In-vitro Antifungal Activity of Some 1,3,5-triazine Derivatives

Udaya Pratap Singh[1(✉)], Prashant Gahtori[2], Ramendra K. Singh[3]

[1]*Department of Pharmaceutical Chemistry, Sam Higginbottom Institute of Agriculture Technology & Sciences (Formerly Allahabad Agricultural Institute)(Deemed-to-be- University), Allahabad 211 007, India*

[2]*Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786 004, India.*

[3]*Nucleic Acids Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad 211002, India*

Abstract*: A series of substituted phenylthiazolyl 1,3,5-triazine derivatives were screened for their in-vitro antifungal activity (MIC and MFC) against four fungi viz. Candida species (C. albicans NCIM-3102, C. glabrata NCIM-3266), Cryptococcus neoformans NCIM-3542, and Aspergillus niger NCIM-620 using modified broth microdilution method recommended by CLSI, with reference to fluconazole and amphotericin B.*

Key words: *fungal infections, 1,3,5-triazine, broth microdilution method, physicochemical parameters.*

Introduction

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy and immunosuppressive drugs.[1] The most common fungal infections of humans are caused by the *Candida*, *Cryptococcus* and *Aspergillus* species.[2,3] In addition, gains in many areas of these disease controls are seriously jeopardized by the emergence of drug-resistant clinical isolates to the available drugs.[4] Treatment of deeply invasive infections has consistently lagged behind chemotherapy; new approaches are urgently needed for improved diagnosis, including species identification, rapid and predictive susceptibility assays, and effective treatment[5]. In view of the above and as part of the our ongoing research on electron rich nitrogen heterocycle system comprises 1,3,5-triazine antimicrobials, which is 5-aza-bioisoster of purine is an important pharmacophore and privileged structure in medicinal chemistry, has been shown to be a favorable scaffold for the design of biologically active antimicrobial compounds, including antibacterial,[6] antimalarial,[7] antiviral[8] and anticancer agents.[9] However, data concerning antifungal activities of these compounds are very limited.[10] The continuous demand to develop efficient synthetic methods has prompted the researchers to use 1,3,5-triazine as a cheap reactive for many group transformations.[11]

Thiazole is another considerable pharmacophore group and has been reported to exhibit a variety of biological activities including antibacterial and antifungal anthelmintic.[12] On the basis of immense pharmacological action triazine and thiazole, it is noteworthy to synthesize these leads by clubbing together and to screen them for potential antifungal activity.

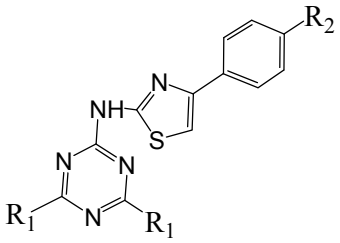
Furthermore, after extensive literature search, it was observed that, till date enough efforts have not been made to combine these two vital moieties as a single molecular scaffold and to study its antifungal activity. In our earlier work, we have developed some amino triazine derivatives bearing thiazole pendant, via nucleophilic substitution reaction of 1,3,5-triazine with various aromatic and substituted phenyl thiazole amines, as a novel potent antibacterial agent.[13,14,15] Prompted by these reports, and continuing to our interest in exploration of chemical and biological activities of 1,3,5-triazine derivatives, we

For correspondence (✉):
Udaya Pratap Singh, Phone: +91-9795142616; +91-532-2695828. Fax: +91-532-2461005.
E-mail: udaysingh98@gmail.com

herein, report *in-vitro* antifungal activities of these constitutive compounds, against few fungal species *viz. Candida*, *Cryptococcus* and *Aspergillus*.

Results and discussion

As depicted from table 1, compounds exhibits numerous degree of inhibition pattern on tested fungal species. Among the synthesized compound (**1-6)**, moderate to no activity was observed with halogen substituted aromatic amines fragments along with substituted phenyl thiazole amines. Out of which, molecule having di-(4-chloro phenyl amine) with 4-nitro phenyl thiazole amine side chains (**3**), posses no activity against fungal species. Significant activity was achieved in case of analogue having di-halogen phenyl amine fragment **1** and **2** (3-chloro,4-phenyl amine). And further 4-chloro phenyl thiazole amine **1** was found more active (MFC=2.50 for *A. niger* equivalent to fluconazole) than their corresponding 4-nitro phenyl thiazole amine derivative **2**. Compound **6**, posses no activity against all tested microorganism although it contain 4-nitro phenyl thiazole amine side chain.



These finding indicates that, presence of hydrophobic fragments and electron withdrawing groups could modulate the antifungal activity of test compounds. The reason for this result may be explained by electron density of the compounds. It has been reported that electron-donating groups increase the electron density which makes the compounds effective against microorganisms and enhances the antibacterial activity.[16] However, high electron density causes more difficult diffusion through the fungal cell and substantial activity loss may occur.[17] Thus, for a compound an optimum electron density is inevitable so as to gain a significant antibacterial activity.

Conclusion

During the course of study, varyingly substituted 1,3,5-triazines were screened for their antifungal activity. It obvious from Structure Activity Profile that, antifungal activity of these tri-substituted triazines is associated with their individual triazine nucleus as well as the phenyl thiazole motifs in their hybrid skeletons.

In the light of above said we can say that for effective triazine antifungal agent, it should have di hydrophobic fragment with electron withdrawing atom on phenyl of thiazole side chain and showed the importance of electronic environment on antimicrobial activity.

Findings from the SAR studies have encouraged us to make some modifications on basic skeleton of the obtained leads to achieve efficacious and potent and derivatives in ongoing studies. In addition, for further investigations these findings can have a good approach on scientists to synthesize similar analogues.

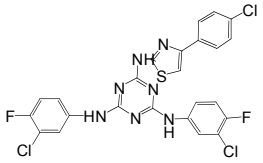
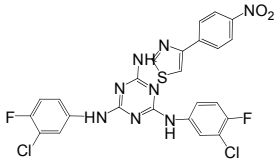
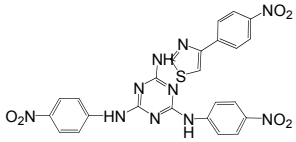
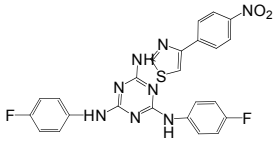
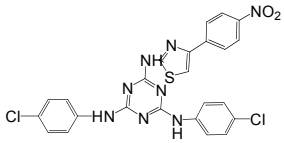
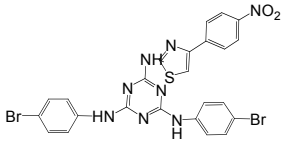
Experimental

Antifungal activity

The synthesized compounds, tri-substituted 1,3,5-triazine derivatives were screened for their antifungal activity (MIC and MFC) against four fungi *viz. Candida* species (*C. albicans* NCIM-3102, *C. glabrata* NCIM-3266), *Cryptococcus neoformans* NCIM-3542, and *Aspergillus niger* NCIM-620 using modified broth microdilution method recommended by CLSI,[18,19] using fungistatic fluconazole and fungicidal amphotericin B as standard controls. The *Candida* spp. and *C. neoformans* strains were subculture on sabouraud dextrose agar at 35^{o}C±1°C for 24 h and 48 h respectively, while *A. niger* strains were subcultured 35^{o}C±1°C for 4 days.

The *Candida* spp. and *C. neoformans* suspensions were diluted with modified broth, RPMI 1640 medium at pH 4.5 in comparison to 0.5 McFarland standard to afford final target inocula of 5.0×10^3 and 5.0×10^5 for *Candida* spp. and *C. neoformans* respectively and *A. niger* inoculum was made to 4.0×10^4 colony forming units (CFU)/ml at pH 7.3 with the same RPMI 1640 medium in 5% Alamar blue. The fungal inocula were added to the samples to achieve a final volume of 200 µL. Eight serial dilutions, starting with 20.0000 µg/ml of the compounds (dissolved in dimethyl sulfoxide, DMSO) were made using 20% dimethyl sulfoxide in normal saline to afford least concentration of 0.1563 µg/ml (=0.16 µg/ml) and transferred in duplicate to 96-well flat-bottom microplates. All organisms were examined at 630 nm prior to and after incubation (*Candida* spp. at 37°C±1°C, 18 to 24 h; while for *C. neoformans* and *A. niger* at 35°C±1°C, 72 h). The lowest test concentration that allowed no detectable growth (or no more than 20% growth for Fluconazole, for *A. niger*, no color change from blue to pink) was defined as the minimum inhibitory concentration (MIC). Minimum fungicidal concentrations (MFCs) were determined by removing 5 µl from each clear (or blue) well, transferring to relevant agar, and incubating as previously mentioned. The MFC was defined as the lowest test concentration that allows no growth of the organism on agar.

Table 2: *In-vitro* antifungal activity (MIC/MFC µg/ml) of tri-substituted-1,3,5-triazine derivatives.

Compound	Structure	*C. albicans*		*C.glabrata*		*C. neoformans*		*A. niger*	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1		1.25	5.00	2.50	5.00	5.00	20.00	2.50	2.50
2		2.50	10.00	5.00	-	2.50	10.00	5.00	-
3		5.00	-	10.00	-	2.50	-	5.00	10.00
4		10.00	-	5.00	-	-	-	-	-
5		-	-	5.00	-	-	-	5.00	10.00
6		10.00	20.00	10.00	10.00	-	-	5.00	20.00
	Fluconazole	0.63	-	0.63	-	-	-	-	-
	Amphotericin B	0.16	0.63	0.32	0.63	1.25	1.25	0.63	2.50

- Not active at the highest test concentration of 20 µg/ml.

References:

1. Garber G. An overview of fungal infections, Drugs 2001;61:1-12.
2. Russell EL, Michael EK, Michael AP. Combination systemic antifungal therapy for *cryptococcosis*, *candidiasis*, and *aspergillosis*, J Infect Dis Pharmacother 1999;3:61-84.
3. Pirofski L. Fungal infections in children with human immunodeficiency virus infection. Semin Pediatr Infect Dis 2001;12:288-295.
4. Mohamed AA, Wright A. Global anti-tuberculosis drug resistance, Clin Infect Dis 2005;41:S258-S262.
5. Sangamwar AT, Deshpande UD, Pekamwar SS. Antifungal: Need to search for a new molecular target, Indian J Pharm Sci 2008;70:423-430.
6. Roland ED. Comprehensive survey of combinatorial library synthesis. J Comb Chem 2000;2:383-433.
7. Katiyar SB, Srivastava K, Puri SK, Chauhan PMS. Synthesis of 2-[3,5-substituted pyrazol-1-yl]-4,6-trisubstituted triazine derivatives as antimalarial agents. Bioorg Med Chem Lett 2005;15:4957-4960.
8. Kreutzberger A, Rose U. Aliphatic substituted chlordihexylamino-1,3,5-triazine Arzneimittelforschung 1986;36:626-629.
9. Z. Brzozowskia Z, Czewski FS, Gdaniec M. Synthesis, structural characterization and antitumor activity of novel 2,4-diamino-1,3,5-triazine derivatives. Eur J Med Chem 2000;35:1053-1064.
10. Wang L, Zhang P, Zhang X, Zhang Y, Li Y, Wang Y. Synthesis and biological evaluation of a novel series of 1,5-benzothiazepine derivatives as potential antimicrobial agents Eur J Med Chem 2009;44:2815-2821.
11. Giacomelli G, Porcheddu A, de Luca L. [1,3,5]-triazine: A Versatile heterocycle in current applications of organic chemistry. Curr Org Chem 2004;8:1497-1519.
12. Amnerkar ND, Bhusari KP. Synthesis of some thiazolyl aminobenzothiazole derivatives as potential antibacterial, antifungal and anthelmintic agents. J Enzyme Inhib Med Chem 2011;26:22-28.
13. Gahtori P, Das A, Bhatt H. Synthesis and antibacterial assessment of N-[4-(4-substituted phenyl)-1,3-thiazol-2-yl]-1,3,5-triazin-2-amine. Indian J Pharm Sci 2009;7:79-82.
14. Gahtori P, Singh A, Ghosh SK, Das A, Archana U. Synthesis of some substituted phenylthiazolyl 1,3,5-triazine derivatives. Asian J Chem 2010;23:1189-1192.
15. Singh UP, Singh RK, Bhat HR, Subhaschandra YP, Kumar V, Kumawat MK, Gahtori P. Synthesis and antibacterial evaluation of series of novel tri substituted-*s*-triazine derivatives. Med Chem Res (*In Press*, DOI: 10.1007/s00044-010-9446-7).
16. Kumar P, Narasimhan B, Sharma D, Judge V, Narang R. Hansch analysis of substituted benzoic acid benzylidene/furan-2-yl-methylene hydrazides as antimicrobial agents Eur.J.Med.Chem. 2009;44:1853-1863.
17. Hania M.M. Eur J Chem 2009;6:S508-S514.
18. Clinical and Laboratory Standards Institute (CLSI): Reference method for broth dilution antifungal susceptibility testing of yeasts, as the document is M27-A2., Wayne, PA. USA.
19. Clinical and Laboratory Standards Institute (CLSI): Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, as the document is M38-A., Wayne, PA. USA.