Bioorganic & Medicinal Chemistry Letters 20 (2010) 1504–1506

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Antiproliferative activity of novel benzo[*b*][1,6]naphthyridines in human solid tumor cell lines

Simonas Rudys [a], Carla Ríos-Luci [b], Eduardo Pérez-Roth [b], Inga Cikotiene [a,*], José M. Padrón [b,c,*]

[a] *Department of Organic Chemistry, Faculty of Chemistry, Vilnius University, Naugarduko 24, Vilnius LT-03225, Lithuania*
[b] *BioLab, Instituto Universitario de Bio-Orgánica 'Antonio González' (IUBO-AG), Universidad de La Laguna, C/Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*
[c] *Instituto Canario de Investigación del Cáncer (ICIC), C/Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain*

ARTICLE INFO

Article history:
Received 21 October 2009
Revised 18 January 2010
Accepted 20 January 2010
Available online 25 January 2010

Keywords:
Multicomponent reaction
Benzob[1,6]naphthyridines
1*H*-Pyrano[4,3-*b*]quinolines
Antitumor drugs
Antibacterial drugs
Antifungal drugs

ABSTRACT

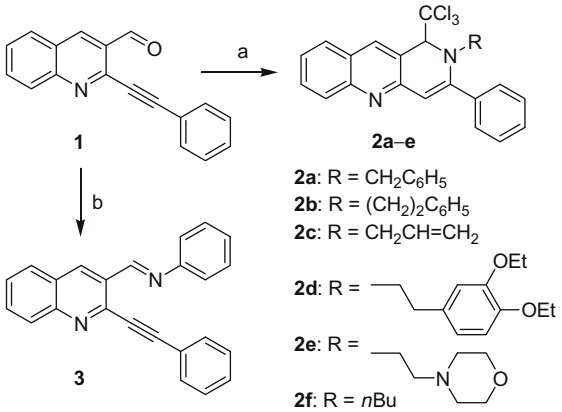
A series of 2-substituted 1,2-dihydro-3-phenyl-1-(trichloromethyl)benzo[*b*][1,6]naphthyridines were synthesized and their in vitro antiproliferative activities were examined against human solid tumor cell lines and relevant strains of bacteria and *Candida*. The compounds induced considerably growth inhibition in all cancer cell lines, whilst showed inactive against microbial strains. Furthermore, we found analog 2-ethoxy-1*H*-pyrano[4,3-*b*]quinoline as selective inhibitor of microbial strains.

Different families of nitrogen-containing heterocycles are currently used in cancer chemotherapy. For instance, the quinazolines[1] gefitinib, erlotinib, and canertinib are EGFR tyrosine kinase inhibitors indicated for the treatment of colorectal, lung, breast, pancreatic, renal, head and neck, gynecologic, and prostate cancer. Quinolines[2] and naphthyridines[3]—structural analogues of quinazolines—are also being explored for cancer chemotherapy with a number of compounds (EKB-569, HKI-272, and SNS-595) in different phases of clinical trials.

Within our program directed at the discovery of bioactive substances for cancer treatment, we have paid attention to nitrogen-containing heterocycles.[4] Recently, several reports dealing with three-component reactions between *o*-alkynylbenzaldehydes, amines, and different pronucleophiles have been reported.[5] As part of our research interests on the use of arylethynylazines in the synthesis of fused nitrogen-nucleus containing heterocycles,[6] we have focused our attention in the three-component reaction between 2-phenylethynyl-quinoline-3-carbaldehyde (**1**), primary aliphatic amines, and chloroform.

Herein, we wish to report on an three-component reaction for the formation of 2-substituted 1,2-dihydro-3-phenyl-1-(trichloromethyl)benzo[*b*][1,6]naphthyridi-nes (**2a**–**e**). The biological activity against human solid tumor cell lines together with the antimicrobial activity in bacterial and fungal strains is also reported.

Direct heating of aldehyde **1** with 1.2 equiv of the corresponding amine in chloroform and in the presence of 3 Å MS allowed us obtaining the target compounds **2a**–**f** in 39–56% yield (Scheme 1).[7,8] It should be pointed out that the synthesis is carried out without the addition of catalysts. The method tolerates well aliphatic amines.



Scheme 1. Reagents and conditions: (a) RCH_2NH_2, $CHCl_3$, reflux, 24–48 h, 39–56%; (b) $C_6H_5NH_2$, $CHCl_3$, reflux, 2 h, 98%.

* Corresponding authors. Tel.: +34 922316502x6126; fax: +34 922318571 (J.M.P.).
E-mail addresses: inga.cikotiene@chf.vu.lt (I. Cikotiene), jmpadron@ull.es (J.M. Padrón).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.01.112

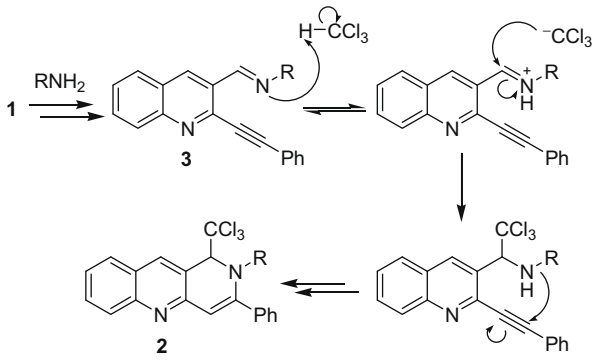


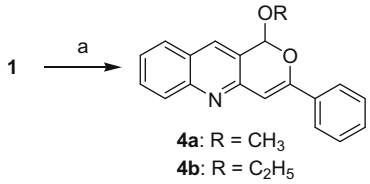
Fig. 1. Proposed mechanism of the three-component reaction.

However, when we used the aromatic amine aniline the cyclization reaction was unsuccessful and the formation of the stable Schiff base **3** as a sole reaction product was observed.

We speculate that the addition of chloroform might start with the protonation of the imine **3** by the chloroform (or even traces of HCl in the solvent). Then, addition of the carbanion followed by attack of the nitrogen on the alkyne function would lead to **2**. The proposed mechanism (Fig. 1) is consistent with the observed failure of aniline. Aromatic amines are weak bases compared with alkyl amines, which facilitates their protonation by the weak acid chloroform.

In order to study the influence of the newly introduced amine fragment on the biological activity, analogues of general structure 1-alkoxy-3-phenyl-1*H*-pyrano[4,3-*b*]quinolines were envisioned. Therefore, compounds **4a**–**b** were synthesized according to literature procedures[9,10] by means of a tandem acetalization–cyclization reaction of **1** with primary alcohols (Scheme 2). Thus, compound **1** heated in methanol or ethanol in the presence of 5 mol % of silver nitrate leads to 1-alkoxy-3-phenyl-1*H*-pyrano[4,3-*b*]quinolines (**4a**–**b**) in high yields. The process is highly regioselective leading to the formation of the sole 6-*endo*-dig cyclization product.[11] This regioselectivity is in agreement with analogous reactions reported in the literature and with our recent investigations.[12]

The biological activity of compounds **2a**–**f** and **4a**–**b** was studied in three whole-cell screening models: a representative panel



Scheme 2. Reagents and conditions: (a) $AgNO_3$ (5 mol %), ROH, reflux, 88% for **4a**, 83% for **4b**.

of human solid tumor cell lines and a set of clinically relevant microbial species. The selected microbes included 10 strains of Gram-positive and Gram-negative bacteria, and five yeasts (*Candida* spp.).

As a model for the anticancer activity we used the human solid tumor cell lines HeLa (cervix), Ishikawa (endometrial), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The in vitro antiproliferative activity was evaluated after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[13] The results expressed as GI_{50} (50% growth inhibition)[14] values are shown in Table 1.

Overall, the data shows that compounds **2a**–**f** are active against all cell lines with GI_{50} values in the range 3.1–71 μM. The most active product of the series was **2f**, with selectivity for HeLa, Ishikawa and SW1573 cells. The lung cancer cell line was the most sensitive to the compounds, followed by HeLa cells. In this particular context, the presence of an aryl group on the side chain (**2a**–**b**, **2d**) favors the antiproliferative activity in SW1573 cells. On the other side, the breast and the colon cancer cell lines were less affected by the exposure to the products. In contrast, compounds **4a**–**b** were inactive against the cell lines.

Compounds were screened against strains of the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, the Gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and against five *Candida* strains, namely *Candida albicans*, *Candida glabrata*, *Candida nivariensis*, *Candida krusei*, and *Candida parapsilosis*.[15] All benzo[*b*][1,6]naphthyridines **2a**–**f** were inactive against the tested microbial strains. Better results were obtained for 2-alkoxy-1*H*-pyrano[4,3-*b*]quinolines **4a**–**b** and are given in Table 2. In particular, compound **4a** was active only against one strain of *S. aureus* (29 μM). More interestingly, compound **4b** was active against all microbial strains, with IC_{50} values in the range 4.5–63 μM. The more resistant strains were *Klebsiella*, whilst the most sensitive to **4b** was the fungus *C. krusei* (4.5 μM). Moreover, analog **4b** was able to inhibit the growth of EMRSA-16, an epidemic methicillin resistant *S. aureus* responsible for a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia.[16] It is noteworthy that the substitution of a methyl (**4a**) for an ethyl group (**4b**) produces such a change in the activity profile.

Even though the results are preliminary, we found a clear trend in the structure activity relationship. On the one hand, benzo[*b*][1,6]naphthyridines **2a–f** represent molecular scaffolds with antiproliferative activity against human tumor cells. On the other, the corresponding 1-alkoxy-1*H*-pyrano[4,3-*b*]quinolines **4a**–**b** are candidates for further investigating antimicrobial activity.

In summary, we have developed a novel and fast synthetic method for preparing 2-substituted 1,2-dihydro-3-phenyl-1-(trichloromethyl)benzo[*b*][1,6]-naphthyridines by means of a three-component reaction between 2-phenylethynylquinoline-3-carbaldehyde, primary aliphatic amines and chloroform. Our synthesized

Table 1
In vitro antiproliferative activity against human solid tumor cells[a]

Compd	Cell line (type)				
	HeLa (cervix)	Ishikawa (endometrial)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
2a	27 (±9.8)	15 (±7.4)	5.1 (±1.4)	71 (±5.8)	46 (±4.7)
2b	38 (±5.2)	25 (±3.3)	5.5 (±1.4)	60 (±13)	57 (±8.9)
2c	32 (±8.9)	31 (±3.6)	46 (±8.2)	48 (±5.9)	42 (±3.4)
2d	5.6 (±2.4)	4.1 (±1.0)	4.3 (±2.3)	54 (±9.4)	37 (±3.0)
2e	6.5 (±0.1)	17 (±0.6)	31 (±5.4)	26 (±3.9)	20 (±0.2)
2f	6.1 (±0.9)	3.1 (±0.4)	6.5 (±0.6)	15 (±0.7)	16 (±2.0)
4a	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100

[a] Values expressed as GI_{50} are given in μM and are means of two to six experiments, standard deviation is given in parentheses.

Table 2
In vitro antimicrobial activity of compounds **4a–b**[a]

Microbial strain	Compound	
	4a	**4b**
Escherichia coli		
ATCC 25922	>100	20 (±0.4)
ATCC 35218	>100	27 (±2.5)
Enterococcus faecalis		
ATCC 29212	>100	25 (±3.1)
Klebsiella oxytoca		
ATCC 700324	>100	63 (±2.1)
Klebsiella pneumoniae		
ATCC 700603	>100	56 (±1.7)
Pseudomonas aeruginosa		
ATCC 27853	>100	22 (±1.7)
Staphylococcus aureus		
ATCC 25923	>100	20 (±0.6)
ATCC 29213	29 (±6.2)	23 (±0.7)
EMRSA-16	>100	17 (±2.6)
NRS 107	>100	24 (±1.5)
Candida albicans		
ATCC 90028	>100	20 (±1.0)
Candida glabrata		
ATCC 90030	>100	18 (±1.3)
Candida krusei		
ATCC 6258	>100	4.5 (±1.6)
Candida nivariensis		
5937-63	>100	16 (±1.1)
Candida parapsilosis		
ATCC 22019	>100	20 (±0.6)

[a] Values expressed as IC_{50} are given in μM and are means of three to six experiments, standard deviation is given in parentheses.

benzo[*b*][1,6]naphthyridines showed relevant selectivity for further development as candidates for cancer therapy. We also found that 1-alkoxy-1*H*-pyrano[4,3-*b*]quinolines show as promising scaffolds for the development of antimicrobial agents, able to inhibit the growth of clinically relevant microbial strains. Extension of these investigations is currently underway and the results together with the biological evaluation will be fully reported in due course.

Acknowledgments

This research was supported by the Lithuanian State Science and Studies Foundation (Reg. No. T-09027, Grant No. T-67/09). Financial support co-financed by the EU FEDER: the Spanish MICIIN (CTQ2008-06806-C02-01/BQU) and MSC (RTICC RD06/0020/1046); the Canary Islands ACIISI (PI 2007/021) and FUNCIS (PI 01/06 and 35/06). E.P.R. thanks the Spanish MSC-FIS for a postdoctoral contract. J.M.P. thanks the Spanish MEC-FSE for a Ramón y Cajal contract.

References and notes

1. (a) Sirisoma, N.; Pervin, A.; Zhang, H.; Jiang, S.; Willardsen, J. A.; Anderson, M. B.; Mather, G.; Pleiman, C. M.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. *J. Med. Chem.* **2009**, *52*, 2341; (b) Krug, M.; Hilgeroth, A. *Mini-Rev. Med. Chem.* **2008**, *8*, 1312; (c) Pédeboscq, S.; L'Azou, B.; Passagne, I.; De Giorgi, F.; Ichas, F.; Pometan, J. P.; Cambar, J. *J. Exp. Ther. Oncol.* **2008**, *7*, 99; (d) Zhang, Y.; Xu, W. *Anticancer Agents Med. Chem.* **2008**, *8*, 698; (e) Jantova, S.; Repicky, A.; Paulovicova, E.; Letasiova, S.; Cipak, L. *Exp. Oncol.* **2008**, *30*, 139; (f) Welch, S. A.; Moore, M. J. *Future Oncol.* **2007**, *3*, 247; (g) Herbst, R. S.; Kies, M. S. *Clin. Adv. Hematol. Oncol.* **2003**, *1*, 466; (h) Denny, W. A. *Farmaco* **2001**, *56*, 51.
2. (a) Srivastava, S. K.; Jha, A.; Agarwal, S. K.; Mukherjee, R.; Burman, A. C. *Anticancer Agents Med. Chem.* **2007**, *7*, 685; (b) Sissi, C.; Palumbo, M. *Curr. Med. Chem. Anticancer Agents* **2003**, *3*, 439; (c) Hradil, P.; Hlavác, J.; Soural, M.; Hajdúch, M.; Kolár, M.; Vecerová, R. *Mini-Rev. Med. Chem.* **2009**, *9*, 696; (d) Xia, Y.; Yang, Z. Y.; Morris-Natschke, S. L.; Lee, K. H. *Curr. Med. Chem.* **1999**, *6*, 179.
3. (a) Kumar, V.; Jaggi, M.; Singh, A. T.; Madaan, A.; Sanna, V.; Singh, P.; Sharma, P. K.; Irchhaiya, R.; Burman, A. C. *Eur. J. Med. Chem.* **2009**, *44*, 3356; (b) 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; (c) Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X. H.; Yang, J. M.; Li, T. K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061; (d) Li, T. K.; Houghton, P. J.; Desai, S. D.; Daroui, P.; Liu, A. A.; Hars, E. S.; Ruchelman, A. L.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **2003**, *63*, 8400.
4. (a) León, L. G.; Carballo, R. M.; Vega-Hernández, M. C.; Martín, V. S.; Padrón, J. I.; Padrón, J. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2681; (b) Padrón, J. M.; Tejedor, D.; Santos-Expósito, A.; García-Tellado, F.; Martín, V. S.; Villar, J. *Lett. Drug Des. Discov.* **2005**, *2*, 529; (c) Padrón, J. M.; Tejedor, D.; Santos-Expósito, A.; García-Tellado, F.; Martín, V. S.; Villar, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2487.
5. (a) Asao, N.; Iso, K.; Yudha, S. S. *Org. Lett.* **2006**, *8*, 4149; (b) Ding, Q.; Wang, B.; Wu, J. *Tetrahedron* **2007**, *63*, 12166; (c) Sun, W.; Ding, Q.; Sun, X.; Fan, R.; Wu, J. *J. Comb. Chem.* **2007**, *9*, 690; (d) Iso, K.; Yudha, S. S.; Menggenbateer; Asao, N. *Heterocycles* **2007**, *74*, 649.
6. (a) Cikotiene, I.; Kairys, V.; Buksnaitiene, R.; Morkunas, M.; Motiejaitis, D.; Rudys, S.; Brukstus, A.; Fernandes, M. X. *Tetrahedron* **2009**, *65*, 5760; (b) Cikotiene, I.; Morkunas, M.; Motiejaitis, D.; Rudys, S.; Brukstus, A. *Synlett* **2008**, 1693; (c) Cikotiene, I.; Morkunas, M. *Synlett* **2009**, 284; (d) Cikotiene, I. *Tetrahedron Lett.* **2009**, *50*, 2570.
7. *General procedure for the synthesis of 2-substituted 1,2-dihydro-3-phenyl-1-(trichloromethyl)benzo[b]-[1,6]naphthyridines* (**2**) *and N-[(1E)-[2-(phenylethynyl)-3-quinolinyl]methylene]benzen-amine* (**3**): To a mixture of **1** (0.1 g, 0.39 mmol) and 3 Å MS (0.3 g) in chloroform (3 mL) the corresponding amine (0.39 mmol) was added. The reaction mixture was refluxed for 24–48 h. After reaction completion as observed by TLC, the solvent was evaporated to leave the crude product, which was purified by basic silica gel column chromatography using a mixture of toluene and ethyl acetate as an eluent to give **2a–f** and **3**.
8. *1,2-Dihydro-3-phenyl-2-(phenylmethyl)-1-(trichloromethyl)benzo[b][1,6]naphthyridine* (**2a**): Yield 48%, yellow solid, mp 146–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.54 (1H, d, J^2 = 15.9 Hz, PhCH), 4.86 (1H, d, J^2 = 15.9 Hz, PhCH), 5.27 (1H, s, CH), 6.49 (1H, s, CH), 6.98–6.99 (2H, m, ArH), 7.08–7.10 (3H, m, ArH), 7.41–7.51 (4H, m, ArH), 7.69–7.81 (4H, m, ArH), 7.97 (1H, s, CH), 8.04 (1H, d, J = 8.4 Hz, ArH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 59.2, 74.9, 104.9, 109.2, 119.0, 124.9, 126.6, 127.3, 127.6, 128.0, 128.4, 128.6, 128.8, 129.5, 130.3, 136.9, 137.5, 137.9, 148.7, 152.8 ppm. Anal. Calcd for $C_{26}H_{19}Cl_3N_2$: C, 67.04; H, 4.11; N, 6.01. Found: C, 67.01; H, 4.25; N, 5.97.
N-[(1E)-[2-(Phenylethynyl)-3-quinolinyl]methylene]benzenamine (**3**): Yield 98%, yellowish solid, mp 118–120 °C (octane). IR (KBr) ν 2118 (C≡C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34–7.39 (3H, m, ArH), 7.45–7.53 (5H, m, ArH), 7.61–7.66 (1H, m, ArH), 7.69–7.72 (2H, m, ArH), 7.80–7.86 (1H, m, ArH), 7.99 (1H, d, J = 8.7 Hz, ArH), 8.19 (1H, d, J = 8.7 Hz, ArH), 9.10 (1H, s, C(4)–H), 9.29 (1H, s, CH=N) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 86.5, 94.6, 121.1, 126.7, 127.1, 127.8, 128.2, 128.5, 128.9, 129.2, 129.4, 129.5, 129.9, 131.5, 132.2, 134.7, 137.1, 143.7, 149.2, 151.7, 157.1 ppm. Anal. Calcd for $C_{24}H_{16}N_2$: C, 86.72; H, 4.85; N, 8.43. Found: C, 86.91; H, 4.77; N, 8.49.
9. Godet, T.; Vaxelaire, C.; Michel, C.; Millet, A.; Belmont, P. *Chem. Eur. J.* **2007**, *13*, 5632.
10. *1-Methoxy-3-phenyl-1H-pyrano[4,3-b]quinoline* (**4a**): Yield 88%, yellow solid, mp 122–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (3H, s, OCH_3), 6.35 (1H, s, CH), 7.00 (1H, s, CH), 7.44–7.49 (4H, m, ArH), 7.69 (1H, t, J = 7.2 Hz, ArH), 7.79 (1H, d, J = 7.8 Hz, ArH), 7.89–7.92 (2H, m, ArH), 8.03–8.07 (2H, m, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.9, 100.1, 102.7, 125.4, 125.6, 127.9, 128.4, 128.5, 128.6, 129.2, 129.9, 130.3, 133.2, 133.7, 148.8, 149.7, 155.8 ppm. Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 79.00; H, 5.25; N, 4.92.
11. Formation of 5-*endo*-dig cyclization product requires the presence of a base. See: (a) Kanazawa, C.; Ito, A.; Terada, M. *Synlett* **2009**, 638; (b) Wei, L. L.; Wei, L. M.; Pan, W. B.; Wu, M. J. *Synlett* **2004**, 1497. and Refs. 6b and 12.
12. (a) Cikotiene, I.; Buksnaitiene, R.; Rudys, S.; Morkunas, M.; Motiejaitis, D. *Tetrahedron* **2010**, *66*, 251; b The more detailed investigation of regioselectivity of acetalisation-cyclization reactions of 2-alkynylquinoline-3-carbaldehydes together with data of NMR and molecular modeling will be published in due course.
13. Miranda, P. O.; Padrón, J. M.; Padrón, J. I.; Villar, J.; Martín, V. S. *ChemMedChem* **2006**, *1*, 323.
14. Monks, A.; Scudiero, D. A.; Skehan, P.; Shoemaker, R. H.; Paull, K. D.; Vistica, D. T.; Hose, C.; Langley, J.; Cronice, P.; Vaigro-Wolf, M.; Gray-Goodrich, M.; Campbell, H.; Mayo, M. R. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
15. The bacterial and fungal strains were grown on nutrient agar plates at 35–37 °C. After 24 h of incubation, cells were suspended in normal saline at a concentration of approximately 5.0×10^5 cfu mL^{-1} for bacterial strains and $1.0–5.0 \times 10^3$ cfu mL^{-1} for *Candida* spp. by matching with 0.5 McFarlands standards. Whole cell antimicrobial activity of compounds was determined in 96-well microtiter plates by a broth microdilution procedure using Mueller Hinton broth (Becton Dickinson, USA) for bacteria and RPMI 1640 (Sigma) buffered with MOPS (Sigma) for fungi. Proper growth and sterile screening controls were included. Pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration, that is, 100 μM. Each compound was tested in duplicates at eight different 10-fold serial dilutions ranging from 100 to 0.05 μM. The microtiter plates were incubated at 35-37 °C in a moist dark chamber. After incubation, plates were shaken and the optical density values were recorded spectrophotometrically. Bacterial plates were read at 630 nm after 24 h of incubation. Fungal plates were read at 490 nm after 48 h of incubation. The IC_{50} was established as the concentration of compound that inhibited 50% growth when compared to untreated cells.
16. DeLeo, F. R.; Chambers, H. F. *J. Clin. Invest.* **2009**, *119*, 2464.