Bioorganic & Medicinal Chemistry Letters 20 (2010) 722–725

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Fluconazole analogues containing 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moieties, a novel class of anti-*Candida* agents

Hanumant B. Borate [a,*], Suleman R. Maujan [a], Sangmeshwer P. Sawargave [a], Mohan A. Chandavarkar [b], Sharangi R. Vaiude [b], Vinay A. Joshi [b], Radhika D. Wakharkar [a], Ramki Iyer [b], Ramesh G. Kelkar [a], Subhash P. Chavan [a], Sunita S. Kunte [a]

[a] *Division of Organic Chemistry, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India*
[b] *FDC Ltd, 142-148, S. V. Road, Jogeshwari (W), Mumbai 400 102, India*

ARTICLE INFO

Article history:
Received 9 July 2009
Revised 10 October 2009
Accepted 13 November 2009
Available online 4 December 2009

Keywords:
Antifungal
Fluconazole
Benzothiazinone
Benzoxazinone

ABSTRACT

As a part of our program to develop new antifungal agents, a series of fluconazole analogues was designed and synthesized wherein one of the triazole moieties in fluconazole was replaced with 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moiety. The new chemical entities thus synthesized were screened against various fungi and it was observed that the compounds **4a** and **4i** are potent inhibitors of *Candida* strains. The structure–activity relationship for these compounds is discussed.

Complications in the fungal infections have increased in the number and severity in recent years as a result of an increasing number of immunocompromised hosts, such as patients suffering from tuberculosis, infected with HIV and undergoing organ transplantations and cancer chemotherapy, due to the use of immunosuppressant agents. Though there are effective antifungal agents available in the market, they have quite a few shortcomings such as toxicity, limited range of activity for the fungal strains, high price and limited penetration through central nervous system.

Fluconazole (**1**)[1,2] is an important antifungal agent used against various fungal strains, but its extensive use has increased the number of fluconazole-resistant fungi due to mutations (see Fig. 1). Amphotericin B (**2**) is the antifungal agent of choice used in fluconazole-resistant fungal infections but it has a higher degree of toxicity than that of fluconazole.[3] The immunocompromised patients have to take long term antifungal therapy to prevent relapses and this causes the development of resistance in fungal strains. This compels the synthetic chemists to design and synthesize new chemical entities in an effort to come up with new drugs which can be used in place of current drugs like fluconazole or amphotericin B against the mutated fungal strains.

Various fluconazole derivatives and analogues have been synthesized and checked for the antifungal activity against different fungal strains. This included derivatisation at tertiary alcohol of fluconazole as ethers, esters, phosphates and so on or the substitution of one of the triazole unit by different moieties.[4] In some of the cases, difluorophenyl ring in fluconazole was replaced with other moieties such as 1,4-benzothiazinones to obtain compounds **3a**.[5] The analogues having general structure **3b** were also synthesized and screened against *Candida albicans*.[6] In all the above 1,4-benzothiazinones and 1,4-benzoxazinones, amide nitrogen was protected as alkyl chain such as methyl or ethyl (see Fig. 2).

As a part of our efforts[7] to develop new antifungal drugs, we synthesized fluconazole analogues wherein one of the triazole moieties in fluconazole was replaced with 2*H*-1,4-benzothiazin-3(4*H*)-one or 2*H*-1,4-benzoxazin-3(4*H*)-one moiety and we report herein the synthesis and in vitro antifungal activity of a series of fluconazole derivatives against various fungi.

The synthesis of compounds **4** was achieved from epoxide **5** and (un)substituted benzothiazinone or benzoxazinone moieties **6** as shown in Schemes 1 and 2.

Epoxide **5a**[8,9] was prepared from ketone **9** by Corey–Chaykovsky epoxidation method. Ketone **9** was in turn obtained from 1,2-difluorobenzene via its acylated intermediate **8** as shown in Scheme 1. Other epoxides such as **5b**, **5c** and **5d** were synthesized[10] using same strategy from their respective starting materials.

The synthesis of benzoxazinone **6a**[11] and benzothiazinone **6b**[11] was achieved from commercially available 2-aminophenol **10a** and 2-aminothiophenol **10b**, respectively, as shown in Scheme 1. Stir-

* Corresponding author. Tel.: +91 20 25902546; fax: +91 20 25902629.
E-mail address: hb.borate@ncl.res.in (H.B. Borate).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.071

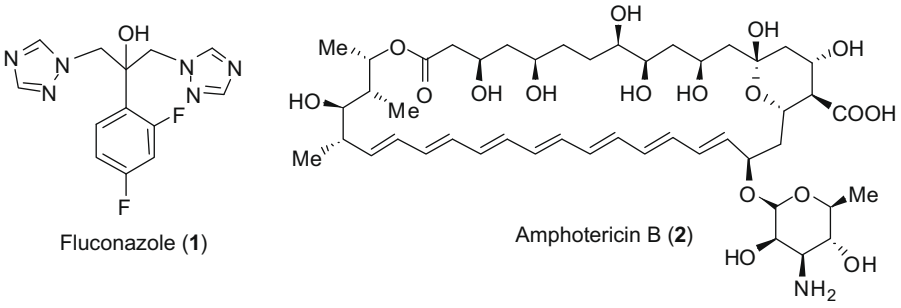


Figure 1. Structures of fluconazole and amphotericin B.

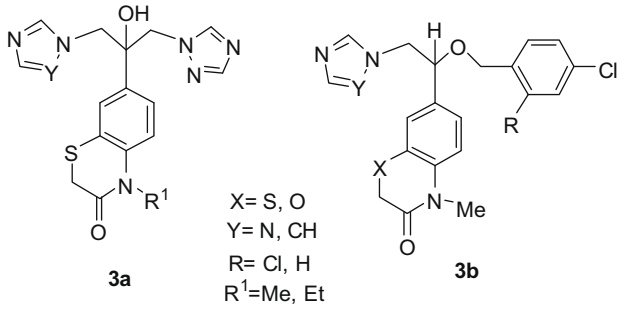
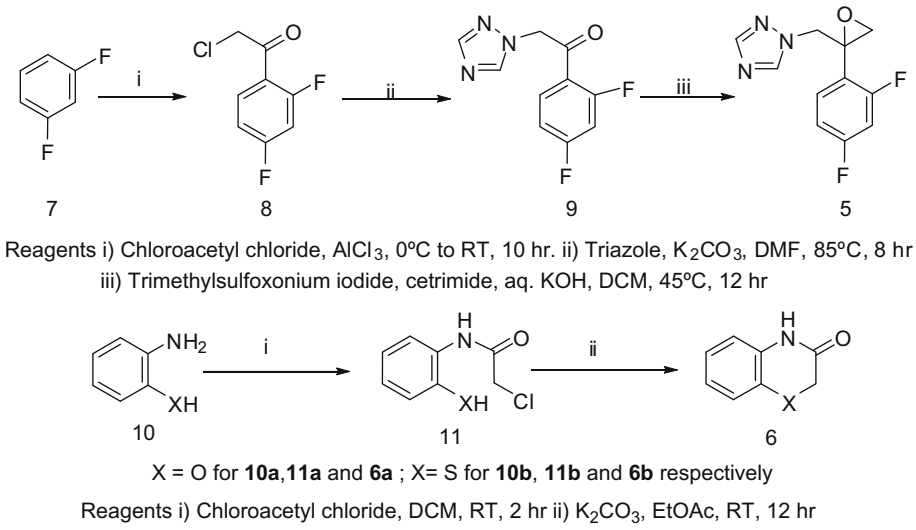


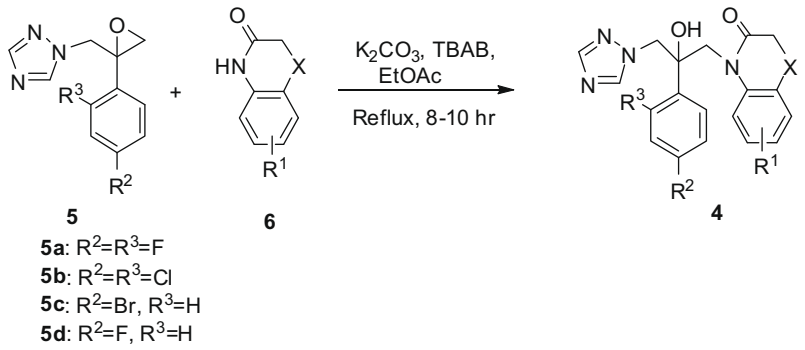
Figure 2. Structures of azole analogues containing benzothiazinone and benzoxazinone moieties.

ring 2-aminophenol **10a** with chloroacetyl chloride in DCM at room temperature for 2 h yielded N-acylated intermediate **11a** which on treatment with potassium carbonate in ethyl acetate at refluxing temperature yielded benzoxazinone **6a**. The same reaction sequence using 2-aminothiophenol **10b** afforded benzothiazinone **6b**. Rest of the benzoxazinones and benzothiazinones were either prepared by the synthetic sequence given in Scheme 1 or were commercially available.

Benzoxazinone and benzothiazinone so prepared were reacted with epoxides **5** in presence of potassium carbonate and *tetra*-butylammonium bromide (TBAB) in ethyl acetate at refluxing temperature for 10–12 h to yield desired compounds **4**[12] in good yields as shown in Scheme 2.



Scheme 1. Synthesis of epoxide intermediate (**5**) and benzothiazinone (**6b**) and benzoxazinone (**6a**) moieties.



Scheme 2. Synthesis of compounds **4** from epoxide **5** and benzothiazinone or benzoxazinone moieties **6**.

Table 1
MIC obtained for compounds **4** by broth macro-dilution method

Sr. no.	Compound no.	Structure **4**	Activity against organisms MIC in μg/mL*		
			C. albicans ATCC 24433	*A. niger* ATCC 16404	*F. proliferatum* ATCC 10052
1	Fluconazole		1	128	>128
2	Amphotericin B		0.25	1	2
3	**4a**	X = S, R^1 = H, R^2 = R^3 = F	0.25	NI	NI
4	**4b**	X = S, R^1 = 7-Cl, R^2 = R^3 = F	1	NI	NI
5	**4c**	X = S, R^1 = 7-Br, R^2 = R^3 = F	1	NI	NI
6	**4d**	X = S, R^1 = 7-OMe, R^2 = R^3 = F	4	NI	NI
7	**4e**	X = S, R^1 = 7-Me, R^2 = R^3 = F	2	NI	NI
8	**4f**	X = S, R^1 = 7-Cl, R^2 = F, R^3 = H	2	NI	NI
9	**4g**	X = S, R^1 = 7-OMe, R^2 = Br, R^3 = H	2	NI	NI
10	**4h**	X = S, R^1 = 7-OMe, R^2 = F, R^3 = H	8	NI	NI
11	**4i**	X = O, R^1 = H, R^2 = R^3 = F	0.5	NI	NI
12	**4j**	X = O, R^1 = 6-Cl, R^2 = R^3 = F	8	NI	NI
13	**4k**	X = O, R^1 = 6-Br, R^2 = R^3 = F	16	NI	NI
14	**4l**	X = O, R^1 = 6-NO_2, R^2 = R^3 = F	32	NI	NI
15	**4m**	X = O, R^1 = 6-Ac, R^2 = R^3 = F	NI	NI	NI
16	**4n**	X = O, R^1 = 6-Cl, R^2 = Br, R^3 = H	4	NI	NI
17	**4o**	X = O, R^1 = 6-Cl, R^2 = F, R^3 = H	16	NI	NI
18	**4p**	X = O, R^1 = 6-Ac, R^2 = Br, R^3 = H	32	NI	NI

NI: no inhibition.
* For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 80% inhibition as compared to the positive control while for amphotericin B, MIC is recorded as the concentration exhibiting complete inhibition.

All the newly synthesized compounds **4** were tested for antifungal activity against various fungi including *C. albicans* ATCC 24433, *Aspergillus niger* ATCC 16404 and *Fusarium proliferatum* ATCC 10052 (Table 1, Sr. no. 3–18). In vitro evaluation of antifungal activity was performed by determining the minimum inhibitory concentration (MIC) following broth dilution methods.[13,14] Broth dilution testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) documents M-27 A2 and M-38 A with RPMI 1640 medium buffered to pH 7.0 with MOPS buffer. Known antifungal agents, fluconazole and amphotericin-B, were used as positive control. End points were determined after 48 h visually and by using spectrophotometer wherever necessary. The activity parameters are enumerated in Table 1.

The compounds **4** prepared in the present work exhibited antifungal activity while the corresponding starting materials **5** and **6** did not exhibit any antifungal activity. The antifungal activity exhibited by the compounds **4** was confirmed by secondary screening of compounds **4a** and **4i** against various strains of *Candida* and it was observed that the activity of **4a** is excellent against the *C. albicans* ATCC24433, *C. albicans* ATCC90028 and *C. glabrata* ATCC90030 when compared with amphotericin B and fluconazole as shown in Table 2.

The above results indicated following points regarding the structure–activity relationship of the compounds studied in the present work.

Table 2
MIC obtained for compounds **4a** and **4i** by broth micro-dilution method

Fungus	MIC in μg/mL*			
	Amphotericin B	Fluconazole	**4a**	**4i**
C. albicans ATCC24433	0.25	0.25	0.03	0.12
C. albicans ATCC10231	0.5	1.0	0.12	0.5
C. albicans ATCC2091	0.5	0.5	0.12	0.25
C. albicans ATCC90028	0.5	0.5	0.03	0.12
C. glabrata ATCC90030	0.25	4	0.06	0.25
C. krusei ATCC6258	0.5	64	4	16
C. tropicalis ATCC750	0.5	2	0.5	2

* For fluconazole and the synthetic compounds, MIC is recorded as the concentration exhibiting 50% inhibition as compared to the positive control; for amphotericin B, MIC is recorded as the concentration exhibiting complete inhibition.

1. The compounds containing benzothiazinone moiety are more active than those containing benzoxazinone (compound no **4a** vs **4i**).
2. In the benzothiazinone series, replacement of fluorine by hydrogen reduces the activity (compound nos **4b** v/s **4f** and **4d** v/s **4h**). The same is true for benzoxazinone series (compound no **4j** v/s **4o**).
3. In the benzothiazinone series, halogen substituent at C7 position is tolerated with slight decrease in activity (compound no **4a** v/s **4b** or **4c**) while substituents like methoxy or methyl decrease the activity considerably (compound no **4a** v/s **4d** or **4e**).
4. In case of benzoxazinone series, no substituent at C6 is tolerated and all compounds having substituents like Cl, Br, NO_2 or Ac lost the activity (compound no **4i** v/s **4j**, **4k**, **4l** or **4m**).

Thus, a novel class of anti-*Candida* agents was synthesized by a short synthetic sequence and in good to excellent yields. Some of the compounds exhibited good antifungal activity against *C. albicans* and two of the compounds (**4a** and **4i)** exhibited excellent anti-*Candida* activity. These observations will be very useful in designing newer molecules in the pursuit of more effective antifungal agents.

Acknowledgements

S.R.M. and S.P.S. thank CSIR, New Delhi for the award of senior research fellowship. Financial supports from Department of Science and Technology, New Delhi and FDC Ltd, Mumbai are gratefully acknowledged.

Supplementary data

Experimental procedures and spectral data for all compounds prepared for antifungal activity testing are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.071.

References and notes

1. Richardson, K.; Brammer, K. W.; Marriott, M. S.; Troke, P. F. *Antimicrob. Agents Chemother.* **1985**, *27*, 832.
2. Kauffman, C. A.; Carver, P. L. *Drugs* **1997**, *53*, 539.

3. Sheehan, J. D.; Hitchcock, C. A.; Sibley, C. M. *Clin. Microbiol. Rev.* **1999**, *12*, 40.
4. (a) Bartroli, J.; Turmo, E.; Alguero, M.; Boncompte, E.; Vericat, M. L.; Garcia-Rafanell, J.; Forn, J. *J. Med. Chem.* **1995**, *38*, 3918. and references cited therein; (b) Bartroli, J.; Turmo, E.; Alguero, M.; Boncompte, E.; Vericat, M. L.; Conte, L.; Ramis, J.; Merlos, M.; Garcia- Rafanell, J.; Forn, J. *J. Med. Chem.* **1998**, *41*, 1855. and references cited therein; (c) Park, K.-S.; Kang, H.-L.; Lee, J. W.; Paik, Y.-K. *J. Microbiol. Biotechnol.* **2004**, *4*, 693; (d) Heravi, M. M.; Motamedi, R. *Phosphorus, Sulfur, Silicon* **2004**, *179*, 2329; (e) Upadhyaya, R. S.; Sinha, N.; Jain, S.; Kishore, N.; Chandra, R.; Arora, S. K. *Bioorg. Med. Chem.* **2004**, *12*, 2225; (f) Nam, N.-H.; Sardari, S.; Selecky, M.; Parang, K. *Bioorg. Med. Chem.* **2004**, *12*, 6255; (g) Lebouvier, N.; Pagniez, F.; Duflos, M.; Pape, P. L.; Na, Y. M.; Baut, G. L.; Borgne, M. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3686; (h) Zhao, Q.-J.; Hu, H.-G.; Li, Y.-W.; Song, Y.; Cai, L.-Z.; Wu, Q.-Y.; Jiang, Y.-Y. *Chem. Biodivers.* **2007**, *4*, 1472; (i) Lebouvier, N.; Pagniez, F.; Duflos, M.; Pape, P. L.; Na, Y. M.; Bauta, G. L.; Borgne, M. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3686; (j) Katritzky, A. R.; Slavov, S. H.; Dobchev, D. A.; Karelson, M. *Bioorg. Med. Chem.* **2008**, *16*, 7055; (k) Uchida, T.; Somada, A.; Kagoshima, Y.; Konosu, T.; Oida, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6538; (l) Uchida, T.; Kagoshima, Y.; Konosu, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2013; (m) Giraud, F.; Guillon, R.; Logé, C.; Pagniez, F.; Picot, C.; Le Borgne, M.; Le Pape, P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 301.
5. (a) Fringuelli, R.; Schiaffella, F.; Bistoni, F.; Pitzurra, L.; Vecchiarelli, A. *Bioorg. Med. Chem.* **1998**, *6*, 103. and references cited therein; (b) Fringuelli, R.; Schiaffella, F.; Navarro, M. P. U.; Milanese, L.; Santini, C.; Rapucci, M.; Marchetti, C.; Riccardi, C. *Bioorg. Med. Chem.* **2003**, *11*, 3245.
6. Fringuelli, R.; Pietrella, D.; Schiaffella, F.; Guarraci, A.; Perito, S.; Bistoni, F.; Vecchiarelli, A. *Bioorg. Med. Chem.* **2002**, *10*, 1681.
7. (a) Gurjar, M. K.; Wakharkar, R. D.; Borate, H. B.; Kelkar, R. G.; Murugan, A.; Chandavarkar, M. A.; Joshi, S. V.; Vaiude, S. R. U.S. Patent US 0259948, 2007 A-1; Ind. Patent 223238, 2008.; (b) Borate, H. B.; Maujan, S. R.; Sawargave, S. P.; Chandavarkar, M. A.; Joshi, S. V.; Vaiude, S. R. Ind. Patent 438/MUM/2008; PCT International PCT/IN08/000571.; (c) Borate, H. B.; Sawargave, S. P.; Maujan, S. R.; Chandavarkar, M. A; Vaiude, S. R ; Joshi, V. A. Ind. Patent 1595/MUM/2007.; (d) Borate, H. B.; Maujan, S. R.; Sawargave, S. P.; Kelkar, R. G.; Wakharkar, R. D.; Chandavarkar, M. A; Vaiude, S. R. ; Joshi, V. A. Ind. Patent 2306/MUM/2008.; (e) Chandavarkar, M. A.; Kulkarni, V. M.; Shivkumar, P.; Shetty, R. S.; Bapat, U. R. WO 03/068142, 2003.
8. Richardson, K. UK Patent 2099818, 1982; *Chem. Abstr.* **1983**, *99*, 38467.
9. Upadhayaya, R. S.; Jain, S.; Sinha, N.; Kishore, N.; Chandra, R.; Arora, S. K. *Eur. J. Med. Chem.* **2004**, *39*, 579.
10. Lebouvier, N.; Giraud, F.; Corbin, T.; Na, Y. M.; Le Baut, G.; Marchanda, P.; Le Borgnea, M. *Tetrahedron Lett.* **2006**, *47*, 6479.
11. Huang, X.; Chan, C.-C. *Synthesis* **1984**, 851.
12. The experimental procedures and spectral data for all compounds prepared for antifungal activity testing in this article are given as Supplementary data. General procedure for the synthesis of fluconazole analogues **4**: To a flame dried K_2CO_3 (0.18 mol), *tetra*-butylammonium bromide (TBAB, 0.09 mol) was added followed by the addition of compound **6** (0.09 mol) in dry ethyl acetate (200 mL). Reaction mixture was stirred at reflux for 30 min. Then epoxide **5** (0.09 mol) dissolved in dry ethyl acetate (200 mL) was added to the refluxing mixture drop wise over a period of 10 min and stirring was continued for further 12 h at the same temperature. It was then cooled to room temperature, diluted with water (800 mL), extracted with ethyl acetate (3×400 mL), dried over Na_2SO_4, concentrated and purified by column chromatography to give pure compound. Various compounds were prepared by using the general procedure described above and spectral data for all the compounds prepared are given as Supplementary data. Spectral data for representative compound are given below: 4-[2-(2,4-Difluorophenyl)-2-hydroxy-3-[1,2,4]triazol-1-yl-propyl]-4*H*-benzo[1,4]thiazin-3-one (**4a**): Yield: 78%; Pale yellow solid, mp 123 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.17 (d, J = 14 Hz, 1H), 3.24 (d, J = 14 Hz, 1H), 4.43 (d, J = 14 Hz, 1H), 4.48 (d, J = 14 Hz, 1H), 4.60 (s, 2H), 5.52 (br s, 1H), 6.41–6.47 (m, 1H), 6.68–6.73 (m, 1H), 6.92–6.98 (m, 1H), 7.13–7.26 (m, 3H), 7.50–7.58 (m, 1H), 7.76 (s, 1H), 8.15 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$,): 31.9, 52.3, 56.1, 76.4, 103.3 (t), 111.3 (d), 119.1, 122.8, 123.9, 125.2, 126.9, 128.2, 130.1, 139.4, 144.6, 151.0, 157.4 (d), 162.8 (d), 168.4. IR (Chloroform): 1647, 3332 cm^{-1}. MS (ESI) m/z: 403.3074 (M+1), 425.2999 (M+Na).
13. CLSI: Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, second edition M27-A2, 2002.
14. CLSI: Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard M38-A, 2002.