

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/235603349>

Synthesis and anti-Candida activity of novel 2-hydrazino-1,3-thiazole derivatives

ARTICLE in BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · MARCH 2013

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2013.01.039 · Source: PubMed

CITATIONS

11

READS

65

9 AUTHORS, INCLUDING:



Sebastien Bertout

Université de Montpellier

39 PUBLICATIONS 337 CITATIONS

SEE PROFILE



Fatih Demirci

Anadolu University

155 PUBLICATIONS 1,466 CITATIONS

SEE PROFILE



Jean Martinez

Université de Montpellier

956 PUBLICATIONS 12,116 CITATIONS

SEE PROFILE



Nicolas Masurier

Université de Montpellier

28 PUBLICATIONS 194 CITATIONS

SEE PROFILE



Synthesis and anti-*Candida* activity of novel 2-hydrazino-1,3-thiazole derivatives

Ludovic T. Maillard^{a,*}, Sébastien Bertout^b, Ophélie Quinonéro^a, Gülşen Akalin^d, Gülhan Turan-Zitouni^c, Pierre Fulcrand^a, Fatih Demirci^e, Jean Martinez^a, Nicolas Masurier^a

^a Institut des Biomolécules Max Mousseron, UMR 5247, CNRS, Universités Montpellier I et II, UFR des Sciences Pharmaceutiques et Biologiques, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France

^b UMI 233 IRD-UMI-UY1-UCAD 'TransVIHMI' Equipe Infections parasitaires et fongiques liées au VIH UFR Sciences Pharmaceutiques et Biologiques, 15 Avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France

^c Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

^d Department of Biochemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

^e Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey

ARTICLE INFO

Article history:

Received 30 October 2012

Revised 8 January 2013

Accepted 12 January 2013

Available online 23 January 2013

Keywords:

Thiazole

Hydrazone

Candida spp.

Antifungal activity

ABSTRACT

Eighteen new hydrazino-1,3-thiazole derivatives were evaluated against 8 strains of multi-resistant *Candida* spp. Introduction of an indolyl moiety linked to the hydrazone function enhanced the in vitro anti-*Candida* activity, with an activity spectrum towards *Candida albicans* strains. Introduction of a (S)-2-aminoethyl chain on the thiazole nucleus largely enhanced the in vitro antifungal activity, with a selectivity oriented towards non-*C. albicans* species.

© 2013 Elsevier Ltd. All rights reserved.

Candida albicans is a diploid opportunistic fungal pathogen present in the gastrointestinal and genitourinary flora of most healthy humans and other mammals. Among opportunistic fungal pathogens, *C. albicans* is the main causative agent of mucosal and deep-seated mycoses. However, over the last two decades it was gradually replaced by other *Candida* species in mucosal and systemic infections.¹ One of the principal problems in the treatment of *Candida* infections is the spread of antifungal drug resistance mainly in patients chronically subjected to antimycotic therapy and more especially in severely immuno-compromised subjects, cancer, transplants as well as surgery patients. The frequency of invasive fungal infections as well as the antifungal resistance dramatically increase despite the introduction of new drugs, like new azole or echinocandin derivatives.^{2,3} In particular, a significant number of *Candida* species (i.e., *Candida glabrata* and *Candida krusei*) that exhibit high minimum inhibitory resistance (MIC) to azole-based agents can acquire echinocandin resistance.^{4,5} As consequences, new drugs are urgently needed to enhance the therapeutic arsenal to overcome the rapid development of multi-resistant strains.

In ongoing work to identify new antifungal derivatives, we have preliminary screened a library of heterocyclic compounds toward a

set of multi-resistant *Candida* strains (data not shown). Among the tested compounds, the substituted 2-hydrazino-1,3-thiazole **1a** has shown an inhibitory activity (MIC of 16 µg/mL) against one resistant strain of *Candida krusei* (Fig. 1 and Table 1). Few other 2-hydrazino-1,3-thiazole analogues that exhibit significant antimicrobial activity have been recently reported in the literature.^{6–9} Those bearing a bicyclic or heterocyclic ring on the hydrazone function and a phenyl at C-4 position of the thiazole nucleus are particularly active and present micromolar or submicromolar MIC against several *Candida* spp. strains.^{6–8} Thus, such template could be seen as a starting point for further optimization of novel antifungal agents.

In order to develop new potent anti-fungal compounds, a new set of 2-hydrazino-1,3-thiazoles based on compound **1a** was synthesized and their anti-*Candida* activity were studied. Two series of 2-hydrazino-1,3-thiazoles were designed. In the first series (series A, Fig. 1), the disubstituted phenyl ring was preserved and the indane moiety on the hydrazino-part was replaced by unsaturated and saturated carbocycles (cyclohexyl and naphthyl), oxo-heterocycles (furyl and benzofuryl) and aza-heterocycles (pyrrolyl, imidazolyl, indolyl, imidazo[1,2-*a*]pyridinyl and imidazo[2,1-*b*]thiazolyl). The choice of such aza-heterocycles was due to our particular interest in this field.¹² In series B, substituted

* Corresponding author. Tel.: +33 4 11 75 96 04; fax: +33 4 11 75 96 41.

E-mail address: ludovic.maillard@univ-montp1.fr (L.T. Maillard).

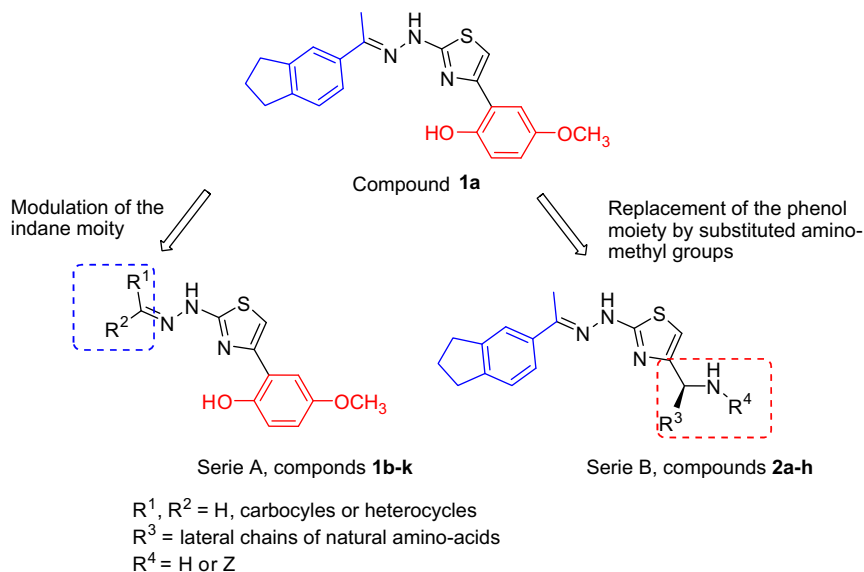


Figure 1. Structures of lead compound **1a** and new synthesized compounds.

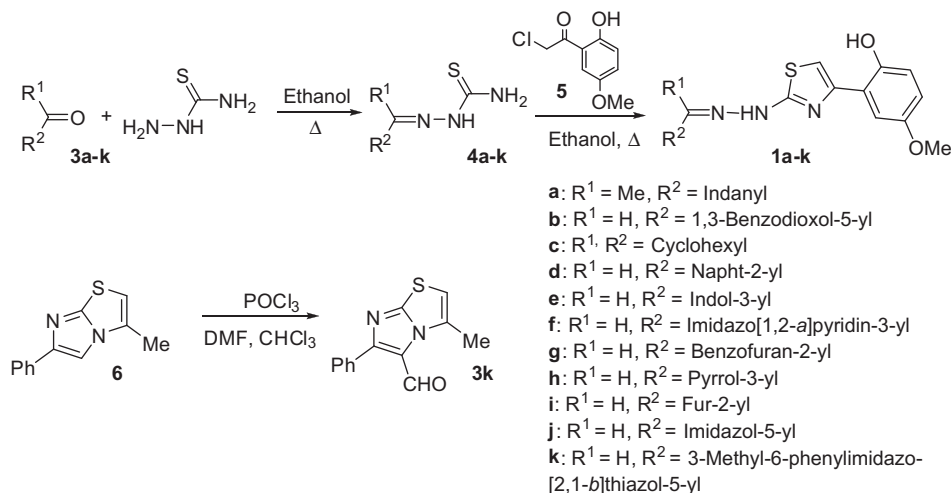
aminomethyl groups were introduced instead of the phenol part to increase the solubility of indanyl-thiazolyhydrazones.

The 2-hydrazino-1,3-thiazole scaffold was easily accessed by Hantzsch cyclization between appropriate thiosemicarbazones and α -chloromethylketones.^{6–8,10,11} To synthesize series A, appropriate carbonyl compounds **3a-k** were reacted with thiosemicarbazide in refluxing ethanol. Compounds **3a-j** were commercially available, while compound **3k** was prepared by Vilsmeier–Hack formylation of 3-methyl-6-phenylimidazo[2,1-*b*][1,3]thiazole **6**, according to the method of Andreani (Scheme 1).¹³ The selectivity of the formylation was attested by ¹H NMR analysis: disappearance of the 7.57 ppm singlet signal of H-5 of compound **6** and presence of a singlet at 6.50 ppm of H-2 of the thiazole nucleus. Finally, the thiosemicarbazones **4a-k** were subsequently condensed with the 2-chloro-1-(2-hydroxy-5-methoxyphenyl)-ethanone **5**¹⁴ to offer thiazolyhydrazones **1a-k**, which were isolated in 37–92% yields (Scheme 1).

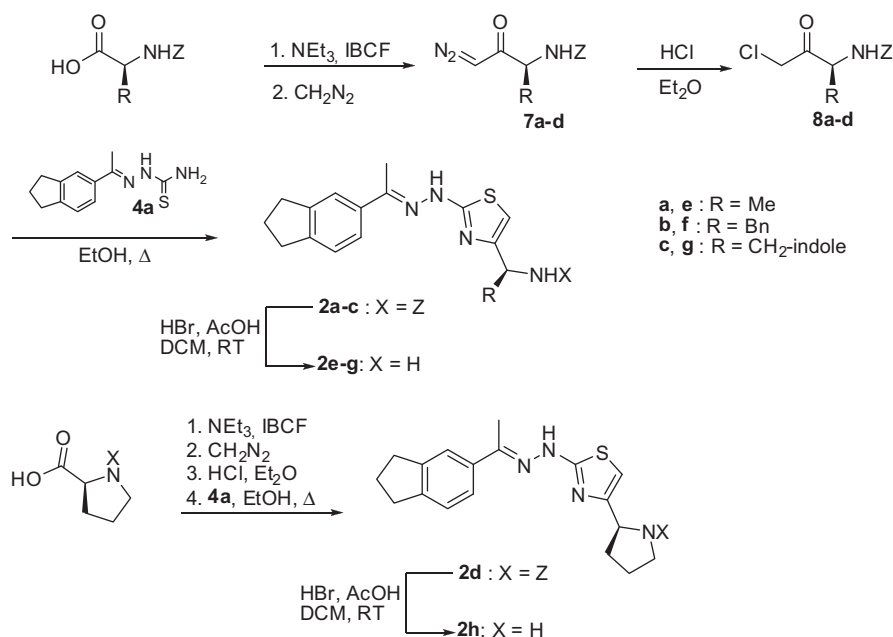
For series B, α -chloromethylketones **8a-d** were synthesized from natural *N*-benzyloxycarbonyl (Z) protected amino-acids, that were, alanine, phenylalanine, tryptophan or proline (Scheme 2), according to literature procedures.^{15–17} Briefly,

diazoketones **7a-d** were obtained by carboxylic acid activation with isobutyl chloroformate (IBCF) in the presence of triethylamine at 0 °C followed by subsequent diazomethane treatment. Compounds **7a-d** conversion into the corresponding α -chloromethylketones **8a-d** was performed by treatment with HCl in diethyl ether. Thereafter, **8a-d** were successively condensed with thiosemicarbazone **4a** to access 2-hydrazino-1,3-thiazoles **2a-d** in 53–81% yields. Final deprotection of the benzyloxycarbonyl group was initially envisioned by hydrogenolysis with activated palladium on barium sulfate. However, under these conditions, deprotection failed. Finally, treatment of compounds **2a-d** with 33% HBr in acetic acid and subsequent treatment by HCl in diethyl ether offered the corresponding amino derivatives **2e-h** as hydrochloride salts. Compounds **2e, f, h** were isolated in good yields (67–93%) while **2g** was obtained in only 20% yield. For this reason, this compound was not evaluated for its antifungal activity. As expected the introduction of a basic nitrogen on the scaffold enhanced the water solubility of compounds **2e-h** as compared to **1a** (Table 1).

All synthesized compounds were fully characterized by analytical and spectral data. Hydrazones **1a-k** and **2a-h** could exist as



Scheme 1. Synthesis of compounds **1a-k** (series A).



Scheme 2. Synthesis of compounds 2a–h (series B).

couples of diastereoisomers *E/Z*. However, in each case only one isomer was detected by HPLC, ¹H and ¹³C NMR analysis. Configuration of 1a–k and 2a–h could not be unambiguously assigned by

NMR studies. However analytical studies of the *E/Z* hydrazine interconversion reported in the literature indicates that the (*E*)-form dominates on (*Z*)-form.¹⁸

Table 1

Minimal inhibitory concentration (MIC) of compounds 1a–k, 2a–f and 2h, amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin against eight strains of *Candida* spp.

Compound	clogD ^a	Maximum aqueous solubility ^b	Tested fungi (MIC values µg/mL)							
			<i>C. albicans</i>		<i>C. glabrata</i>		<i>C. krusei</i>		<i>C. parapsilosis</i>	
			5.O.A. ^c	7.O.A. ^d	R ^e	724 ^f	730 ^g	6258 ^h	ANF8 ⁱ	22019 ^j
1a	4.29	<0.05 mM	>16	>16	>16	>16	16	>16	>16	>16
1b	2.45	ND	>16	>16	>16	>16	>16	>16	>16	>16
1c	2.91	ND	>16	>16	>16	>16	8	16	>16	16
1d	4.38	ND	>16	2	>16	>16	>16	>16	>16	>16
1e	3.77	ND	0.25	0.5	2	>16	0.5	>16	0.25	>16
1f	3.65	ND	>16	>16	>16	>16	>16	>16	>16	>16
1g	3.72	ND	>16	>16	>16	>16	>16	>16	>16	>16
1h	2.03	ND	16	>16	16	8	16	>16	>16	16
1i	2.77	ND	>16	>16	>16	>16	>16	>16	>16	>16
1j	1.53	ND	>16	>16	>16	>16	>16	>16	>16	>16
1k	5.19	ND	>16	>16	>16	>16	2	>16	4	>16
2a	4.79	ND	>16	>16	>16	>16	>16	>16	>16	>16
2b	5.89	ND	>16	>16	>16	>16	>16	>16	>16	>16
2c	5.96	ND	>16	>16	>16	>16	8	>16	>16	8
2d	5.57	ND	>16	>16	>16	>16	>16	>16	>16	>16
2e	2.64	0.7 mM	>16	>16	16	8	8	0.125	0.5	8
2f	4.29	0.15 mM	>16	>16	>16	>16	>16	>16	>16	>16
2h	3.11	0.9 mM	>16	>16	>16	>16	16	>16	>16	>16
Amphotericin B	—	—	0.5	0.5	0.5	0.5	1	1	0.5	1
Fluconazole	—	—	32	>64	0.125	64	64	16	1	64
Itraconazole	—	—	0.5	>16	0.03	2	1	0.5	0.03	0.5
Voriconazole	—	—	0.25	>16	0.25	8	16	2	0.06	0.25
Caspofungin	—	—	0.5	0.25	0.5	0.5	2	2	8	0.5

^aCalculated logD (pH 7.4) of compounds 1 and 2 (data obtained from ACD/Labs, ACD/cLogD, Advanced Chemistry Development, Inc., Toronto, Canada, 2010).

^bDetermined in PBS buffer at pH 7.0.

^cClinical fluconazole resistant *C. albicans* isolate.

^dClinical azoles resistant *C. albicans* isolate.

^e*C. albicans* caspofungin resistant laboratory strain.

^fClinical voriconazole resistant *C. glabrata* isolate.

^gClinical voriconazole resistant isolate.

^h*C. glabrata* fluconazole resistant reference strain.

ⁱ*C. glabrata* laboratory strain of *C. parapsilosis*, anidulafungin resistant.

^jReference strain of *C. parapsilosis* sensible to reference drugs.

Table 2
Cytotoxicity of selected compounds to mouse fibroblast (NIH/3T3) cell line

	Concentration ($\mu\text{g/mL}$) ^a			
	0.5	1	31.3	62.5
1d	83.7 \pm 1.3	69.2 \pm 0.2	45.3 \pm 2.4	52.7 \pm 3.9
1e	91.9 \pm 10.7	80.6 \pm 8.2	70.6 \pm 3.2	50.2 \pm 4.6
1k	94.6 \pm 6.3	90.0 \pm 0.1	64.1 \pm 2.1	59.5 \pm 2.9
2e	93.6 \pm 4.0	92.6 \pm 1.8	36.2 \pm 4.1	32.4 \pm 1.2

^a Values represent mean \pm standard deviation of triplicate determinations.

Compounds **1a–k**, **2a–f** and **2h** were evaluated for their anti-fungal activity against 8 *Candida* spp. strains (*C. albicans*, *C. glabrata*, *C. krusei* and *Candida parapsilosis*) and compared with five reference drugs, that were, amphotericin B, itraconazole, fluconazole, voriconazole and caspofungin (Table 1). Several resistant strains to one or several reference drugs (see Table 1) were chosen to evaluate the potential activity of synthesized compounds against multi-resistant *Candida* spp. Prior testing, each *Candida* spp. isolate was subcultured on a qualified medium to ensure purity and optimal growth. The susceptibility assays were determined by the microbroth dilution method performed in sterile flat-bottom 96-well microplates (Difco Laboratories, Detroit, USA) as described in CLSI guidelines, M27-A3 document.¹⁹ Compounds **1d**, **1e**, **1k** and **2e** that achieved the strongest anti-*Candida* activity were also analyzed to estimate their cytotoxic effects (Table 2). Cytotoxicity was evaluated using the MTT cell proliferation/viability assay on mouse 3T3/NIH fibroblast cells, which were originally obtained from the American Type Culture Collection (ATCC, USA). The level of cellular MTT reduction was quantified as previously described in literature with small modifications.^{20–22}

In an initial screening program to determine potential anti-*Candida* activities of 2-hydrazino-1,3-thiazoles, compound **1a** was chosen as the first hit for further derivatization (MIC of 16 $\mu\text{g/mL}$ against a fluconazole/voriconazole resistant *C. krusei* strain). Replacement of the indanyl nucleus by various aliphatic or aromatic carbocycles or heterocycles was initially studied (series A compounds). Surprisingly when the indanyl nucleus was replaced by the structurally related 1,3-benzodioxol-5-yl moiety (compound **1b**, Scheme 1, Table 1), the anti-*Candida* activity was lost. Introduction of a cyclohexyl or a naphthyl ring was previously used to design 2-hydrazino-1,3-thiazoles with good anti-*Candida* activities.⁷ In our case, insertion of a cyclohexyl (compounds **1c**) or a naphthyl ring (compound **1d**) led to only a weak activity enhancement. These disappointing results led us to switch to other modulations consisting in the introduction of various aza-heterocycles. The best activities were obtained when the indanyl substituent was replaced by an indole ring: compound **1e** presented a good anti-*Candida* activity, with a selectivity oriented towards *C. albicans*. In particular, this compound showed similar or better activity than reference drugs for several *Candida* strains. We assumed that the biological activity was enhanced by the presence of a NH hydrogen bond donor in the carbocyclic part. This hypothesis was confirmed when we introduced an imidazo-[1,2-*a*]pyridinyl substituent (compound **1f**), which is usually viewed as an aza-indolyl isostere.^{23,24} Imidazo[1,2-*a*]pyridine does not have any hydrogen bond donating group making **1f** inactive toward all *Candida* strains. That was also the case when indole was replaced by benzofurane (compound **1g**). Similar results were obtained with series containing a five-membered heteroaromatic substituent: the pyrrole derivative **1h** was more active than its furyl counterpart **1i**. Surprisingly, although imidazole had a hydrogen bond donor, compound **1j** was totally inactive whatever the strain considered. This might be due to a higher hydrophilicity of the molecule ($\text{clogD} = 1.53$) compared to compound **1h** ($\text{clogD} = 2.03$). The

hydrophobicity balance was already proved to be important in such series to obtain anti-*Candida* activity.⁸ Finally, some activity was recovered for the substituted imidazo[2,1-*b*]thiazolyl derivative (compound **1k**). This compound showed a relative high clogD (5.19), which suggested the partial influence of this parameter to obtain activity in this series.

In the second series (series B, compounds **2a–h**, Scheme 2, Table 2), we replaced the phenol moiety at C-4 of the thiazole ring in **1a** by different aminoalkyl substituents. When the amino group was protected with a Z group, no significant anti-*Candida* activity was detected (compounds **2a–d**). Among the unprotected derivatives (compounds **2e–f** and **2h**), compound **2e** showed an interesting activity against several *Candida* spp., with a selectivity oriented towards non-*Candida albicans* species. In particular, a high activity was detected against the *C. krusei* 6258 strain, which presents a multi-resistant profile against several reference drugs. To determine the selectivity of 2-hydrazino-1,3-thiazoles towards fungi, the most active compounds **1d**, **1e**, **1k** and **2e** were finally evaluated for their cytotoxic activity against mouse fibroblast cells. The cytotoxic profile (Table 2) showed a weak toxicity at 0.5 $\mu\text{g/mL}$ for all tested compounds, with more than 83% of cell viability.

In conclusion, we have synthesized a series of hydrazino-1,3-thiazoles based on compound **1a**. Their in vitro anti-*Candida* activity was evaluated against 8 strains of multi-resistant *Candida* spp. Preliminary structure activity relationships have shown that substitution of the hydrazine by a hydrogen bond donating heterocycle enhanced the anti-*Candida* activity. In particular, introduction of an indolyl substituent instead of the indanyl led to interesting compounds, especially active against *C. albicans* strains. Moreover, replacement of the phenol moiety in compound **1a** by a (S)-2-aminoethyl chain (compound **2e**) largely enhanced the antifungal activity, with a selectivity oriented towards non *Candida albicans* species. Based on these results, derivatives **1e** and **2e** represent good starting points for the development of novel anti-*Candida* spp. agents. Synthesis of next generation compounds combining modifications on both sides of the scaffold will be reported in due course.

Acknowledgments

The authors are grateful to G. Tambutet (Université Montpellier 2, France), C. Lamoureux and M. Aussedat (Faculté de Pharmacie de Montpellier, France) for technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2013.01.039>.

References and notes

- Rodloff, C.; Koch, D.; Schaumann, R. *Eur. J. Med. Res.* **2011**, *16*, 187.
- Pfaller, M. A. *Am. J. Med.* **2012**, *125*, S3.
- Mathew, B. P.; Nath, M. *ChemMedChem* **2009**, *4*, 310.
- Munro, C. A. *Expert Rev. Anti-Infect.* **2012**, *10*, 115.
- Perlin, D. S. *Drug Resist. Updat.* **2007**, *10*, 121.
- Chimenti, F.; Bizzarri, B.; Bolasco, A.; Secci, D.; Chimenti, P.; Granese, A.; Carradori, S.; D'Ascenzio, M.; Lilli, D.; Rivanera, D. *Eur. J. Med. Chem.* **2011**, *46*, 378.
- Chimenti, F.; Bizzarri, B.; Maccioni, E.; Secci, D.; Bolasco, A.; Fioravanti, A.; Chimenti, P.; Granese, A.; Carradori, S.; Rivanera, D.; Lilli, D.; Zicari, A.; Distinto, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4635.
- Secci, D.; Bizzarri, B.; Bolasco, A.; Carradori, S.; D'Ascenzio, M.; Rivanera, D.; Mari, E.; Polletta, L.; Zicari, A. *Eur. J. Med. Chem.* **2012**, *53*, 246.
- De Logu, A.; Saddi, M.; Cardia, M. C.; Borgna, R.; Sanna, C.; Saddi, B.; Maccioni, E. *J. Antimicrob. Chemother.* **2005**, *55*, 692.
- Turan-Zitouni, G.; Ozdemir, A.; Kaplancikli, Z. A.; Benkli, K.; Chevallet, P.; Akalin, G. *Eur. J. Med. Chem.* **2008**, *43*, 981.

11. Ozdemir, A.; Turan-Zitouni, G.; Kaplancikli, Z. A.; Demirci, F.; Iscan, G. *J. Enzyme Inhib. Med. Chem.* **2008**, *23*, 470.
12. Chaubet, G.; Maillard, L. T.; Martinez, J.; Masurier, N. *Tetrahedron* **2011**, *67*, 4897.
13. Andreani, A.; Rambaldi, M.; Mascellani, G.; Rugarli, P. *Eur. J. Med. Chem.* **1987**, *22*, 19.
14. Toyoda, T.; Sasakura, K.; Sugawara, T. *J. Org. Chem.* **1981**, *46*, 189.
15. Garcia-Lopez, M. T.; Gonzalez-Mufliz, R.; Harto, J. R. *Tetrahedron* **1988**, *44*, 5131.
16. Albeck, A.; Persky, R. *Tetrahedron* **1994**, *50*, 6333.
17. Fittkau, S.; Jahreis, G.; Peters, K.; Balaspiri, L. *J. Prakt. Chem.* **1986**, *328*, 529.
18. Cirilli, R.; Ferretti, R.; La Torre, F.; Secci, D.; Bolasco, A.; Carradori, S.; Pierini, M. *J. Chromatogr., A* **2007**, *1172*, 160.
19. *Determination of antifungal activity.* Isolates were inoculated at 35 °C and observed at 24 and 48 h. Five colonies greater than 1 mm in diameter were selected, suspended in saline solution and adjusted to a final concentration of 0.5×10^3 to 2.5×10^3 in RPMI 1640 medium equivalent to a 0.5 McFarland standard solution buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid. Synthesized agents were used versus standard antifungal agents. Five antifungal agents were used in the susceptibility tests. Tested compounds **1a–k**, **2a–f** and **2h**, itraconazole (ITZ), Voriconazole (VCZ) and Amphotericin B were dissolved in dimethylsulfoxide (DMSO). Fluconazole (FCZ) and Caspofungin (CAS) were dissolved in sterile distilled water. The drugs were prepared at the following concentrations: 1280 µg/mL for FCZ and 1600 µL/mL for all other compounds. The solutions were diluted in RPMI medium and final drugs concentrations ranged from 16 to 0.03 µg/mL for tested compounds, ITZ, VCZ and AMB; from 64 to 0.125 µg/mL for FCZ and from 8 to 0.015 µg/mL for CAS. After 24 h and 48 h of incubation at 35 °C, MIC (minimum inhibitory concentration) were determined visually by comparing its turbidity with the drug-free growth control well. The MIC values were defined as lower drug concentration which resulted in reduction of 100% in the turbidity in comparison with the drug-free growth control well.
20. Keiser, K.; Johnson, C. C.; Tipton, D. A. *J. Endod.* **2000**, *26*, 288.
21. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
22. *MTT cytotoxicity assay.* NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Gibco, Paisley, Scotland), 100 IU/mL penicillin (Gibco) and 100 mg/mL streptomycin (Gibco) at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the tested substances (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in DMSO and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability). The tested compounds were added to give final concentration in the range 0.5–1000 µg/mL and the cells were incubated for 24 h. Thereafter, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37 °C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 µL DMSO to each well and absorbance was read at 540 nm (Bio-Tek plate reader). Every concentration was repeated in three wells and IC₅₀ values were defined as the drug concentrations that reduced absorbance to 50% of control values. MTT cytotoxicity assay was repeated twice.
23. Masurier, N.; Debiton, E.; Jacquemet, A.; Bussière, A.; Chezal, J. M.; Ollivier, A.; Tetegan, D.; Andaloussi, M.; Galmier, M. J.; Lacroix, J.; Canitrot, D.; Teulade, J. C.; Gaudreault, R. C.; Chavignon, O.; Moreau, E. *Eur. J. Med. Chem.* **2012**, *52*, 137.
24. Andaloussi, M.; Moreau, E.; Masurier, N.; Lacroix, J.; Gaudreault, R. C.; Chezal, J. M.; El Laghdach, A.; Canitrot, D.; Debiton, E.; Teulade, J. C.; Chavignon, O. *Eur. J. Med. Chem.* **2008**, *43*, 2505.