In Vitro Activities of Novel Azole Compounds (ATTAF-1 and ATTAF-2) Against Fluconazole-Susceptible and -Resistant Isolates of *Candida* species

Hamed Fakhim [a,b], Saeed Emami[c], Afsane Vaezi [a,b], Seyedeh Mahdieh Hashemi[c], Leila Faeli [a,b], Kambiz Diba[d], Eric Dannaoui[e] , Hamid Badali[b, f]*

Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran [a]; Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran [b]; Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran [c]; Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran [d]; Université Paris-Descartes, Faculté de Médecine, APHP, Hôpital Européen Georges Pompidou, Unité de Parasitologie-Mycologie, Service de Microbiologie, Paris, France [e,] Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran [f]

***Corresponding authors:** H. Badali, PhD Tel: +989128413720; E-mail: badalii@yahoo.com

Running title: Activities of Novel Azole Against *Candida* spp.

Keywords: *In vitro* susceptibility, triazole derivatives, *Candida* species

In vitro **activities of two novel azole compounds (ATTAF-1 and ATTAF-2) and five comparator antifungal agents against 52 clinical *Candida* isolates from 5 different species were determined. Novel azole compounds had the lowest geometric mean MICs followed by fluconazole. Moreover, combinations of these compounds with fluconazole exhibited synergistic effects against fluconazole-susceptible (22 of 23), -susceptible dose dependent (10 of 13) and -resistant (1 of 16) *Candida* isolates.**

Word count text: 1495

Word count abstract: 74

Candidiasis is a serious life-threatening infection, associated with significant morbidity and mortality rates. The incidence of this infection has increased in recent years, especially among immunocompromised patients (1, 2). *Candida* species are the fourth most common agent of hospital-acquired candidemia (3-5). Guidelines for the management of candidiasis have recommended the use of azoles, polyenes, and echinocandins (6, 7). However, toxic effects of amphotericin B and resistance to azoles and echinocandins in *Candida* species have recently become a serious clinical challenge (8-10). Fluconazole is the most commonly used agent for systemic candidiasis, given its low toxicity, high solubility, and wide tissue distribution (11). In addition, use of fluconazole for prophylaxis and treatment is thought to be a potential risk factor, leading to the gradual development of azole-resistant species (12). Accordingly, there is an urgent need for introducing a novel class of antifungal agents with potent activities and new mechanisms of action to improve the management of *Candida* infections (13).

Replacement of one triazole ring in the fluconazole structure with other heterocyclic moieties with the purpose of introducing and developing new antifungal agents has received particular attention in medical chemistry. We previously designed and synthesized numerous triazole alcohols by replacing the 1,2,4-triazol-1-yl group in the fluconazole structure with 4-amino-5-aryl-3-mercapto-1,2,4-triazole motif (14,15). Since this newly introduced motif represented a new type of side chain in triazole alcohol antifungals, we focused on the structural refinement of the primary lead and removed the amino group from the structure to obtain new entities, namely aryl-1,2,4-triazole-3-yl(thio) analogues of fluconazole (ATTAF). In particular, ATTAF-1 and ATTAF-2 compounds (formerly 10h and 11h, respectively), containing (2,4-dichlorophenyl)-

1,2,4-triazole-thiol moiety, were found to be potential agents against *Candida* species with no significant cytotoxicity against HepG2 cell line (Figure 1) (15). Although ATTAF-1 and ATTAF-2 are triazole alcohol-derived analogues, their increased antifungal activity in comparison with fluconazole might be attributed to the presence of (2,4-dichlorophenyl)-1,2,4-triazole-thiol scaffold as an additional pharmacophoric structure with a mechanism of action distinct from fluconazole. Therefore, we aimed to describe the *in vitro* activity of ATTAF-1 and ATTAF-2 in comparison with five clinically important antifungal drugs against fluconazole-susceptible and -resistant *Candida* isolates. Moreover, we investigated the combination of these compounds with fluconazole.

Compounds ATTAF-1 and ATTAF-2 were synthesized and characterized according to our previous study (15). Fluconazole (Pfizer, Groton, CT, USA), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer, Central Research, Sandwich, United Kingdom), amphotericin B (Sigma, St. Louis, MO, USA), and anidulafungin (Pfizer) were obtained as reagent-grade powders from the respective manufacturers and used for preparation of the CLSI microdilution trays.

Fifty-two *Candida* isolates from five different species including, fluconazole-susceptible (n=23), -susceptible dose dependent (n=13) and -resistant (n=16), according to the new CLSI species-specific clinical breakpoints (CBPs) for fluconazole against *Candida* species (16), were obtained from the reference culture collection of the Invasive Fungi Research Center (IFRC), Sari, Iran (Table 1). Isolates has been previously identified by sequencing of the ITS rDNA region. Antifungal susceptibility testing was performed according to CLSI guidelines M27-A3

and M27-S4 documents (17, 18) after 24 h of incubation at 35°C. The antifungal agents were prepared at a final concentration of 0.016–16 µg/ml for amphotericin B, itraconazole and voriconazole, 0.063–64 µg/ml for fluconazole, ATTAF-1 and ATTAF-2 and 0.008–8 µg/ml for anidulafungin. The MIC endpoint was defined as 100% of inhibition for amphotericin B and > 50% of inhibition for the other drugs. For calculations, high off-scale MICs were raised to the next $\log_2$-dilution step, while the low off-scale MICs were left unchanged (19, 20). Differences of the mean values were determined by using Kruskal-Wallis and Mann-Whitney Test with the statistical SPSS package (version 7.0). P values of < 0.05 were considered statistically significant. In addition, the interactions of ATTAF-1 and ATTAF-2 with fluconazole were investigated using a microdilution checkerboard technique in 96-well microtitre plates (21). The range of the concentration depended on the MIC results of each isolates, i.e., the maximum concentration was twofold the MIC and then serial diluted. In vitro combination of fluconazole with voriconazole against 11 *Candida* isolates from 5 different species (fluconazole –susceptible (n=5), -susceptible dose dependent (n=3) and –resistant (n=3)) were chosen as controls the interactions of newly synthesized azole compounds with fluconazole. To assess the interaction of combinations of drugs, further analysis was conducted using the fractional inhibitory concentration index (FICI). The interaction was defined as synergistic if the FICI was ≤0.5, indifferent if >0.5 – ≤4.0, and antagonistic if >4 (21).

Table 1 summarizes the MIC range, mode, geometric mean (GM) MIC, MIC_{50}, and MIC_{90} of ATTAF-1 and ATTAF-2 and five comparators against 52 clinical *Candida* isolates from 5 different species. In terms of GM MICs, anidulafungin, followed by the newly synthesized azole

compounds, exhibited potent activity against all *Candida* isolates (n=52). Interestingly, the widest range and highest MIC_{90} values for *C. albicans* against fluconazole were 0.5-128 µg/ml and 128 µg/ml, respectively. The GM MICs against *C. albicans* were 0.01, 0.21, 0.22, 0.25, 0.46, 0.74, and 2 µg/ml for anidulafungin, ATTAF-1, ATTAF-2, voriconazole, itraconazole, amphotericin B, and fluconazole, respectively. GM MICs of ATTAF-1 and ATTAF-2 were lower than fluconazole against *C. glabrata* and MIC_{50} of ATTAF-1 (0.25 µg/ml) was 5 log_2-dilution steps less than fluconazole (8 µg/ml). The checkerboard analysis of the tested compounds is summarized in Table 2. FICI results revealed synergistic effects against fluconazole-susceptible (22 of 23), -susceptible dose dependent (10 of 13) and -resistant (1 of 16) *Candida* isolates when ATTAF-1 and ATTAF-2 were combined with fluconazole. In addition, no antagonistic effect was observed against *Candida* isolates with these combinations. Remarkably, ATTAF-1 and ATTAF-2 were more active than fluconazole against *C. albicans* isolates and showed synergistic activity against 16 (76.1%) isolates (Table 2). Moreover, synergistic activity against *C. glabrata*, *C. parapsilosis, C. krusei,* and *C. tropicalis* was observed in 5 (50%), 5 (62.5%), 4 (44.4%), and 4 (100%) strains, respectively. Overall, no antagonistic effects were observed against *Candida* isolates with these combinations. Remarkably combinations of fluconazole with voriconazole as controls revealed an unfavorable antifungal effect against 11*Candida* isolates with high FICI range 1.5 – 4 in comparison with 0.25 - 2 and 0.31-2 FICI range for ATTAF1 and ATTAF2, respectively.

Based on the findings, there was no significant difference in the activity of ATTAF-1 and ATTAF-2 against specific *Candida* isolates ($P > 0.05$).

123 Considering the advances in modern medicine, leading to the availability and indiscriminate
124 use of chemotherapeutic, immunosuppressive, and broad-spectrum antifungal agents, increased
125 incidence of severe candidiasis has been recently attributed to the large population of high-risk
126 individuals (1, 2). Although fluconazole is the drug of choice for prophylaxis and treatment of
127 candidiasis, prolonged use of this agent has contributed to the development of drug resistance in
128 *Candida* isolates (20). Accordingly, novel therapeutic strategies, such as combination therapy,
129 are essential for increasing the efficacy and reducing the toxicity of antifungal agents. Major
130 attempts have been made to develop potent and safe antifungal agents with unique mechanisms
131 of action (20). Fluconazole analogues with a triazole-modified scaffold display enhanced activity
132 against *Candida* and *Cryptococcus* species, compared to filamentous fungi (15, 22). In the
133 current study, ATTAF-1 and ATTAF-2 as two promising novel azole compounds revealed that
134 either used alone or in combination with fluconazole, could show potent activity against all
135 *Candida* species. In line with the present results, Shi et al. (23) and Ramírez et al. (24) showed
136 that the newly synthesized azole-based compounds were more active than fluconazole, and
137 combination of these compounds with fluconazole could exert synergistic effects. Moreover, Ji et
138 al. (25) synthesized triazole derivatives, based on the structure of lanosterol 14α-demethylase
139 (CYP51) and revealed that these compounds have better activity against *C. albicans*, compared
140 to fluconazole. ATTAF-1 and ATTAF-2 share general structural features with the triazole
141 alcohol class of antifungal agents, while exhibiting novel and distinct characteristics. In fact, the
142 increased antifungal potency of these compounds might be due to the secondary activities or
143 actions within *Candida* isolates, not shared by fluconazole. In previous studies, the mechanism

of azole resistance, including decreased intracellular concentration of the target enzyme, changes in the drug target, and increased production of lanosterol 14α-demethylase, has been identified in different *Candida* isolates (26). The mechanisms of action in azole compounds and their derivatives have been precisely determined and established. Although our newly synthesized azole compounds showed more potent antifungal activities compared to fluconazole, the involved mechanism of action might differ from fluconazole; moreover, synergistic activities apparently did not have major potential significance since these interactions were observed mostly for isolates that are non-resistant to fluconazole and the synergistic mechanisms remained unclear. Therefore, we need to determine which subset of events and mechanisms is primarily responsible for the observed growth inhibition in the synergistic use of azole compounds. Further analysis of the diversity between different compounds and fluconazole could elucidate the underlying mechanism of action. In conclusion, although ATTAF-1 and ATTAF-2 exhibited potent activities against clinical *Candida* isolates, their effectiveness, alone or in combination with fluconazole, in the treatment of *Candida* infection needs to be determined; in addition, the underlying mechanism of action should be investigated.

Acknowledgments: This study was financially supported by a grant (nr: 2321) from the School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran which we gratefully acknowledge.

Conflict of interest: No potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

164 **References**

165 1. **Guo F, Yang Y, Kang Y, Zang B, Cui W, Qin B, Qin Y, Fang Q, Qin T, Jiang D.** 2013.
166 Invasive candidiasis in intensive care units in China: a multicentre prospective observational
167 study. J Antimicrob Chemother **68:**1660-1668.

168 2. **Hu L, Du X, Li T, Song Y, Zai S, Hu X, Zhang X, Li M.** 2015. Genetic and phenotypic
169 characterization of *Candida albicans* strains isolated from infectious disease patients in
170 Shanghai. J Med Microbiol **64:**74-83.

171 3. **Pfaller MA, Messer SA, Moet GJ, Jones RN, Castanheira M.** 2011. *Candida* bloodstream
172 infections: comparison of species distribution and resistance to echinocandin and azole
173 antifungal agents in Intensive Care Unit (ICU) and non-ICU settings in the SENTRY
174 Antimicrobial Surveillance Program (2008–2009). Int J Antimicrob Agents **38:**65-69.

175 4. **Pfaller M, Diekema D.** 2007. Epidemiology of invasive candidiasis: a persistent public health
176 problem. Clin Microbiol Rev **20:**133-163.

177 5. **Bergamasco M, Garnica M, Colombo A, Nucci M.** 2013. Epidemiology of candidemia in
178 patients with hematologic malignancies and solid tumours in Brazil. Mycoses **56:**256-263.

179 6. **Cornely O, Bassetti M, Calandra T, Garbino J, Kullberg B, Lortholary O, Meersseman**
180 **W, Akova M, Arendrup M, Arikan-Akdagli S.** 2012. ESCMID* guideline for the diagnosis
181 and management of Candida diseases 2012: non-neutropenic adult patients. Clin Infect Dis
182 **18:**19-37.

7. **Ullmann A, Akova M, Herbrecht R, Viscoli C, Arendrup M, Arikan-Akdagli S, Bassetti M, Bille J, Calandra T, Castagnola E.** 2012. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). Clin Infect Dis **18:**53-67.

8. **Chandrasekar P.** 2011. Management of invasive fungal infections: a role for polyenes. J Antimicrob Chemother**. 66:**457-465.

9. **Kothavade RJ, Kura MM, Valand AG, Panthaki MH.** 2010. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol **59:**873-880.

10. **Beyda ND, Lewis RE, Garey KW.** 2012. Echinocandin resistance in *Candida* species: mechanisms of reduced susceptibility and therapeutic approaches. Ann Pharmacother **46:**1086-1096.

11. **Brammer K, Farrow P, Faulkner J.** 1990. Pharmacokinetics and tissue penetration of fluconazole in humans. Rev. Infect Dis **12:**S318-S326.

12. **Rogers TR.** 2006. Antifungal drug resistance: limited data, dramatic impact? Int J Antimicrob Agents **27:**7-11.

13. **Shalini K, Kumar N, Drabu S, Sharma PK.** 2011. Advances in synthetic approach to and antifungal activity of triazoles. Beilstein. J. Org. Chem. **7:**668-677.

14. **Hashemi SM, Badali H, Faramarzi MA, Samadi N, Afsarian MH, Irannejad H, Emami S.** 2015. Novel triazole alcohol antifungals derived from fluconazole: design, synthesis, and biological activity. Mol Divers **19:**15-27.

15. **Hashemi SM, Badali H, Irannejad H, Shokrzadeh M, Emami S.** 2015. Synthesis and biological evaluation of fluconazole analogs with triazole-modified scaffold as potent antifungal agents. Bioorg Med Chem **23:**1481-1491.

16. **Pfaller MA, Diekema DJ.** 2012. Progress in antifungal susceptibility testing of *Candida* spp. by use of Clinical and Laboratory Standards Institute broth microdilution methods, 2010 to 2012. J Clin Microbiol **50**:2846-56.

17. **Clinical and Laboratory Standards Institute** (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition, M27–A3. Wayne: CLSI; 2008.

18. **Clinical and Laboratory Standards Institute** (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts: fourth informational supplement M27-S4. Wayne: CLSI; 2012.

18. **Cuenca-Estrella M, Gomez-Lopez A, Alastruey-Izquierdo A, Bernal-Martinez L, Cuesta I, Buitrago MJ, Rodriguez-Tudela JL.** 2010. Comparison of the Vitek 2 antifungal susceptibility system with the Clinical and Laboratory Standards Institute (CLSI) and European

220 Committee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution reference
221 methods and with the Sensititre YeastOne and Etest techniques for in vitro detection of
222 antifungal resistance in yeast isolates. J Clin Microbiol **48:**1782-1786.

223 19. **Pfaller MA, Watanabe N, Castanheira M, Messer SA, Jones RN.** 2011. Pre-clinical
224 development of antifungal susceptibility test methods for the testing of the novel antifungal agent
225 E1210 versus *Candida*: comparison of CLSI and European Committee on Antimicrobial
226 Susceptibility Testing methods. J Antimicrob Chemother **66:**2581-2584.

227 20. **Mane A, Vidhate P, Kusro C, Waman V, Saxena V, Kulkarni-Kale U, Risbud A.** 2016.
228 Molecular mechanisms associated with Fluconazole resistance in clinical *Candida albicans*
229 isolates from India. Mycoses **59:**93-100.

230 21. **Odds FC.** 2003. Synergy, antagonism, and what the chequerboard puts between them. J
231 Antimicrob Chemother **52**:1-1.24.

232 22. **Babazadeh-Qazijahani M, Badali H, Irannejad H, Afsarian MH, Emami S.** 2014.
233 Imidazolylchromanones containing non-benzylic oxime ethers: Synthesis and molecular
234 modeling study of new azole antifungals selective against *Cryptococcus gattii*. Eur J Med Chem
235 **76:**264-273.

236 23. **Shi C, Liu C, Liu J, Wang Y, Li J, Xiang M. 2015.** Anti-*Candida* Activity of New Azole
237 Derivatives Alone and in Combination with Fluconazole. Mycopathologia **180:**203-207.

24. **Ramírez J, Rodríguez MV, Quiroga J, Abonia R, Sortino M, Zacchino SA, Insuasty B. 2014.** Efficient Synthesis of Novel 3-Aryl-5-(4-chloro-2-morpholinothiazol-5-yl)-4, 5-dihydro-1H-pyrazoles and Their Antifungal Activity Alone and in Combination with Commercial Antifungal Agents. Arch Pharm **347:**566-575.

25. **Ji D, Lu J, Lu B, Xin C, Mu J, Li J, Peng C, Bao X. 2013.** Efficient synthesis and antimicrobial activity of some novel S-β-d-glucosides of 5-aryl-1,2,4-triazole-3-thiones derivatives. Bioorg. Med Chem Lett **23**:1997-2000.

26. **Gonçalves SS, Souza ACR, Chowdhary A, Meis JF, Colombo AL.** 2016. Epidemiology and molecular mechanisms of antifungal resistance in *Candida* and *Aspergillus*. Mycoses doi: 10.1111/myc.12469.

Legends:

Figure 1. Chemical structures of fluconazole, **ATTAF-1** and **ATTAF-2** compounds

Table 1. *In vitro* susceptibilities of five antifungal drugs and two novel azole compounds (**ATTAF-1 and ATTAF-2**) against 52 *Candida* isolates from five different species. MIC range, geometric mean MIC, MIC_{50}, and MIC_{90} values are expressed in µg/ml.

Table 2. Interactions between fluconazole and the novel compounds (**ATTAF-1** and **ATTAF-2**) against *Candida* isolates

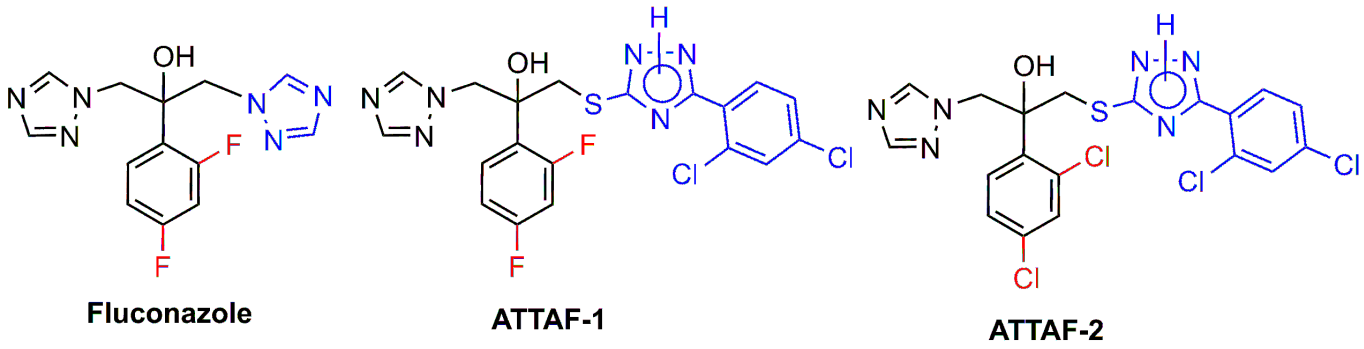

Figure 1. Chemical structures of fluconazole, ATTAF-1 and ATTAF-2 compounds

Table 1. In vitro susceptibilities of five antifungal drugs and two novel azole compounds (**ATTAF-1 and ATTAF-2**) against 52 *Candida* isolates from five different species. MIC range, geometric mean MIC, MIC_{50}, and MIC_{90} values are expressed in µg/ml.

	MICs (µg/ml)																		
Strains (no.)	**≤0.008**	**0.016**	**0.031**	**0.063**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**	**Range**	**MIC_{50}/MIC_{90}**	**Mode**	**G mean**
drugs or compounds																			
***C. albicans* (n=21)**																			
ATTAF-1			1	**11**	2	1	1	1	1		2	1				0.031-16	0.063/8	0.063	0.21
ATTAF-2				**14**	1	1		1	1			1	2			0.063-32	0.063/16	0.063	0.22
Fluconazole							**8**	5	1	4					3	0.5-128	1/128	0.5	2
Itraconazole				2		7	**7**	2	1	1	1					0.063-8	0.5/2	0.5	0.46
Voriconazole				2	6	**8**	2	1	2							0.063-2	0.25/1	0.25	0.25
Anidulafungin	**11**	8	1	1												0.008-0.063	0.008/0.016	0.008	0.01
Amphotericin B						1	**11**	5	4							0.25-2	0.5/2	0.5	0.74
***C. glabrata* (n=10)**																			
ATTAF-1				1	3	**4**						1	1			0.063-32	0.25/32	0.25	0.5
ATTAF-2				**5**	1	1	1						1	1		0.063-64	0.125/64	0.063	0.35
Fluconazole									1	3	2				**4**	2-128	8/128	128	17.14
Itraconazole						1	**4**	2	1	2						0.25-4	1/4	0.5	0.93
Voriconazole					2	1	**5**		2							0.125-2	0.5/2	0.5	0.46
Anidulafungin	**6**	3	1													0.008-0.031	0.008/0.031	0.008	0.01
Amphotericin B			1	1	2	2	1	**2**	1							0.031-2	0.25/2	1	0.25

C. krusei **(n=9)**																		
ATTAF-1				1	1	4			1		1	1			0.063-16	ND	ND	ND
ATTAF-2			1	1		1	3	1					2		0.031-64	ND	ND	ND
Fluconazole								1	3	2	1			2	1-128	ND	ND	ND
Itraconazole					1	2	3	1				2			0.125-16	ND	ND	ND
Voriconazole					3	1	2	1		1	1				0.125-8	ND	ND	ND
Anidulafungin	6	1		1	1										0.008-0.125	ND	ND	ND
Amphotericin B				2	1	2	1	2	1						0.063-2	ND	ND	ND
C. parapsilosis **(n=8)**																		
ATTAF-1			3	2	2	1									0.031-0.25	ND	ND	ND
ATTAF-2			3		4	1									0.031-0.25	ND	ND	ND
Fluconazole							3	1	1	3					0.5-4	ND	ND	ND
Itraconazole				4	1	2	1								0.063-0.5	ND	ND	ND
Voriconazole			2	3	2		1								0.031-0.5	ND	ND	ND
Anidulafungin			1	7											0.031-0.063	ND	ND	ND
Amphotericin B	6	2													0.008-0.016	ND	ND	ND
C. tropicalis **(n=4)**																		
ATTAF-1				3	1										0.063-0.125	ND	ND	ND
ATTAF-2				3	1										0.063-0.125	ND	ND	ND
Fluconazole							2	1	1						0.5-2	ND	ND	ND
Itraconazole				2		1	1								0.063-0.5	ND	ND	ND
Voriconazole				2		1	1								0.063-0.5	ND	ND	ND
Anidulafungin	3	1													0.008-0.016	ND	ND	ND

Amphotericin B	2	1	1	0.063-0.5	ND	ND	ND

Abbrevations: MIC_{50} concentration at which 50 % of the isolates were inhibited, MIC_{90} concentration at which 90 % of the isolates were inhibited, ND not determined,

Numbers in boldfaces indicate the modal value.

Table 2. Interactions between novel compounds (**ATTAF-1** and **ATTAF-2**) and fluconazole against *Candida* isolates

Species and isolate	MIC (µg/ml)				MIC (µg/ml)			
	FLC	**ATTAF-1**	FLC/ **ATTAF-1**	FICI/INT	FLC	**ATTAF-2**	FLC/ **ATTAF-2**	FICI/INT
***C. albicans* (n=21)**								
IFRC 25	0.5	0.063	0.031/0.016	0.31/SYN	0.5	0.125	0.125/0.031	0.5/SYN
IFRC 27	0.5	0.063	0.063/0.016	0.37/SYN	0.5	0.063	0.063/0.016	0.37/SYN
IFRC 37	0.5	0.063	0.063/0.016	0.37/SYN	0.5	0.25	0.125/0.031	0.37/SYN
IFRC 600	0.5	0.063	0.031/0.016	0.31/SYN	0.5	0.063	0.031/0.016	0.31/ SYN
IFRC 604	0.5	0.063	0.031/0.016	0.25/SYN	0.5	0.063	0.016/0.016	0.28/ SYN
IFRC 120	1	0.25	0.125/0.031	0.25/SYN	1	0.125	0.125/0.031	0.37/SYN
IFRC 614	1	0.063	0.031/0.016	0.28/ SYN	1	0.125	0.063/0.016	0.19/ SYN
IFRC 1055	1	0.063	0.25/0.016	0.5/ SYN	1	0.125	0.125/0.016	0.25/SYN
IFRC 10	1	0.25	0.125/0.063	0.37/SYN	1	0.125	0.125/0.031	0.37/SYN
IFRC 13	1	0.125	0.125/0.031	0.37/SYN	1	0.125	0.063/0.031	0.31/SYN
IFRC 15	1	0.125	0.063/0.031	0.31/SYN	1	0.25	0.063/0.031	0.18/SYN
IFRC 24	2	0.063	0.063/0.016	0.28/SYN	2	0.25	0.125/ 0.063	0.31/SYN
IFRC 14	2	0.25	0.5/0.125	0.75/IND	2	0.125	0.25/0.063	0.63/IND
IFRC 18	2	0.125	0.125/0.031	0.31/SYN	2	0.125	0.125/0.031	0.31/SYN
IFRC 38	4	1	0.25/0.063	0.12/SYN	4	1	0.25/0.125	0.18/ SYN
IFRC 26	4	0.5	0.5/0.063	0.25/SYN	4	0.125	0.25/0.031	0.31/SYN
IFRC 603	4	1	1/ 0.5	0.75/ IND	4	4	2/2	1/ IND
IFRC 616	4	0.25	0.063/0.063	0.26/ SYN	4	1	0.25/0.125	0.18/SYN
IFRC 1260	≥64	8	16/4	0.62/IND	≥64	32	16/16	0.62/IND
IFRC 1261	≥64	16	16/16	1.12/IND	≥64	32	16/16	0.62/IND
IFRC 1262	≥64	8	16/4	0.62/IND	≥64	16	32/8	0.75/IND
***C. glabrata* (n=10)**								
IFRC 1276	2	0.125	0.125/0.031	0.31/SYN	2	0.125	0.5/0.031	0.5/ SYN
IFRC 1274	4	0.25	1/0.031	0.37/SYN	4	0.5	1/0.063	0.37/SYN
IFRC 1275	4	0.125	0.5/0.031	0.37/SYN	4	0.25	0.5/0.031	0.25/SYN
IFRC 671	4	0.25	0.5/0.063	0.25/SYN	4	0.063	0.25/0.016	0.31/SYN
IFRC 680	8	0.25	2/0.125	1.25/IND	8	0.063	2/0.063	1.25/IND
IFRC 339	8	0.125	4/0.063	1/IND	8	0.063	4/0.063	1.25/IND
IFRC 648	≥64	32	32/8	0.5/ SYN	≥64	64	32/16	0.5/ SYN
IFRC 1063	≥64	16	64/16	1.5/IND	≥64	16	64/16	1.5/IND
IFRC 1065	≥64	32	64/8	0.72/IND	≥64	32	32/16	0.72/IND
IFRC 704	≥64	16	64/16	1.5/IND	≥64	16	64/16	1.5/IND
***C. krusei* (n=9)**								
IFRC 1251	4	0.125	1/0.031	0.5/ SYN	4	0.25	1/0.031	0.37/SYN
IFRC 1052	4	0.25	1/0.031	0.37/SYN	4	0.5	1/0.063	0.37/SYN
IFRC 1058	4	1	1/0.125	0.37/SYN	4	1	1/0.063	0.31/SYN
IFRC 85	4	4	1/1	0.5/ SYN	4	2	0.5/0.125	0.18/SYN
IFRC 1013	4	4	1/2	0.75/IND	4	4	1/2	0.75/IND
IFRC 1012	4	1	1/0.5	0.75/IND	4	2	1/1	0.75/IND
IFRC 1014	16	4	4/2	0.75/IND	16	2	4/1	0.75/IND
IFRC 1280	≥64	8	32/4	0.72/IND	≥64	64	64/64	1.5/IND
IFRC 1281	≥64	16	32/16	1.25/IND	≥64	64	64/64	1.5/IND
***C. parapsilosis* (n=8)**								
IFRC 1015	0.5	0.125	0.031/0.031	0.31/SYN	0.5	0.125	0.125/0.031	0.5/SYN
IFRC 1269	0.5	0.125	0.031/0.031	0.31/SYN	0.5	0.125	0.063/0.031	0.37/SYN
IFRC 1270	0.5	0.125	0.031/0.031	0.31/SYN	0.5	0.125	0.125/0.031	0.5/ SYN
IFRC 1271	1	0. 25	0.125/0.031	0.25/SYN	1	0.25	0.25/0.031	0.37/SYN
IFRC 1059	2	0.125	0.25/0.031	0.37/SYN	2	0.25	0.5/0.063	0.5/ SYN
IFRC 261	4	0.5	2/0.25	1/IND	4	0.5	2/0.125	0.75/IND
IFRC 1017	4	0.125	4/0.125	2/IND	4	0.25	4/0.25	2/IND
IFRC 1016	4	0.25	2/0.125	1/IND	4	0.5	4/0.5	2/IND
***C. tropicalis* (n=4)**								
IFRC 32	0.5	0.125	0.063/0.031	0.37/ SYN	0.5	0.125	0.063/0.031	0.37/SYN
IFRC 1060	1	0.125	0.125/0.031	0.37/SYN	1	0.125	0.25/0.031	0.5/SYN
IFRC 1057	2	0.25	0.5/0.063	0.37/SYN	2	0.25	0.5/0.063	0.5/ SYN
IFRC 1058	2	0.5	0.25/0.063	0.25/SYN	2	0.125	0.25/0.031	0.37/SYN

FLC Fluconazole; FICI Fractional Inhibitory Concentration Index; IND Indifference; SYN synergy; MIC minimal inhibitory concentration; INT interpretation; No number of isolates