Bioorganic & Medicinal Chemistry Letters 19 (2009) 759–763

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and antifungal activity of 1,2,3-triazole containing fluconazole analogues

Nilkanth G. Aher [a], Vandana S. Pore [a,*], Nripendra N. Mishra [b], Awanit Kumar [b], Praveen K. Shukla [b], Aanchal Sharma [c], Manoj K. Bhat [c]

[a] Organic Chemistry Division, National Chemical Laboratory, Pune 411 008, India
[b] Division of Fermentation Technology, Central Drug Research Institute, Lucknow 226001, India
[c] National Centre for Cell Science, Pune University Campus, Pune 411 007, India

ARTICLE INFO

Article history:
Received 28 July 2008
Revised 10 November 2008
Accepted 5 December 2008
Available online 10 December 2008

Keywords:
1,2,3-Triazole
Conjugates
Bile acid
Fluconazole
Antifungal agent

ABSTRACT

Fluconazole based novel mimics containing 1,2,3-triazole were designed and synthesized as antifungal agents. Their antifungal activities were evaluated in vitro by measuring the minimal inhibitory concentrations (MICs). Compounds **12, 15**, and **16** were found to be more potent against *Candida* fungal pathogens than control drugs fluconazole and amphotericin B. The studies presented here provide structural modification of fluconazole to give 1,2,3-trazole containing molecules. Furthermore, these molecules were evaluated in vivo against *Candida albicans* intravenous challenge in Swiss mice and antiproliferative activities were tested against human hepatocellular carcinoma Hep3B and human epithelial carcinoma A431. It was found that compound **12** resulted in 97.4% reduction in fungal load in mice and did not show any profound proliferative effect at lower dose (0.001 mg/ml).

During passed two decades fungal infections have emerged as a major cause of disease and mortality, in part as a consequence of the increase in acquired immunodeficiency syndrome (AIDS), the greater use of immunosuppressive drugs in transplantation and chemotherapeutic agents in cancer, long term use of corticosteroids and even the indiscriminate use of antibiotics.[1] Clinically, candidosis, aspergillosis, cryptococcosis are the three major infections in the immunocompromised individuals.[2] The common antifungal agents currently used in clinic are polyenes (such as amphotericin B[3a] and nystatin[3b]), echinocandins (such as caspofungin and micafungin),[3c] allylamines (such as niftifine and terbinafine),[3d] and azoles (such as fluconazole, ketoconazole, itraconazole, etc.).[3e]

The current antifungal drugs are either highly toxic, for example, amphotericin B or are becoming ineffective due to appearance of resistant strains, for example, flucytosine and azoles. In spite of significant research on antifungal agents, the azoles remain the mainstay of therapy for systemic life threatening fungal infections as they have fungistatic, orally active, and broad-spectrum activities against most yeasts and filamentous fungi. Fluconazole is a 1,2,4-triazole based drug that has established an exceptional therapeutic record for *Candida* infections, including oropharyngeal and esophageal candidiasis, vulvovaginal candidiasis, candidemia, and disseminated candidiasis. It is an antifungal agent of choice for the treatment of infections by *Candida albicans* and *Cryptococcus neoformans* due to its potent activity, excellent safety profile, and favorable pharmacokinetic characteristics.[4] However, fluconazole is not effective against invasive aspergillosis and is not fungicidal. In addition, extensive use of fluconazole has increased the number of fluconazole-resistant *C. albicans* isolates.[5] Itraconazole is an improvement of fluconazole in its broad-spectrum activity and better toleration but shows low bioavailability and oral absorption. Therefore, great efforts have been made to modify the chemical structure of fluconazole, in order to broaden its antifungal spectrum of activity and to increase its potency. Several new azoles, containing 1,2,4-triazole and 1,3-difluorobenzene moieties, such as voriconazole,[6a] posaconazole,[6b] ravuconazole,[6c] etc. are marketed or in the late stages of clinical trials (Fig. 1).

Azoles exert antifungal activity through inhibition of CYP51 by a mechanism in which the heterocyclic nitrogen (N-3 of imidazole or N-4 of 1,2,4-tiazole) binds to the sixth coordination of heme iron atom of the porphyrin in the substrate binding site of the enzyme.[7] Based on the structure of the active site of CYP51 and the extensive investigation of the structure–activity relationships of azole antifungals, it was found that 1,2,4-triazole ring and 2,4-difluorophenyl group are essential for the high antifungal activity.[8]

Several reports on the synthesis and biological activity of structurally modified new analogues of fluconazole are known in the literature.[9–13] We focused our attention on replacement of one of the

* Corresponding author. Tel.: +91 20 25902327; fax: +91 20 25902624.
E-mail address: vs.pore@ncl.res.in (V.S. Pore).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.12.026

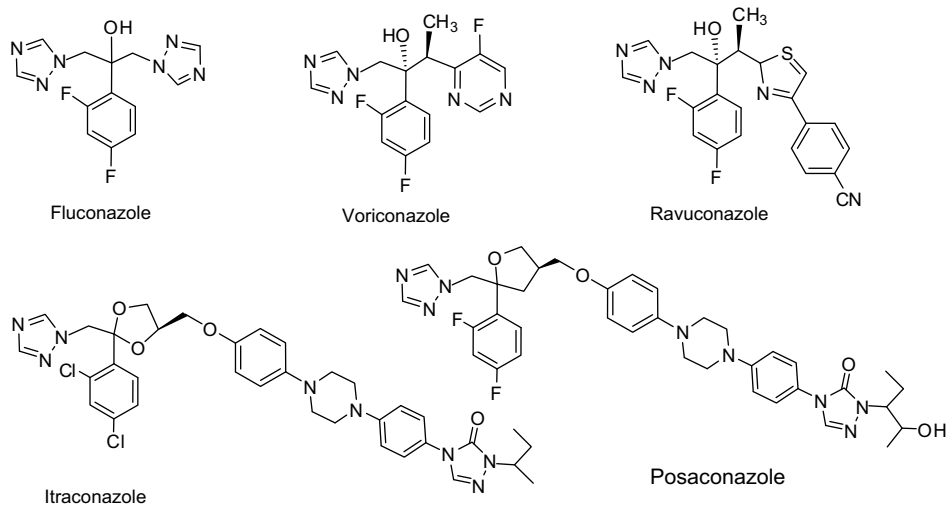


Figure 1. Azole antifungals containing 1,2,4-triazole.

1,2,4-triazole rings of fluconazole with monosubstituted or 1,4-disubstituted 1,2,3-triazoles. 1,2,3-Triazole moieties are stable to metabolic degradation and are capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and for solubility.[14] 1,2,3-Triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole unit show diverse biological activities including antibacterial, herbicidal, fungicidal, antiallergic, and anti-HIV.[15] Huisgen's 1,3-dipolar cycloaddition of terminal acetylenes and organic azides gives a mixture of 1,4- and 1,5-disubstituted-1,2,3-triazoles.[16a] Cu(I) catalyzed 1,3-dipolar cycloaddition has been a method of choice for the synthesis of 1,4-disubstituted-1,2,3-triazoles.[16b–d] Our preliminary work on development of new antifungal triazoles included synthesis of fluconazole/bile acid conjugates **1**–**4** via Cu(I) catalyzed 1,3-dipolar cycloaddition (Fig. 2).[17]

These molecules **1–4** having epimeric mixture at C-2 atom of fluconazole moiety showed moderate antifungal activity against *Candida* species (MIC ranging from 3.12 to 6.25 μg/ml).

In this letter, we describe the synthesis and biological evaluation of fluconazole analogues containing monosubstituted (**11, 13**) and 1,4-disubstituted (**12, 14**, and **15**) 1,2,3-triazoles. Synthesis of ester linked bile acid fluconazole conjugates (**16** and **17**) is also described in which, amphiphilicity of bile acid moiety was thought to be helpful as drug transporter.[18]

The two common intermediates, terminal acetylene **8** and azide **10** have been used for the synthesis of new fluconazole analogues **11–17**. The terminal acetylene **8** was synthesized according to our earlier report[17] from 1,3-diflurobenzene **5** through intermediate **6** and ketone **7** (Scheme 1).

Propargylation of the ketone **7** by using propargyl bromide and zinc dust in DMF/THF gave racemic compound **8** in 95% yield. Another intermediate, namely azide **10** was synthesized from ketone **7**. Reaction of ketone **7** with trimethylsulfoxonium iodide in the presence of sodium hydride afforded oxirane **9** in 91% yield.[12] Opening of oxirane **9** with NaN_3 in DMF at 60–65 °C resulted into azide **10** in 75% yield as a racemic mixture.[19]

Acetylenic compound **8** on cycloaddition reaction with azidotrimethylsilane under microwave irradiation at 245 W in DMF/H_2O using catalytic amount of copper sulfate and sodium ascorbate gave trimethylsilyl deprotected[20] monosubstituted 1,2,3-triazole containing fluconazole analogue **11** in 87% yield (Scheme 2).

The reaction of the same acetylenic compound **8** with 1-azidooctane, under similar reaction conditions afforded 1,4-disubstituted 1,2,3-triazole analogue **12** having long alkyl chain, in 94% yield. The azide **10** was reacted with trimethylsilyl acetylene (microwave irradiation at 175 W because of the low boiling point of trimethylsilyl acetylene) to give trimethylsilyl deprotected monosubstituted 1,2,3-triazole compound **13** in 74% yield along with trimethylsilyl containing compound **14** in 21% yield. Reaction of azide **10** with 1-octyne afforded compound **15** having long alkyl chain attached to 1,2,3-triazole ring. Similar reaction of the azide **10** with propargyl ester of deoxycholic acid, which was synthesized according to our earlier report,[21] yielded diastereomeric mixture of bile acid–fluconazole conjugate **16** in 95% yield. Similarly cycloaddition reaction of **10** with propargyl esters of cholic acid yielded conjugate **17** in 94% yield. All the compounds **11**–**17** were characterized by spectroscopic data as well as elemental analysis.

All the compounds **11**–**17** were tested for the in vitro antifungal activity. The antifungal activity was evaluated against different fungal strains such as *Candida albicans*, *Candida neoformans*, *Sporothrix schenckii*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Candida parapsilosis* (ATCC 22019). Minimum inhibitory concentration (MIC) and IC_{50} values were determined using standard broth microdilution technique as per NCCLS guidelines.[22] Fluconazole, amphotericin B, and ketoconazole were used as standard drugs for the comparison of antifungal activity. All the biological data of the tested compounds are depicted in Table 1 as MIC and IC_{50} values.

All these newly synthesized compounds were found to show good antifungal activity. From the biological data (Table 1), it was observed that compounds **11** and **13** having monosubstituted 1,2,3-triazole ring which are isosteres of fluconazole and com-

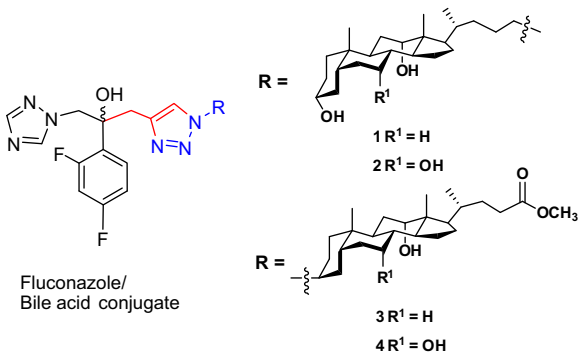
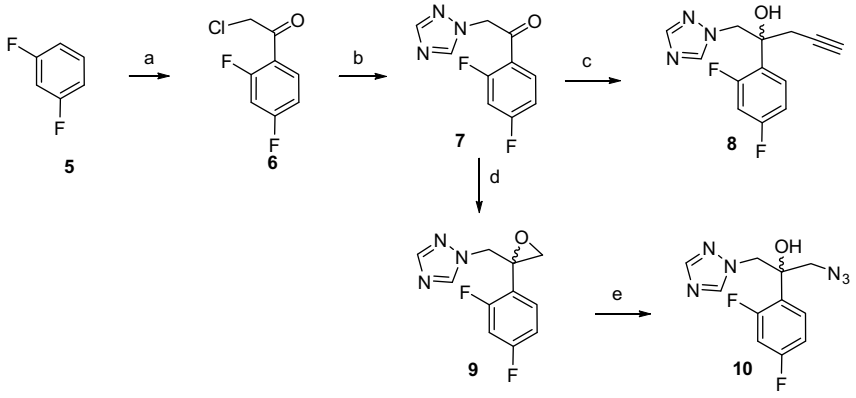
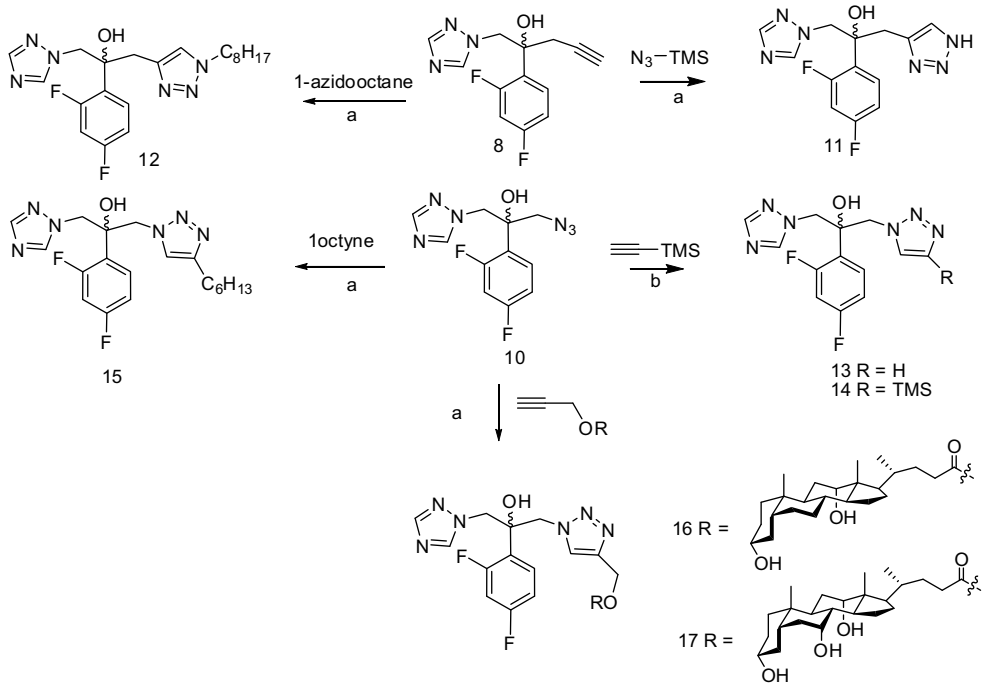


Figure 2. Fluconazole/bile acid conjugates.



Scheme 1. Reagents and conditions: (a) $AlCl_3$, 1,2-dichloroethane, chloroacetyl chloride, 25 °C, 7 h; (b) 1,2,4-triazole, $NaHCO_3$, toluene, reflux, 4 h (overall 55% in two step); (c) Zn, propargyl bromide, DMF/THF, 25 °C, 5 h, 95%; (d) trimethylsulfoxonium iodide, NaH, DMSO/THF, 25 °C, 2 h, 91%; (e) NaN_3, DMF, 60–65 °C, 12 h, 75%.



Scheme 2. Reagents and conditions: (a) $CuSO_4{\cdot}5H_2O$ (5 mol%), sodium ascorbate (40 mol%), DMF/H_2O (4:1), microwave (245 W), 10 min; (b) $CuSO_4{\cdot}5H_2O$ (5 mol%), sodium ascorbate (40 mol%), DMF/H_2O (4:1), microwave (175 W), 10 min.

Table 1
In vitro antifungal activities of compounds **11–17** and standard antifungal drugs fluconazole, ketoconazole, and amphotericin B (MIC and IC_{50} in μg/ml)

Compound	Inhibitory concentration in μg/ml against											
	CA		CN		SS		TM		AF		CP	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
11	3.16	2.74	3.12	0.69	12.4	4.43	49.03	46.99	>50	>50	29.10	16.83
12	**0.002**	**0.001**	**0.002**	**<0.006**	**0.036**	**0.008**	**0.480**	**0.249**	**1.12**	**0.8**	**0.01**	**0.002**
13	1.48	0.72	0.78	0.03	10.62	5.77	12.16	6.45	>50	>50	12.82	6.83
14	3.26	3.04	1.56	1.01	3.16	3.11	15.56	8.57	49.81	43.19	44.53	33.10
15	**0.032**	**0.018**	**0.02**	**<0.01**	**0.14**	**0.043**	**6.72**	**2.46**	**6.9**	**5.0**	**0.39**	**0.12**
16	**<0.001**	**<0.001**	**0.01**	**<0.001**	**0.84**	**0.76**	**>50**	**46.47**	**>50**	**6.24**	**0.396**	**0.38**
17	0.442	0.35	0.19	0.03	12.56	11.27	18.41	8.9	>50	31.24	5.15	2.93
Flu.	0.5	0.13	1.0	0.45	2.0	1.45	1.0	0.6	2.0	1.06	1.0	0.21
Keto.	0.002	0.001	0.001	0.001	0.031	0.026	4.0	2.12	2.0	0.008	0.031	0.024
Amp. B	0.12	0.09	0.06	0.04	0.12	0.08	0.12	0.02	0.5	0.38	0.12	0.11

Bold values indicate the values of more active compounds in the series.
Abbreviations used: CA, *Candida albicans*; CN, *Cryptococcus neoformans*; SS, *Sporothrix schenckii*; TM, *Trichophyton mentagrophytes*; AF, *Aspergillus fumigatus*; CP, *Candida parapsilosis* (*ATCC 22019*); Flu., fluconazole; Keto., ketoconazole; Amp. B, amphotericin B.

pound **14** containing trimethylsilyl group, showed good in vitro antifungal activity against fungal pathogens *C. albicans, C. neoformans*, and *S. schenckii*, which was comparable with fluconazole but these compounds were less active than amphotericin B and ketoconazole (imidazole based current antifungal drug). These molecules were found to be less active against *T. mentagrophytes, A. fumigatus, and C. parapsilosis* (ATCC 22019).

1,4-Disubstituted 1,2,3-triazole containing compounds **12** and **15** with long alkyl chains showed very good antifungal activity against all the tested fungal pathogens. Compound **12** showed much better activity for *C. albicans* (IC_{50} 0.001 μg/ml), *C. neoformans* (IC_{50} <0.006 μg/ml), and *C. parapsilosis* (IC_{50} 0.002 μg/ml) as compared with fluconazole, amphotericin B, and ketoconazole, while compound **15** was found to be less active than compound **12** but showed better activity against *C. albicans* (IC_{50} 0.018 μg/ml), *C. neoformans* (IC_{50} <0.01 μg/ml), and *C. parapsilosis* (IC_{50} 0.043 μg/ml) as compared with fluconazole and amphotericin B. Compounds **16** and **17** which are ester linked bile acid fluconazole conjugates were found to be more potent against *Candida* strains. Compound **16** showed much better activity for *C. albicans* (IC_{50} <0.001 μg/ml), *C. neoformans* (IC_{50} <0.001 μg/ml), *S. schenckii* (IC_{50} <0.043 μg/ml), and *C. parapsilosis* (IC_{50} 0.12 μg/ml) as compared with fluconazole and amphotericin B.

Compounds **16** and **17** were found to be more potent than our earlier reported fluconazole–bile acid conjugates **1**–**4**.[17]

We also evaluated the in vivo activity of more potent compound **12, 15**, and **16** against *C. albicans* intravenous challenge in Swiss mice (Table 2).

Table 2
In vivo efficacy of **12**, **15**, and **16** against systemic challenge of *Candida albicans* in mouse[a]

Sr. No.	Animal groups	Average CFU/g kidney tissue	% Reduction in CFU load
1	Control	3.4×10^6	Nil
2	**12**, 50 mg/kg (OD)	8.8×10^4	97.4
3	**12**, 25 mg/kg (OD)	2×10^6	41.2
4	**15**, 50 mg/kg (OD)	1.7×10^5	95.0
5	**15**, 25 mg/kg (OD)	ND	ND
6	**16**, 50 mg/kg (OD)	2.1×10^6	38.2

[a] Five mice per group were used; the test compounds were given orally, once a day in PEG 200 for 7 days. On day 9 the mice were sacrificed and CFU in kidney tissue were determined; ND, not detected.

Compound **12** resulted in 97.4% reduction while compound **15** resulted in 95% reduction in fungal load in mice at a dose of 50 mg/kg po. Although the bile acid conjugate **16** showed good activity in vitro, it did not exhibit significant in vivo activity (38.2% reduction in fungal load) in this model.

The lead compounds **12, 15** and conjugates **16, 17** were tested for their antiproliferative activity against human cancer cells Hep3B and A431. All the four compounds as well as standard drugs fluconazole, amphotericin B, and ketoconazole were less toxic at lower dose (0.001 mg/ml) while at higher dose (1 mg/ml), they were toxic to both Hep3B and A431 cells (Fig. 3).

All the four compounds **12, 15, 16**, and **17** were more toxic than fluconazole and were less toxic than ketoconazole in both the cell lines. In Hep3B cell lines compound **15** was more toxic than fluconazole but less toxic than other compounds as well as amphotericin B and ketoconazole. Compounds **16** and **17** were comparable with amphotericin B in their toxicity.

In A431 cells, compounds **12** and **15** were found to be more toxic than fluconazole but were less toxic than other compounds as well as amphotericin B and ketoconazole. The antiproliferative activities of compounds **16** and **17** were comparable to amphotericin B in A431 cells.

The results therefore suggest that the antiproliferative activity of compound **12** was similar to fluconazole in A431 cells, while it was toxic for Hep3B cells than fluconazole. The compound **15** was less toxic than ketoconazole and amphotericin B but moderately toxic than flucanozole in both the cell lines. Taken together, these results indicated that the lead compounds **12** and **15** do not have any profound effect on proliferation of human cancer cell lines at lower concentrations and moreover their activity is comparable to that of standard drugs.

In conclusion, fluconazole based novel mimics containing 1,2,3-triazole were designed and synthesized. These molecules were tested for their antifungal activity. Out of these easily accessible molecules, 1,4-disubstituted-1,2,3-triazole compounds **12** and **15**, containing long alkyl chains, showed very good antifungal activity against *Candida* species when compared with the standard drugs fluconazole, ketoconazole, and amphotericin B and did not show any profound proliferative effect. The in vivo assay also indicated that these lead molecules are very good candidates for drug design. A large library of compounds can be easily synthesized from the intermediates **8** and **10** for extensive structure–activity relation studies so that one can reach to the more appropriate drug candidate. Synthesis of pure enantiomers is in progress in our laboratory. These are expected to show better activity than racemic

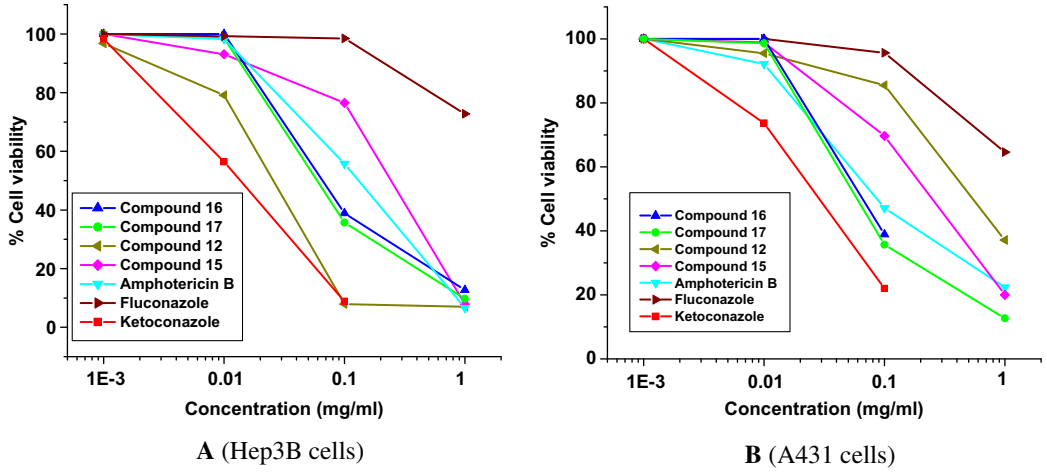


Figure 3. Graphical representation for antiproliferative activities of compounds in Hep3B cells (A) and A 431 cells (B).

compounds. 1,2,3-Triazole containing molecules may be considered as new entry to azole antifungals.

Acknowledgments

V.S.P. thanks Director NCL for in-house project funding (MLP 013126). N.G.A. thanks CSIR-UGC, New Delhi, for award of Senior Research Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.026. Experimental procedures as well as materials and methods for bioevaluation and spectroscopic data of compounds **7**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**.

References and notes

1. (a) Fridkin, S. K.; Jarvis, W. R. *Clin. Microbiol. Rev.* **1996**, *9*, 499; (b) Larocco, M. T.; Burgert, S. J. *Clin. Microbiol. Rev.* **1997**, *10*, 277; (c) Patel, R.; Paya, C. V. *Clin. Microbiol. Rev.* **1997**, *10*, 86.
2. (a) Latge, J. P. *Clin. Microbiol. Rev.* **1999**, *12*, 310; (b) Steenbergen, J. N.; Casabevall, A. *J. Clin. Microbiol.* **2000**, *38*, 1974.
3. (a) Gallis, H. A.; Drew, R. H.; Pickard, W. W. *Rev. Infect. Dis.* **1990**, *12*, 308; (b) Arikan, S.; Rex, J. H. *Curr. Opin. Invest. Drugs* **2001**, *2*, 488; (c) Denning, D. W. *J. Antimicrob. Chemother.* **2002**, *49*, 889; (d) Birnbaum, J. E. *J. Am. Acad. Dermatol.* **1990**, *23*, 782; (e) Sheehan, D. J.; Hitchcock, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999**, *12*, 40.
4. (a) Kauffman, C. A.; Carver, P. L. *Drugs* **1997**, *53*, 539; (b) Karyotakis, N. C.; Anaissie, E. J. *Curr. Opin. Inf. Dis.* **1994**, *7*, 658; (c) Zervos, M.; Meunier, F. *Int. J. Antimicrob. Agents* **1993**, *3*, 147.
5. Rex, J. H.; Rinaldi, M. G.; Pfaller, M. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 1.
6. (a) Chandrasekar, P. H.; Manavathu, E. *Drugs Today (Barc.)* **2001**, *37*, 135; (b) Herbrecht, R. *Int. J. Clin. Pract.* **2004**, *58*, 612; (c) Arikan, S.; Rex, J. H. *Curr. Opin. Invest. Drugs* **2002**, *3*, 555.
7. Hitchcock, C. A.; Dickinson, K.; Brown, S. B.; Evans, E. G. V.; Adams, D. J. *Biochem. J.* **1990**, *266*, 475.
8. (a) Michael, S. M.; Kenneth, R. In *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents*; Fromtling, R. A., Ed.; J.R. Prous Science Publishers: SA, 1987; pp 81–92; (b) Aperis, G.; Mylonakis, E. *Expert Opin. Investig. Drugs* **2006**, *15*, 579; (c) Chen, A.; Sobel, J. D. *Expert Opin. Emerg. Drugs* **2005**, *10*, 21.
9. Lebouvier, N.; Pagniez, F.; Duflos, M.; Le Pape, P.; Na, Y. M.; Le Baut, G.; Le Borgne, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3686.
10. Nam, N.-H.; Sardari, S.; Selecky, M.; Parang, K. *Bioorg. Med. Chem.* **2004**, *12*, 6255.
11. Heravi, M. M.; Motamedi, R. *Heterocycl. Commun.* **2005**, *11*, 19.
12. Upadhayaya, R. S.; Jain, S.; Sinha, N.; Kishore, N.; Chandra, R.; Arora, S. K. *Eur. J. Med. Chem.* **2004**, *39*, 579.
13. (a) Park, J. S.; Yu, K. A.; Kang, T. H.; Kim, S.; Suh, Y.-G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3486; (b) Sun, Q.-Y.; Xu, J.-M.; Cao, Y.-B.; Zhang, W.-N.; Wu, Q.-Y.; Zhang, D.-Z.; Zhang, J.; Zhao, H.-Q.; Jiang, Y.-Y. *Eur. J. Med. Chem.* **2007**, *42*, 1226; (c) Nguyen, H. N. *Tap Chi Duoc Hoc* **2005**, *45*, 13; (d) Liang, S.; Liu, C.; Jin, Y.; He, Q. *Zhongguo Yaowu Huaxue Zazhi* **2004**, *14*, 71; (e) Liu, C.; Xu, F.; Liang, S.; Sun, Q.; Jiang, Y. *Zhongguo Yaowu Huaxue Zazhi* **2004**, *14*, 129.
14. (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. *Chem. Res. Toxicol.* **2002**, *15*, 269; (b) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366.
15. (a) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953; (b) Wamhoff, H. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; pp 669–732; (c) Buckle, D. R.; Rockell, C. J. M.; Smith, H.; Spicer, B. A. *J. Med. Chem.* **1986**, *29*, 2262; (d) Alvarez, R.; Velazquez, S.; San-Felix, A.; Aquaro, S.; De Clercq, E.; Perno, C. F.; Karlsson, A.; Balzarini, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185.
16. (a) Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1967**, *100*, 2494; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; (c) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (d) Bock, V. D.; Hiemstra, H.; Van Maarseveen, J. H. *Eur. J. Org. Chem.* **2005**, 51.
17. Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178.
18. (a) Enhsen, A.; Kramer, W.; Wess, G. *Drug Discovery Today* **1998**, *3*, 409; (b) Kannan, A.; De Clercq, E.; Pannecouque, C.; Witvrouw, M.; Hartman, T. L.; Turpin, J. A.; Buckheit, R. W., Jr.; Cushman, M. *Tetrahedron* **2001**, *57*, 9385; (c) Anelli, P. L.; Lattuada, L.; Lorusso, V.; Lux, G.; Morisetti, A.; Morosini, P.; Serleti, M.; Uggeri, F. *J. Med. Chem.* **2004**, *47*, 3629; (d) Bhat, L.; Jandeleit, B.; Dias, T. M.; Moors, T. L.; Gallop, M. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 85.
19. Konosu, T.; Tajima, Y.; Takeda, N.; Miyaoka, T.; Kasahara, M.; Yasuda, H.; Oida, S. *Chem. Pharm. Bull.* **1990**, *38*, 2476.
20. The [3+2] cycloaddition of nonactivated terminal alkynes and trimethylsilyl azide proceeded smoothly in the presence of Cu(I) catalyst to give the corresponding *N*-unsubstituted triazole in good to high yield Jin, T.; Kamijo, S.; Yamamoto, Y. *Eur. J. Org. Chem.* **2004**, 3789.
21. (a) Aher, N. G.; Pore, V. S. *Synlett* **2005**, 2155; (b) Aher, N. G.; Pore, V. S.; Patil, S. P. *Tetrahedron* **2007**, *63*, 12927.
22. (a) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast, Approved Standard. Document M27-A; National Committee for Clinical Laboratory Standards: Wayne, PA, USA, **1997**; (b) National Committee for Clinical Laboratory Standard. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium Forming Filamentous Fungi: Proposed Standard. Document M38-P; National Committee for Clinical Laboratory Standard: Wayne, PA, USA, **1998**; (c) National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Approved Standard, 5th ed.; NCCLS: Villanova, PA, **2000**, pp M7–A5; (d) Yamamoto, N.; Fujita, J.; Shinzato, T.; Higa, F.; Tateyama, M.; Tohyama, M.; Nakasone, I.; Yamane, N. *Int. J. Antimicrob. Agents* **2006**, *27*, 171.