3.56 (s, 2H; SCH_2); 5.34 (s, 2H; CH_2N); 6.88 and 7.12 (s, each 1H; imidazolic H); 7.30 (d, $J = 8.6$ Hz, 1H; H-5); 7.55–7.75 (m, 3H; aromatic and imidazolic H); 12.10 ppm (s, 1H; NOH). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N.

7-[(1*Z*)-*N*-Hydroxy-2-(1*H*-1,2,4-triazol-1-yl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-3b]. It was synthesized starting from ketone **2b**.[3c] White solid, mp 185–187 °C, 61% yield. ^{1}H NMR ([D_6]DMSO): $\delta = 3.29$ (s, 3H; NCH_3); 3.45 (s, 2H; SCH_2); 5.45 (s, 2H; CH_2N); 7.27 (d, $J = 8.7$ Hz, 1H; H-5); 7.63 (dd, $J = 8.7$ and 1.3 Hz, 1H; H-6); 7.66 (d, $J = 1.3$ Hz, 1H; H-8); 7.91 and 8.57 (s, each 1H; triazolic H); 12.02 ppm (bs, 1H; NOH). Anal. ($C_{13}H_{13}N_5O_2S$) C, H, N.

4-Ethyl-7-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-3c]. It was synthesized starting from ketone **2c**.[3c] White solid, mp 205.1–205.8 °C, 72% yield. ^{1}H NMR ([D_6]DMSO): $\delta = 1.13$ (t, $J = 6.8$ Hz, 3H; CH_2CH_3); 3.52 (s, 2H; SCH_2); 3.97 (q, $J = 6.8$ Hz, 2H; CH_2CH_3); 5.32 (s, 2H; $NCCH_2N$); 6.84 and 7.06 (s, each 1H; imidazolic H); 7.34 (d, $J = 8.7$ Hz, 1H; H-5); 7.55–7.75 (m, 3H; aromatic and imidazolic H); 12.12 ppm (s, 1H; NOH). Anal. ($C_{15}H_{16}N_4O_2S$) C, H, N.

4-Butyl-7-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-3d]. It was synthesized starting from ketone **2d**. White solid, mp 212–215 °C, 48% yield. ^{1}H NMR ([D_6]DMSO): $\delta = 0.86$ (t, $J = 7.2$ Hz, 3H; CH_2CH_3); 1.15–1.60 (m, 4H; $CH_2CH_2CH_3$); 3.36 (s, 2H; SCH_2); 3.80–4.00 (m, 2H; NCH_2CH_2); 5.31 (s, 2H; $NCCH_2N$); 6.84 and 7.06 (s, each 1H; imidazolic H); 7.25–7.75 (m, 4H; aromatic and imidazolic H); 12.10 ppm (s, 1H; NOH). Anal. ($C_{17}H_{20}N_4O_2S$) C, H, N.

General method for the preparation of oxime derivatives 3e–g. The procedure is illustrated by the synthesis of 6-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-**3e**]. $NH_2OH{\cdot}HCl$ (0.18 g; 2.60 mmol) was added to a solution of ketone **2e**[3c] (0.51 g, 1.80 mmol) in pyridine (20 mL). The mixture was stirred at 70 °C for 4 h, evaporated to dryness, and the residue suspended in H_2O. The precipitate was filtered off to afford a crude residue constituted from the mixture of (*Z*)-**3e** and (*E*)-**3e** (8.9:1.1) that was chromatographed eluting with $CHCl_3$ to give the isomer (*Z*)-**3e**. It was crystallized from MeOH, 0.22 g, 41% yield, mp 219–220 °C. ^{1}H NMR ([D_6]DMSO): $\delta = 3.32$ (s, 3H; NCH_3); 3.52 (s, 2H; SCH_2); 5.35 (s, 2H; CH_2N); 6.81, 7.04, and 7.65 (s, each 1H; imidazolic H); 7.36–7.42 (m, 3H; aromatic H); 12.10 ppm (s, 1H; NOH). Anal. ($C_{14}H_{14}N_4O_2S$) C, H, N.

2-Butyl-6-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-3f] and 2-butyl-6-[(1*E*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*E*)-3f]. They were prepared starting from ketone **2f**.[3d]

(*Z*)-**3f**, oil, 62.2% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (t, $J = 6.5$ Hz, 3H; CH_2CH_3); 1.25–1.55 (m, 5H; $SCHCHHCH_2CH_2$); 1.75–1.95 (m, 1H; $SCHCHHCH_2$); 3.25–3.50 (m, 1H; $SCHCH_2$); 3.42 (s, 3H; NCH_3); 5.27 (s, 2H; CH_2N); 7.02, 7.10, and 7.86 (s, each 1H; imidazolic H); 7.15–7.37 ppm (m, 3H; aromatic H). Anal. ($C_{18}H_{22}N_4O_2S$) C, H, N.

(*E*)-**3f**, oil, 28.7%, ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (t, $J = 6.6$ Hz, 3H; CH_2CH_3); 1.20–1.59 (m, 5H; $SCHCHHCH_2CH_2$); 1.75–1.95 (m, 1H; $SCHCHHCH_2$); 3.34 (s, 3H; NCH_3); 3.30–3.50 (m, 1H; $SCHCH_2$); 4.98 (s, 2H; CH_2N); 6.89–7.37 (m, 6H; aromatic and imidazolic H); 12.00 ppm (bs, 1H; NOH). Anal. ($C_{18}H_{22}N_4O_2S$) C, H, N.

2-Butoxy-6-[(1*Z*)-*N*-hydroxy-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-3g] and **2-butoxy-6-[(1*E*)-*N*-hydroxy-2-(1*H*-1-imidazo-lyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*E*)-3g].** They were synthesized starting from ketone **2g**.[3e]

(*Z*)-**3g**, amorphous solid, 19.2% yield, ^{1}H NMR ($CDCl_3$): $\delta = 0.75$ (t, $J = 6.5$ Hz, 3H; CH_2CH_3); 0.95–1.40 (m, 4H; $CH_2CH_2CH_2CH_3$); 3.30–3.55 (m, 1H; $OCHHCH_2$); 3.40 (s, 3H; NCH_3); 3.60–3.80 (m, 1H; $OCHHCH_2$); 5.05 (s, 1H; SCHO); 5.25 (s, 2H; CH_2N); 7.00–7.45 (m, 5H; aromatic and imidazolic H); 7.75 (s, 1H; imidazolic H); 13.75 ppm (bs, 1H; NOH). Anal. ($C_{18}H_{22}N_4O_3S$) C, H, N.

(*E*)-**3g**, amorphous solid, 46.1% yield, ^{1}H NMR ($CDCl_3$): $\delta = 0.75$ (t, $J = 6.5$ Hz, 3H; CH_2CH_3); 1.00–1.50 (m, 4H; $CH_2CH_2CH_2CH_3$); 3.30–3.60 (m, 1H; $OCHHCH_2$); 3.40 (s, 3H; NCH_3); 3.65–3.80 (m, 1H; $OCHHCH_2$); 5.00 (s, 1H; SCHO); 5.10 (s, 2H; CH_2N); 6.90–7.40 (m, 5H; aromatic and imidazolic H); 7.65 ppm (s, 1H; imidazolic H). Anal. ($C_{18}H_{22}N_4O_3S$) C, H, N.

General method for the preparation of oxime ether derivatives 4a–g. The procedure is illustrated by the synthesis of 7-[(1*Z*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-**4a**]. NaH (60% mineral oil dispersion, 0.03 g, 0.70 mmol) was added portionwise to a solution of oxime (*Z*)-**3a** (0.20 g, 0.66 mmol) in dry DMF (4 mL) under nitrogen atmosphere. The mixture was stirred for 30 min at room temperature, then a solution of 4-chlorobenzyl chloride (0.12 g, 0.66 mmol) in dry DMF (2 mL) was added dropwise. After 20 h the mixture was poured into water and extracted with $CHCl_3$. The or-

ganic phase was evaporated to dryness and the residue chromatographed eluting with $CHCl_3$ to give (*Z*)-**4a** as a yellowish oil, 0.20 g, 71% yield. ^{1}H NMR ($CDCl_3$): $\delta = 3.40$ (s, 3H; NCH_3); 3.42 (s, 2H; SCH_2); 5.18 (s, 2H; CH_2N); 5.25 (s, 2H; OCH_2Ph); 6.86, 7.00, and 7.50 (s, each 1H; imidazolic H); 7.05 (d, $J = 8.7$ Hz, 1H; H-5); 7.25–7.45 (m, 5H; aromatic H); 7.60 ppm (d, $J = 1.8$ Hz, 1H; H-8). Anal. ($C_{21}H_{19}ClN_4O_2S$) C, H, N.

7-[(1*Z*)-*N*-[(4-Chlorobenzyl)oxy]-2-(1*H*-1,2,4-triazol-1-yl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4b]. It was synthesized starting from (*Z*)-**3b**. Amorphous solid, 70% yield. ^{1}H NMR ($CDCl_3$): $\delta = 3.43$ (s, 3H; NCH_3); 3.45 (s, 2H, SCH_2); 5.26 (s, 2H; CH_2N); 5.39 (s, 2H; OCH_2Ph); 7.10 (d, $J = 8.7$ Hz, 1H; H-5); 7.38 (AA′BB′ system, 4H; aromatic H); 7.67 (dd, $J = 8.7$ and 2.1 Hz, 1H; H-6); 7.82 (d, $J = 2.1$ Hz, 1H; H-8); 7.93 and 8.11 ppm (s, each 1H; triazolic H). Anal. ($C_{20}H_{18}ClN_5O_2S$) C, H, N.

7-[(1*Z*)-*N*-[(4-Chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-ethyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4c]. It was synthesized starting from (*Z*)-**3c**. Amorphous solid, 80% yield. ^{1}H NMR ($CDCl_3$): $\delta = 1.26$ (t, $J = 7.2$ Hz, 3H; CH_2CH_3); 3.38 (s, 2H; SCH_2); 4.02 (q, $J = 7.2$ Hz, 2H; CH_2CH_3); 5.17 (s, 2H; $NCCH_2N$); 5.25 (s, 2H; OCH_2Ph); 6.86, 7.01, and 7.51 (s, each 1H; imidazolic H); 7.12 (d, $J = 7.5$ Hz, 1H; H-5); 7.30–7.45 (m, 5H; aromatic H); 7.61 ppm (d, $J = 1.4$ Hz, 1H; H-8). Anal. ($C_{22}H_{21}ClN_4O_2S$) C, H, N.

4-Butyl-7-[(1*Z*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4d]. It was synthesized starting from (*Z*)-**3d**. Amorphous solid, 65% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.94$ (t, $J = 7.3$ Hz, 3H; CH_2CH_3); 1.20–1.45 (m, 2H; $CH_2CH_2CH_3$); 1.50–1.65 (m, 2H; $CH_2CH_2CH_3$); 3.39 (s, 2H; SCH_2); 3.99 (t, $J = 7.7$ Hz, 2H; NCH_2CH_2); 5.18 (s, 2H; OCH_2Ph); 5.27 (s, 2H; $NCCH_2N$); 6.88, 7.03, and 7.52 (s, each 1H; imidazolic H); 7.10 (d, $J = 8.7$ Hz, 1H; H-5); 7.30–7.45 (m, 5H; aromatic H); 7.62 ppm (d, $J = 2.1$ Hz, 1H; H-8). Anal. ($C_{24}H_{25}ClN_4O_2S$) C, H, N.

6-[(1*Z*)-*N*-[(4-Chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4e]. It was synthesized starting from (*Z*)-**3e**. Oil, 58% yield. ^{1}H NMR ($CDCl_3$): $\delta = 3.39$ (s, 5H; NCH_3 and SCH_2); 5.19 (s, 2H; CH_2N); 5.25 (s, 2H; OCH_2Ph); 6.84, 7.00, and 7.50 (s, each 1H; imidazolic H); 7.14 (dd, $J = 8.0$ and 1.8 Hz, 1H; H-6); 7.23 (d, $J = 1.8$ Hz, 1H; H-8); 7.25–7.40 ppm (m, 5H; aromatic H). Anal. ($C_{21}H_{19}ClN_4O_2S$) C, H, N.

2-Butyl-6-[(1*Z*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4f]. It was synthesized starting from (*Z*)-**3f**. Oil, 47.6% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.87$ (t, $J = 7.3$ Hz, 3H; CH_2CH_3); 1.26–1.50 (m, 5H; $SCHCH\mathit{HCH_2CH_2}$); 1.80–1.95 (m, 1H; $SCHCH\mathit{H}HCH_2$); 3.30–3.50 (m, 1H; $SCH\mathit{H}CH_2$); 3.42 (s, 3H; NCH_3); 5.24 (s, 2H; CH_2N); 5.27 (s, 2H; OCH_2Ph); 6.83, 7.05, and 7.74 (s, each 1H; imidazolic H); 7.14–7.40 ppm (m, 7H; aromatic H). Anal. ($C_{25}H_{27}ClN_4O_2S$) C, H, N.

2-Butyl-6-[(1*E*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*E*)-4f]. It was synthesized starting from (*E*)-**3f**. Oil, 50% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.87$ (t, $J = 7.3$ Hz, 3H; CH_2CH_3); 1.25–1.55 (m, 5H; $SCHCH\mathit{HCH_2CH_2}$); 1.75–1.95 (m, 1H; $SCHCH\mathit{H}HCH_2$); 3.30–3.50 (m, 1H; $SCH\mathit{H}CH_2$); 3.42 (s, 3H; NCH_3); 5.12 (s, 2H; CH_2N); 5.27 (s, 2H; OCH_2Ph); 6.90–7.79 ppm (m, 10H; aromatic and imidazolic H). Anal. ($C_{25}H_{27}ClN_4O_2S$) C, H, N.

2-Butoxy-6-[(1*Z*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*Z*)-4g]. It was synthesized starting from (*Z*)-**3g**. Oil, 91% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.77$ (t, $J = 7.0$ Hz, 3H; CH_2CH_3); 1.05–1.50 (m, 4H; $CH_2CH_2CH_2CH_3$); 3.35–3.46 (m, 1H; $OCH\mathit{H}CH_2$); 3.46 (s, 3H; NCH_3); 3.60–3.70 (m, 1H; $OCH\mathit{H}CH_2$); 5.05 (s, 1H; SCHO); 5.19 (s, 2H; CH_2N); 5.26 (s, 2H; OCH_2Ph); 6.85, 7.01, and 7.56 (s, each 1H; imidazolic H); 7.18–7.39 ppm (m, 7H; aromatic H). Anal. ($C_{25}H_{27}ClN_4O_3S$) C, H, N.

2-Butoxy-6-[(1*E*)-*N*-[(4-chlorobenzyl)oxy]-2-(1*H*-1-imidazolyl)ethanimidoyl]-4-methyl-3,4-dihydro-2*H*-1,4-benzothiazin-3-one [(*E*)-4g]. It was synthesized starting from (*E*)-**3g**. Oil, 63% yield. ^{1}H NMR ($CDCl_3$): $\delta = 0.77$ (t, $J = 7.4$ Hz, 3H; CH_2CH_3); 1.06–1.45 (m, 4H; $CH_2CH_2CH_2CH_3$); 3.35–3.55 (m, 1H; $OCH\mathit{H}CH_2$); 3.46 (s, 3H; NCH_3); 3.65–3.75 (m, 1H; $OCH\mathit{H}CH_2$); 5.06 (s, 1H; SCHO); 5.21 (s, 2H; CH_2N); 5.26 (s, 2H; OCH_2Ph); 6.86, 7.02, and 7.62 (s, each 1H; imidazolic H); 7.10–7.45 ppm (m, 7H; aromatic H). Anal. ($C_{25}H_{27}ClN_4O_3S$) C, H, N.

Computational Method

A chimeric model of the *C. albicans* CYP51 was constructed following the computational procedure described in Rossello et al.[6e] The resulting structure of the enzyme was prepared for docking experiments using the protein preparation wizard script as implemented in Maestro 7.5.[10] Docking experiments were carried out using the default settings of Glide 4.0[11] and the standard precision procedure. The five best poses for each compound were retrieved from an overall search of 50000 possible poses. The search was performed within a grid cage the size of which was calculated in proportion to the crystal structure of CYP51 in complex with fluconazole. During docking experiments, a search constraint was inserted on the iron atom of the heme group.

Susceptibility testing

Susceptibility testing was performed by the M27-A micro-dilution method of the National Committee for Clinical Laboratory Standards[12] in 0.165 M MOPS (morpholinepropanesulfonic acid)-buffered (pH 7) RPMI 1640 medium (Gibco BRL, Paisley, United Kingdom). The activity of compounds against *C. albicans* and *C. krusei* was tested using serial dilutions ranging from 0.9 to 500 $\mu g\,mL^{-1}$. The MIC was the lowest concentration of chemical that produced an 80% reduction in the turbidity compared to chemical-free normal subjects.

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