Azole Antifungal Agents Related to Naftifine and Butenafine

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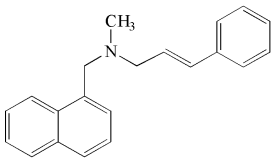
***Key Words**: Azole naftifine analogues; azole butenafine analogues; aminoazole antifungal agents*

Summary

The methyl group of naftifine (**1**) and butenafine (**2**) was replaced by an azolic nucleus to obtain the new compounds **3–8** which exhibit the characteristics of both allylamine (or benzylamine) and azole antifungals. The title compounds were evaluated *in vitro* against several pathogenic fungi responsible for human disease. Among these, compounds **5**, **6**, and **8** were found to inhibit the growth of dermatophytes with a potency comparable to that of naftifine. The synthetic sequence includes the preparation of aminoazole Schiff bases, reduction, and alkylation of the corresponding secondary amines.

Introduction

Only one target, the cytochrome P-450 dependent lanosterol C-14α-demethylase, can be considered a major success in antifungal chemotherapy. Inhibitors of this enzyme, the imidazole and triazole antimycotics, include the most important and successful of today's antifungal drugs endowed with clinical efficacy. On the other hand, an enzyme at the very early stage in the pathway of ergosterol synthesis, squalene epoxidase, is the target of two antimycotics of the allylamine and benzylamine groups, namely naftifine (**1**) and butenafine (**2**) [1].



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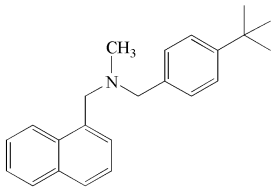

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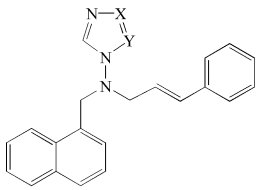
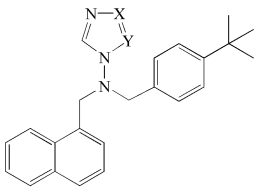
In the present work we report the synthesis of novel structural analogues of naftifine and butenafine, in which the methyl moiety has been substituted with an azolic nucleus.



3; X = CH, Y = CH
4; X = N, Y= CH
5; X = CH, Y = N



6; X = CH, Y = CH
7; X = N, Y = CH
8; X = CH, Y = N

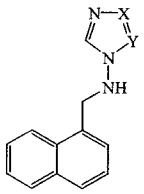
The new compounds **3–8** would thus contain structural elements of both allylamine (or benzylamine) and azole antifungals.

This approach might lead to a mechanistically ideal drug in that it might inhibit two enzymes in a single linear biosynthetic pathway. This property, usually achieved only by drug combination, results in a synergy which enhances activity and makes it more difficult for pathogens to acquire resistance by alteration of the target.

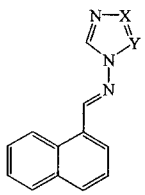
Chemistry

Tertiary amines **3**–**8** were prepared starting from the secondary amines **9**–**11** and the appropriate alkyl bromides. Triazole derivatives **4**, **5**, **7**, and **8** were obtained in anhydrous tetrahydrofuran in the presence of sodium hydride. Imidazole amine **9** did not react under the same conditions. Therefore potassium *tert*-butoxide/18-crown-6 in anhydrous diethyl ether was used in this case. Secondary amines **9**–**11** were produced by reduction of the corresponding Schiff bases **12**–**14** with sodium borohydride in methanol. Schiff base **12** was synthesised by adding imidazole to a neutral solution of hydroxylamine-O-sulfonic acid in water. The crude residue obtained after acidification and elimination of the water was subsequently treated with a solution of naphthalene-1-carbaldehyde in ethanol.

When 1*H*-1,2,4-triazole was used instead of imidazole, a mixture of 4*H*-1,2,4- and 1*H*-1,2,4-triazole derivatives **13** and **14** was obtained and chromatographic separation of isomers was necessary.



9; X = CH, Y = CH
10; X = N, Y= CH
11; X = CH, Y = N



12; X = CH, Y = CH
13; X = N, Y = CH
14; X = CH, Y = N

Attempts to obtain tertiary amines **6–8** by reduction of the amides **15–17** by various methods were unsuccessful. Amine **6**, however, could be obtained (yield: 10%) by reduction of 4-*tert*-butylbenzamide **15** with lithium borohydride in the presence of chlorotrimethylsilane [2]. Deacylated amine **9** and 4-*tert*-butylbenzyl alcohol were also recovered.

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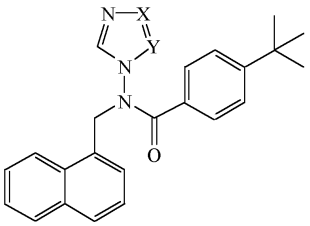

5; X = CH, Y = CH
16; X = N, Y = CH
17; X = CH, Y = N

Amides **15–17** were prepared in anhydrous tetrahydrofuran from secondary amines **9–11** and 4-*tert*-butyl-benzoyl chloride in the presence of triethylamine.

Results

The title compounds were evaluated *in vitro* against several pathogenic fungi responsible for human disease. Test pathogens included representatives of yeasts (*Candida albicans*, *Candida parapsilosis*, *Criptococcus neoformans*), dermatophytes (*Tricophyton verrucosum*, *Tricophyton rubrum*, *Microsporum gypseum*), and molds (*Aspergillus fumigatus*). Naftifine and miconazole were used as reference drugs. The title compounds were also tested for antibacterial activity against representative Gram positive and negative bacteria and for *in vitro* cytotoxicity in a lymphoid cell line (MT-4). Cytotoxicity evaluation was performed in order to determine whether test compounds were endowed with selective antimicrobial activity.

All test compounds were inactive against yeasts (MIC > 200 μM). In the same tests, the azole antifungal miconazole showed a good activity against tested strains (MICs = 1.2–5.0 μM) and the allylamine naftifine was active only against *Candida parapsilosis* (MIC = 12.0 μM).

On the other hand, imidazole derivative **6** and 1*H*-1,2,4-triazole derivatives **5**, **8** inhibited the growth of dermatophytes similarly to naftifine, the imidazole analogous **3** of naftifine was moderately active, whereas the 4*H*-1,2,4-triazole derivatives **4** and **7** were inactive (Table 1).

The presence of a carbonyl group in the amide **15** and **17** abates activity (MIC > 200 μM). All compounds were inactive when used against the mold strain tested (*Aspergillus fumigatus*) and the representative of Gram positive (*Staphylococcus aureus*) and Gram negative (*Salmonella* spp.) bacteria.

When evaluated *in vitro* for antiproliferative activity (Table 1), compounds **3**–**8** inhibited the growth of MT-4 cells with a range of CC_{50} in between that of miconazole (18 μM) and that of naftifine (>200 μM).

Discussion

Replacement of 3-phenyl-2-propenyl moiety in the naftifine structure with a 4-*tert*-butylbenzyl group have led to butenafine that exhibits enhanced activity against a range of human pathogenic fungi, but is moderately effective against Candida species [3].

Table 1. Antimycotic activity of compounds **3–8** against dermathophytes.

Compd.	[a]CC_{50}. MT-4	[b]MIC *T. verrucosum*	*T. rubrum*	*M. gypseum*
3	>200	200	100	100
4	51	>200	>200	>200
5	59	6 (10)	3 (20)	6 (10)
6	28	3 (9)	3 (9)	3 (9)
7	37	>200	>200	>200
8	154	3 (51)	6 (26)	3 (51)
Naftifine	>200	2.4 (>83)	7.4 (>27)	2.4 (>83)
Miconazole	18	0.15 (120)	0.15 (120)	1.2 (15)

a) Compound concentration (μM) required to reduce the viability of MT4 cells by 50%.
b) Minimum inhibitory concentration (μM). Values in parenthesis represent the CC_{50}/MIC ratio (selectivity index). Data represent mean values from three independent determinations.

In the attempt to improve the antimicotic effect of naftifine and butenafine, the methyl group was replaced with an azolic ring, to obtain a simultaneous effect on both fungal 14α-demethylase and squalene epoxidase enzymes.

Interestingly, derivatives **5**, **6**, and **8** showed an antifungal activity against dermatophytes comparable to that of naftifine, although a systematic variation of individual structural elements in naftifine had demonstrated that substitution of methyl group seems to be limited to the *N*-ethyl analogue, which is still highly active *in vitro* and *in vivo*. The *N*-cyclohexyl, the *N*-phenyl, and the *N*-benzyl analogues were completely devoid of activity [4].

Although showing an activity restricted to dermatophytes, the title compounds seem to act similarly to the azole antifungals. This hypothesis is supported by the observation that 4*H*-1,2,4-triazole derivatives **4** and **7** were inactive. Triazole compounds in fact are reported to inhibit the binding of the natural substrate lanosterol to demethylase by coordination of the unhindered N-4 ring nitrogen atom to the sixth coordination position of the iron atom of the enzyme protoporphyrin system [5]. Furthermore, the introduction of a carbonyl group completely abates the activity in the amides **15** and **17**. On the other hand, the 1-naphthamide obtained from naftifine modified at the amino group, displayed a good activity *in vitro* and to a somewhat lesser extent *in vivo* [4].

It is noteworthy that the imidazole analogue **3** of naftifine was less active than the triazole **5**. According to some authors [6], the triazole ring should show increased specificity for fungal enzymes and apparent increased potency (itraconazole *versus* ketoconazole). However, more recently, the use of comparative molecular field analysis in the molecular modelling of azole antifungals revealed the preference of an imidazole over a triazole group in the active site. This result was also supported by the observed *in vitro* inhibitory activity values, which consistently show that imidazole derivatives are more active than the triazoles [7].

Moreover, we assume that the title compounds did not give synergistic effects because the azolyl group that replaced the methyl group in naftifine and butenafine may be too hindered to fit properly into the active site of the squalene epoxidase.

Our efforts to define the importance of the *N*-azolylamine portion in antimycotic chemotherapy are the subject of further investigations.

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Experimental

Chemistry

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. Elemental analyses (C, H, N) were performed by Dr. Emilio Cebulec at the Chemistry Department of the University of Trieste and were within ±0.4% of the calculated values. IR spectra were obtained on a Jasco FT/IR-200 spectrophotometer (KBr). All compounds showed appropriate IR, which are not reported. The ^{1}H-NMR spectra were determined on a Varian 200 instrument using deuteriochloroform as solvent. Chemical shifts are given in δ values downfield from tetramethylsilane as internal standard; coupling constants are given in Hertz. Mass spectra data were determined on a V6-Micromass 7070H mass spectrometer. Silica gel chromatography was performed using Merck silica gel 60 (0.015–0.040 mm); alumina chromatography was performed using Merck aluminium oxide 90 (0.063–0.200 mm). Petroleum ether refers to petroleum ether (40–60 °C).

*N-(1H-Imidazol-1-yl)-N-(naphthalen-1-ylmethylene)-amine (**12**)*

A solution of hydroxylamine-O-sulfonic acid (11.3 g, 100 mmol) in water (100 ml) was made neutral with sodium hydrogen carbonate and imidazole (13.6 g, 200 mmol) was added. After stirring for 20 h at room temperature, the mixture was acidified with 2N hydrochloric acid and then was evaporated under reduced pressure. To a stirring suspension of the residue in 150 ml of absolute ethanol, a solution of naphthalene-1-carbaldehyde (15.6 g, 100 mmol) in the same solvent (50 ml) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure and the residue was taken up with water (200 ml). The insoluble portion was filtered off and the filtrate was extracted with diethyl ether (2×100 ml). The organic phase was eliminated while the aqueous solution was rendered basic with sodium hydrogen carbonate and extracted with chloroform (3×100 ml). The combined extracts were dried with sodium sulfate, the chloroform was evaporated off, and the residue was crystallized from cyclohexane. Yield 38%, mp 87–88 °C. ^{1}H-NMR: 7.22 (s, 1H, 5-H imidazole), 7.51–8.07 (m, 8H, aromatic H, 4-H and 2-H imidazole), 8.73–8.82 (m, 1H, aromatic H), 9.08 (s, 1H, C*H*N). Anal. ($C_{14}H_{11}N_3$) C, H, N.– MS; *m/z* = 221 [M^+].

*Procedure for the Preparation of N-(Naphthalen-1-ylmethylene)-1,2,4-triazolamine **13** and **14***

Prepared from 1*H*-1,2,4-triazole (13.8 g, 200 mmol) following the procedure described above. After stirring for 24 h at room temperature, the solvent was evaporated under reduced pressure and the residue was taken up with water (200 ml). The reaction mixture was rendered basic with sodium hydrogen carbonate and extracted with chloroform (3×100 ml). The combined extracts were dried with sodium sulfate, the chloroform was evaporated, and the residue was separated by alumina column chromatography (chloroform-petroleum ether 1:1). First eluates were discarded, then compound **14** and subsequently compound **13** were recovered.

*N-(Naphthalen-1-ylmethylene)-4H-1,2,4-triazol-4-amine (**13**)*

Yield 13%, mp 143 °C (ethanol) (ref. [8] 142–143 °C). ^{1}H-NMR: 7.55–7.75 (m, 3H, aromatic H), 7.90–8.11 (m, 3H, aromatic H), 8.72 (s, 2H, H triazole), 8.75–8.85 (m, 1H, aromatic H), 9.17 (s, 1H, C*H*N). Anal. ($C_{13}H_{10}N_4$) C, H, N.– MS; *m/z* = 222 [M^+].

*N-(Naphthalen-1-ylmethylene)-1H-1,2,4-triazol-1-amine (**14**)*

Yield 13%, mp 89 °C (petroleum ether). ^{1}H-NMR: 7.54–7.74 (m, 3H, aromatic H), 7.90–8.17 (m, 4H, aromatic H and 5-H triazole), 8.49 (s, 1H, 3-H triazole), 8.77–8.86 (m, 1H, aromatic H), 9.84 (s, 1H, C*H*N). Anal. ($C_{13}H_{10}N_4$) C, H, N.– MS; *m/z* = 222 [M^+].

*General Procedure for the Preparation of Secondary Amines **9**–**11***

A cooled solution of appropriate Schiff base (50.0 mmol) in methanol (200 ml) was treated portionwise with an excess of sodium borohydride powder until the reaction was completed (TLC), then the solvent was removed under reduced pressure. The residue was taken up with water (200 ml) and extracted with chloroform. The organic phase was dried with sodium sulfate and the solvent was evaporated to give a solid which was recrystallized.

*N-(1H-Imidazol-1-yl)-N-(naphthalen-1-ylmethyl)-amine (**9**)*

Yield 99%, mp 102–103 °C (toluene-petroleum ether). ^{1}H-NMR: 4.70 (d, *J* = 5.0 Hz, 2H, C*H*$_2$), 5.05 (t, *J* = 5.0 Hz, 1H, exchanges with deuterium oxide, N*H*), 6.96 (s, 2H, 5-H and 4-H imidazole), 7.24–8.20 (m, 8H, aromatic H and 2-H imidazole). Anal. ($C_{14}H_{13}N_3$) C, H, N.– MS; *m/z* = 223 [M^+].

*N-(Naphthalen-1-ylmethyl)-4H-1,2,4-triazol-4-amine (**10**)*

Yield 99%, mp 173–175 °C (ethanol) (ref. [9] 171 °C). ^{1}H-NMR: 4.69 (d, *J* = 5.0 Hz, 2H, C*H*$_2$), 5.56 (t, *J* = 5.0 Hz, 1H, exchanges with deuterium oxide, N*H*), 7.16–7.67 (m, 4H, aromatic H), 7.80–7.95 (m, 2H, aromatic H), 7.99 (s, 2H, H triazole), 8.03–8.16 (m, 1H, aromatic H). Anal. ($C_{13}H_{12}N_4$) C, H, N.– MS; *m/z* = 224 [M^+].

*N-(Naphthalen-1-ylmethyl)-1H-1,2,4-triazol-1-amine (**11**)*

Yield 99%, mp 88–89 °C (cyclohexane) ^{1}H-NMR: 4.81 (d, *J* = 4.4 Hz, 2H, C*H*$_2$), 5.47 (t, *J* = 4.4 Hz, 1H, exchanges with deuterium oxide, N*H*), 7.23–7.97 (m, 8H, aromatic H, 5-H triazole and 3-H triazole), 8.20–8.30 (m, 1H, aromatic H). Anal. ($C_{13}H_{12}N_4$) C, H, N.– MS; *m/z* = 224 [M^+].

*General Procedure for the Preparation of Tertiary Amines **3** and **6***

Potassium *tert*-butoxide (2.24 g, 20.0 mmol) was added to a solution of 18-crown-6 (0.53 g, 2.00 mmol) in diethyl ether (50 ml), then *N*-(1*H*-imidazol-1-yl)-*N*-(naphthalen-1-ylmethyl)-amine (**9**) (4.47 g, 20.0 mmol) was added in a single portion under stirring. After stirring for 15 min at room temperature under nitrogen, a solution of the opportune bromide (20.0 mmol) in diethyl ether (30 ml) was added dropwise to the cooled reaction mixture over a period of 20 min. Stirring was continued for 4 h then water (100 ml) was added and the layers were separated. Aqueous phase was extracted with diethyl ether (3×50 ml) and the combined extracts were washed with a saturated sodium chloride solution, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by chromatography.

*N-(1H-Imidazol-1-yl)-N-(naphthalen-1-ylmethyl)-(3-phenylallyl)-amine (**3**)*

Chromatography: silica (chloroform). Yield 61%, oil. ^{1}H-NMR: 3.91 (d, *J* = 7.0 Hz, 2H, C*H*$_2$CH), 4.58 (s, 2H, C*H*$_2$Ar), 6.21 (dt, *J* = 15.8/7.0 Hz, 1H, CH$_2$C*H*), 6.52 (d, *J* = 15.8 Hz, 1H, C*H*Ar), 6.95 (s, 1H, 5-H imidazole), 7.13–7.89 (m, 13H, aromatic H, 4-H imidazole and 2-H imidazole), 8.22–8.29 (m, 1H, aromatic H). Anal. ($C_{23}H_{21}N_3$) C, H, N.– MS; *m/z* = 339 [M^+].

*N-(1H-Imidazol-1-yl)-N-(naphthalen-1-ylmethyl)-(4-tert-butylbenzyl)-amine (**6**)*

From *N*-(1*H*-imidazol-1-yl)-*N*-(naphthalen-1-ylmethyl)-amine (**9**), as reported in the General Procedure.

Chromatography: alumina (cyclohexane-chloroform 2:8). Yield 46%, mp 126 °C (benzene-petroleum ether). ^{1}H-NMR: 1.29 (s, 9H, C(CH_3)$_3$), 4.24 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 6.90 (s,1H, 5-H imidazole), 7.01 (s, 1H, 4-H imidazole), 7.10–8.14 (m, 12H, 2-H imidazole and aromatic H). Anal. ($C_{25}H_{27}N_3$) C, H, N.– MS; *m/z* = 369 [M^+].

From *N*-(1*H*-imidazol-1-yl)-*N*-(naphthalen-1-ylmethyl)-(4-*tert*-butyl)-benzamide (**15**)

To a solution of lithium borohydride in tetrahydrofuran (8.0 ml, 2 M solution, 16.0 mmol) was added a solution of chlorotrimethylsilane (3.48 g, 32.0 mmol) in anhydrous tetrahydrofuran (10 ml). After 2 min, a solution of *N*-(1H-imidazol-1-yl)-*N*-(naphthalen-1-ylmethyl)-4-*tert*-butyl)-benzamide (**15**) (1.53 g, 4.00 mmol) in anhydrous tetrahydrofuran (30 ml) was added dropwise over a period of 5 min and the reaction mixture was stirred for 24 h at room temperature. The reaction was treated cautiously with methanol (10 ml) and the solvent was evaporated under reduced pressure. The residue was taken up with chloroform and the resulting inorganic precipitate was filtered. The filtrate was then concentrated under reduced pressure and the residue was separated by alumina column chromatography (cyclohexane-chloroform 4:6). First eluates gave 4-*tert*-butylbenzyl alcohol, then compound **6** (yield 10%) and after desacylamine **9** were recovered.

*General Procedure for the Preparation of Tertiary Amines **4**, **5**, **7**, and **8***

Sodium hydride (0.96 g, 40.0 mmol) was added to a solution of the opportune triazole amine (4.49 g, 20.0 mmol) in anhydrous tetrahydrofuran (200 ml) under dry nitrogen at room temperature. The mixture was stirred for 24 h then a solution of the appropriate bromide (22.0 mmol) in anhydrous tetrahydrofuran (70 ml) was added dropwise over a period of 20 min. After stirring under nitrogen at room temperature for 24 h, methanol (15 ml) was added. The solvent was evaporated and the residue was taken up with water (100 ml) and extracted with chloroform (3×50 ml). The combined organic solution was washed with a saturated sodium chloride solution and dried with sodium sulfate. The solvent was removed by evaporation under reduced pressure and the residue was purified by chromatography.

*N-(Naphthalen-1-ylmethyl)-N-(3-phenylallyl)-4H-1,2,4-triazol-4-amine (**4**)*

Chromatography: alumina (cyclohexane-chloroform 1:1). Yield 79%, mp 131–132 °C (benzene-ligroin). ^{1}H-NMR: 3.95 (d, *J* = 6.9 Hz, 2H, CH_2CH), 4.66 (s, 2H, CH_2Ar), 6.24 (dt, *J* = 15.8/6.9 Hz, 1H, CH_2C*H*), 6.56 (d, *J* = 15.8 Hz, 1H, C*H*Ar), 7.10–7.38 (m, 7H, aromatic H), 7.49–7.65 (m, 2H, aromatic H), 7.78–7.91 (m, 2H, aromatic H), 8.10 (s, 2H, H triazole), 8.14–8.22 (m, 1H, aromatic H). Anal. ($C_{22}H_{20}N_4$) C, H, N.– MS; *m/z* = 340 [M^+].

*N-(Naphthalen-1-ylmethyl)-N-(3-phenylallyl)-1H-1,2,4-triazol-1-amine (**5**)*

Chromatography: alumina (cyclohexane-chloroform 7:3). Yield 71%, mp 77–78 °C (ligroin). ^{1}H-NMR: 4.08 (d, *J* = 6.9 Hz, 2H, CH_2CH), 4.70 (s, 2H, CH_2Ar), 6.21 (dt, *J* = 15.8/6.9 Hz, 1H, CH_2C*H*), 6.53 (d, *J* = 15.8 Hz, 1H, C*H*Ar), 7.08–7.38 (m, 8H, aromatic H and 5-H triazole), 7.46–7.64 (m, 2H, aromatic H), 7.70–7.90 (m, 3H, aromatic H and 3-H triazole); 8.20–8.28 (m, 1H, aromatic H). Anal. ($C_{22}H_{20}N_4$) C, H, N.– MS; *m/z* = 340 [M^+].

*N-(4-tert-Butylbenzyl)-N-(naphthalen-1-ylmethyl)-4H-1,2,4-triazol-4-amine (**7**)*

Chromatography: alumina (cyclohexane-chloroform 4:6). Yield 92%, mp 152 °C (toluene-cyclohexane). ^{1}H-NMR: 1.28 (s, 9H, C(CH_3)$_3$), 4.32 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 7.08–7.61 (m, 8H, aromatic H), 7.74–8.08 (m, 5H, aromatic H and H triazole). Anal. ($C_{24}H_{26}N_4$) C, H, N.– MS; *m/z* = 370 [M^+].

*N-(4-tert-Butylbenzyl)-N-(naphthalen-1-ylmethyl)-1H-1,2,4-triazol-4-amine (**8**)*

Chromatography: alumina (cyclohexane-chloroform 1:1). Yield 85%, mp 107 °C (petroleum ether). ^{1}H-NMR: 1.28 (s, 9H, C(CH_3)$_3$), 4.41 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 7.08–7.34 (m, 7H, aromatic H and 5-H triazole), 7.44–7.62 (m, 2H, aromatic H), 7.70 –7.90 (m, 3H, aromatic H and 3-H triazole), 8.08–8.18 (m, 1H, aromatic H). Anal. ($C_{24}H_{26}N_4$) C, H, N.– MS; *m/z* = 370 [M^+].

*General Procedure for the Preparation of Amides **15–17***

A solution of 4-*tert*-butyl-benzoyl chloride (2.16 g, 11.0 mmol) in anhydrous tetrahydrofuran (30 ml) was added to a solution of secondary amine (10.0 mmol) and triethylamine (1.11 g, 11.0 mmol) in the same solvent (60 ml), then the mixture was refluxed for 4 h. After cooling, the mixture was filtered, the filtrate was evaporated and the residue dissolved with chloroform (100 ml). The organic layer was washed with a saturated sodium chloride solution, dried with anhydrous sodium sulfate and concentrated. After purification by alumina chromatography (chloroform as eluent) the amides were recrystallized from suitable solvent.

*N-(1H-Imidazol-1-yl)-N-(naphthalen-1-ylmethyl)-(4-tert-butyl)-benzamide (**15**)*

Yield 97%, mp 197 °C (ethanol). ^{1}H-NMR: 1.21 (s, 9H, C(CH_3)$_3$); 5.54 (s, 2H, CH_2); 6.51 (s, 1H, 5-H imidazole); 6.74 (s, 1H, 4-H imidazole); 6.79 (s, 1H, 2-H imidazole); 7.06–8.22 (m, 11H, aromatic H). Anal. ($C_{25}H_{25}N_3O$) C, H, N.– MS; *m/z* = 383 [M^+].

*N-(Naphthalen-1-ylmethyl)-N-(4H-1,2,4-triazol-4-yl)-(4-tert-butyl)-benzamide (**16**)*

Yield 38%, mp 197 °C (ethanol). ^{1}H-NMR: 1.22 (s, 9H, C(CH_3)$_3$); 5.54 (s, 2H, CH_2); 7.02–8.12 (m, 13H, aromatic H and H triazole). Anal. ($C_{24}H_{24}N_4O$) C, H, N.– MS; *m/z* = 384 [M^+].

*N-(Naphthalen-1-ylmethyl)-N-(1H-1,2,4-triazol-4-yl)-(4-tert-butyl)-benzamide (**17**)*

Yield 77%, mp 145 °C (ethanol). ^{1}H-NMR: 1.20 (s, 9H, C(CH_3)$_3$); 5.60 (br. s, 2H, CH_2); 6.85 (s, 1H, 5-H triazole); 7.05–8.20 (m, 12H, aromatic H and 3-H triazole). Anal. ($C_{24}H_{24}N_4O$) C, H, N.– MS; *m/z* = 384 [M^+].

Microbiology

Compounds. Test compounds were dissolved in DMSO at an initial concentration of 200 mM and then were serially diluted in culture medium.

Cells. Cell line were from American Type Culture Collection (ATCC); bacterial and fungal strains were either clinical isolates (obtained from Clinica Dermosifilopatica, University of Cagliari) or collection strains from ATCC.

Antibacterial Assays. Staphylococcus aureus, group D *Streptococcus*, *Shighella*, and *Salmonella* spp. were recent clinical isolates. Tests were carried out in nutrient broth, pH 7.2, with an inoculum of 10^3 cells/tube. MICs were determined after 18 h incubation at 37 °C in the presence of serial dilutions of the test compounds.

Antimycotic Assays. Yeast blastospores were obtained from a 30 h old shaken culture incubated at 30 °C in Sabouraud dextrose broth. The dermatophyte inoculum was scraped aseptically with a spatula from a 7 day-old culture on agar and the macerate was finely suspended in Sabouraud dextrose broth using a glass homogenizer. Glicerol, final concentration 10%, was added as a cryoprotective agent to both yeast and dermatophyte suspension, aliquots of which were then stored in liquid nitrogen. Test tubes were inoculated with 10^3 blastospores or colony forming units (CFU)/tube. The minimal inhibitory concentration (MIC) was determined by serial dilutions using Sabouraud dextrose broth (pH 5.7) and incubating at 37 °C. The growth control for yeast was read after 1 day and for dermatophytes after 3 days (5 days for *Cryptococcus neoformans*). The MIC was defined as the compound concentration at which no macroscopic signs of fungal growth were detected. The minimal germicidal concentration (MGC) was determined by subcultivating negative test tubes in Sabouraud dextrose agar.

The cytotoxicity evaluation of compounds was based on the viability of mock-infected cells, as monitored by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [10].

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