Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

SYNTHESIS AND *IN VITRO* ANTIFUNGAL ACTIVITY OF CHALCONE DERIVATIVES LINKED WITH 1, 2, 4 - TRIAZOLE

Sudheer Kumar Dokuparthi[*1], J. Baloji Naik[1], Shabistha Amreen[1], B. Bhanu Priya[1], Mounika R[1], Sabiha Anjum[1], R. Suthakaran[1]

[1*]Department of Pharmacognosy, Vijaya College of Pharmacy, Munaganoor, Hyderabad, India.

ABSTRACT

Since resistance of pathogens towards currently available drug therapies is rapidly becoming a major problem, the design of new compounds to deal with fungus resistance has become one of the most important areas of antibacterial research today. Keeping in view of the above, here in, we have described the synthesis of a series of novel 3-(substituted phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide derivatives (3a, 3b and 3c) as anti-fungal agents. These compounds were prepared by Claisen-Schmidt condensation reaction for substituted benzaldehydes with N-(4*H*-1, 2, 4-triazol-4-yl) acetamide under basic conditions. The synthesized compounds were characterized by detailed spectral analysis of ^{1}H NMR, MS, and IR spectroscopy. The synthesized Chalcone derivatives 3a, 3b and 3c were screened for their anti-fungal activity by using agar diffusion method against *Aspergillus niger and Penicillium notatum*. All the compounds have shown significant anti-fungal activity. The compound 3a has shown good anti-fungal activity compared to other compounds and flucanazolewas taken as standard against both *Aspergillus niger and Penicillium notatum.*

KEYWORDS

1, 2, 4-triazole, Chalcone, Antifungal, *Aspergillus niger and Penicillium notatum.*

Author for Correspondence:

Sudheer Kumar Dokuparthi,
Department of Pharmacognosy,
Vijaya College of Pharmacy,
Munaganoor, Hayathnagar, Hyderabad, India.

Email: skdokuparthi@gmail.com

INTRODUCTION

Fungal infections pose a continuous and serious threat to human health and life[1]. These fungal infections in humans can be classified into (a) allergic reactions to fungal proteins, (b) toxic reactions to toxins present in certain fungi and (c) infections (mycoses). Many fungal infections are caused by opportunistic pathogens that may be endogenous (Candida infections) or acquired from the environment (Cryptococcus, Aspergillus infections). The other type of fungal infection, that

is, invasive fungal infections and dermatomycoses produced by fungal organisms in the individuals with increased vulnerability such as neonates, cancer patients receiving chemotherapy, organ transplant patients, and burns patients, apart from those with acquired immunodeficiency syndrome (AIDS). Other risk factors include corticosteroid and antibiotic treatments, diabetes, lesions of epidermis and dermis, malnutrition, neutropenia and surgery. In recent years, the incidence and severity of fungal diseases has increased, particularly in patients with impaired immunity. The growing number of cases of fungi involved in sepsis is a consistent trend[2]. Clinically, Candidiasis and aspergellosis account for 80-90% in immunocompromised patients. Although, arsenal antifungal drugs have expanded, currently available antifungal drugs do not meet the increasing requirements of managing infection in the complex patient populations. The development of new antifungal drugs has been constantly required in the clinical therapy[3].

Traditionally, small heterocyclic molecules have been a reliable source for discovering novel biologically active molecules. Among the family of heterocyclic compounds, 1, 2, 4-triazole derivatives represent one of the most interesting and important classes of compounds which shows the different biological activities such as anti-fungal[4-6], anti-bacterial[7,8], anti-malarial[9], anti-inflammatory[4,10], anti-cancer[11], analgesic[10], etc.

Different classes of anti-fungal agents are disclosed in the literature. Those are polyenes, imidazoles, triazoles, thiazoles and allylamines. Among the triazolesflucanazole, voricanazole and posaconazole are promising anti-fungal agents[12,13]. While these new classes of compounds are now frequently used in treatment of fungal infections, resistance to these drugs is rising. Since resistance of pathogens towards currently available drug therapies is rapidly becoming a major problem, the design of new compounds to deal with resistance fungus has become one of the most important areas of antibacterial research today.

MATERIAL AND METHODS

General

The fungal strains were obtained from the department of microbiology, Osmania University. They were preserved at 4^0C. All chemicals and solvents used in this experiment were synthetic grade purchased from Sigma-Aldrich and used without further purification except specifically mentioned.

Synthesis of targeted compounds

The compounds were synthesised as mentioned in the Scheme No.1. The novel N-(4H-1, 2, 4-triazol-4-yl) acetamide was synthesized by acetylation process of 4-amino-1, 2, 4-triazole. This intermediates was condensed with various aromatic aldehydes at reflux temperatures to afford 3-(substituted phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide. The synthetic pathway and structural formulae of the novel N3-(substituted phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamidecompounds are depicted in the Scheme No.1. The products were isolated by simple crystallization techniques. The yields of products were found to be moderate to good. The phyd=sical data of the compounds were shown in Table No.1.

All reactions were monitored by thin layer chromatography using UV light as visualizing agent. Purification was done on silica gel column (100-200 mesh) and alumina. Melting points were determined for the purified compounds on a capillary melting point apparatus and are uncorrected. Then the samples were submitted to ^{1}H NMR, Mass and IR for detailed spectral analysis. ^{1}H NMR spectra were recorded in the indicated solvent on Bruker AMX 400 MHz spectrophotometer using TMS as an internal standard. IR spectra were recorded in KBr on Perkin-Elmer FT-IR spectrophotometer. The mass spectra was recorded on waters Quattro micro LC-MS/MS.

Synthesis of 3-(nitrophenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3a)

A solution of N-(4H-1, 2, 4-triazol-4-yl) acetamide (0.01 mol) in absolute ethanol was refluxed with 4-nitro benzaldehyde in the presence of 2% NaOH (5 mL) for 10 h. Then solvent was concentrated,

cooled and poured onto ice. The solids thus obtained were recrystallized from Methanol.

Synthesis of 3-(3, 4-dimethoxy phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3b)

A solution of N-(4H-1, 2, 4-triazol-4-yl) acetamide (0.01 mol) in absolute ethanol was refluxed with 3, 4-dimethoxy benzaldehyde in the presence of 2% NaOH (5 mL) for 12 h. Then solvent was concentrated, cooled and poured onto ice. The solids thus obtained were recrystallized from Methanol.

Synthesis of 3-(3, 4-dihydoxyphenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3c)

A solution of N-(4H-1, 2, 4-triazol-4-yl) acetamide (0.01 mol) in absolute ethanol (50 mL) was refluxed with 3, 4-dihydroxy benzaldehyde in the presence of 2% NaOH (5 mL) for 18 h. Then solvent was concentrated, cooled and poured onto ice. The solids thus obtained were recrystallized from ethanol.

***In-vitro* antifungal activity**

The antifungal activity of chalcones was evaluated by the agar well diffusion method. All the cultures were adjusted to approximately 1.5×108 cfu/mL. The 30mL of Sabouraud's dextrose agar (SDA) was poured into each Petri plate and the agar plates were swabbed with 100 µL inoculate of each test fungi and kept for 15 min for adsorption. Using sterile cork borer of 8mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 5.0 mg/mL of each compound reconstituted in the Dimethylsulphoxide (DMSO). All the plates were incubated at 25^{o}C for 2 days. Antifungal activity, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8 mm. The experiments were performed in triplicate. DMSO was used as a negative control whereas fluconazole (1000 µg/mL) was used as a positive control. The results were showed in Table No.2.

RESULTS

Synthesis

Three novel triazole derivatives were synthesised in accordance with the scheme described in the Figure No.1. The structures were confirmed by the detailed spectral data and by comparing the parent molecule. The details were described below.

3-(nitrophenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3a)

White crystals, Yield: 72%, m.p:165^{0}C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.0 (s, 1H), 8.12 (d, 2H), 8.31 (d, 2H), 8.46 (d, 2H), 10.23 (s, 2H), IR (KBr, cm^{-1}): 3091, 1710, 1519, 1347, and 1105.

3-(3, 4-dimethoxy phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3b)

White crystals, Yield: 65%, m.p: 184^{0}C, ^{1}H NMR ($CDCl_3$, 400MHz) : δ 3.82 (s, 3H), 4.32 (s, 3H), 6.0 (s, 1H), 7.98 (s, 1H), 8.35 (d, 2H), 8.43 (d, 2H, , 10.52 (s, 2H), IR (KBr,cm^{-1}): 2982, 1696, 1375, 1261 and 1079.

3-(3, 4-dihydoxyphenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide (3c)

White crystals,Yield: 74%,m.p: 202^{0}C,^{1}H NMR ($CDCl_3$, 400 MHz) : δ 6.01 (s, 1H), 6.42 (br s, 1H), 6.75(br.s, 1H), 7.96 (s, 1H), 8.32 (d, 2H *),* 8.56 (d, 2H), 10.52 (s,2H).IR (KBr,cm^{-1}): 3425, 2963, 1696, 1371, 1261, and 1070.

DISCUSSIONS

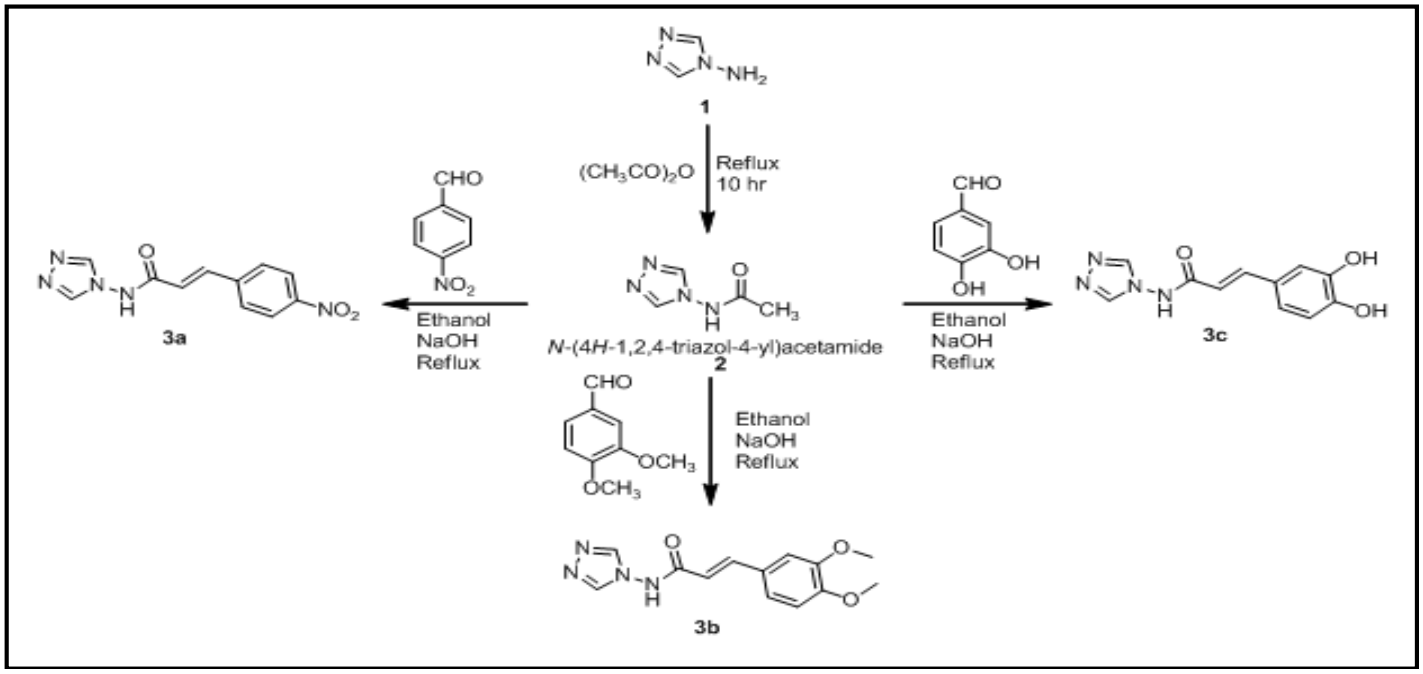
The earlier studies on triazoles revealed that they are most effective as antifungal agents. In the present case, three novel compounds which are chalcone derivatives linked with 1, 2, 4-triazoleswere synthesised by Claisen-Schmidt condensation of acetamide with various aldehydes in the presence of NaOH. The prepared hybrids of chalcones are also expected to have anti-fungal activity. Our results showed that all the three compounds (3a-c) having significant antifungal activity. Among the three compounds the compound 3a is showing the better antifungal activity. The possible mechanism involved is due to the presence of nitro group in the structure of compound 3a, it may form hydrogen bond with the targeted enzyme 14α-demethylase, which is a key enzyme in the biosynthesis of ergo sterol from lanosterol. Inhibition of this step causes the depletion of ergo sterol levels in the cell wall which leads to the poor integrity of the fungal cell wall finally leads to the death of the fungus.

Table No.1: Physical data of synthesized compounds 3a-c

S.No	Compound	Mol. Formula	Yield (%)	m.p(^{0}C)	Recrystallization solvent
1	3a	$C_{11}H_9N_5O_3$	72	165	Methanol
2	3b	$C_{13}H_{14}N_4O_3$	65	184	Methanol
3	3c	$C_{11}H_{10}N_4O_3$	75	202	Ethanol

Table No.2: The antifungal activity of compounds 3a-c

S.No	Compound	Concentration (µg/ml)	Zone of Inhibition (In mm)	
			Aspergillus Niger	*Penicillium notatum*
1	3a	10	40	24
2	3b	10	21	12
3	3c	10	35	23
4	Fluconazole	10	50	30
5	Control(DMSO)	-	10	10



Scheme No.1: Synthesis of compounds 3a-c

CONCLUSION

A Series of 3-(substituted phenyl)-N-(4H-1, 2, 4-triazol-4-yl) acrylamide derivatives 3a-c were synthesized and characterized by NMR, MS and IR spectral data. The synthesized Chalcone derivatives3a-c were screened for their anti-fungal activity by agar diffusion method against *Aspergillus niger and Penicillum notatum.* From the above results, it is concluded that all the compounds have shown promising anti-fungal activity. The compound 3a had better anti-fungal activity compared to other compounds and fluconazole was taken as standard. Some more advanced models are needed to confirm the molecular mechanism of antifungal activity for these synthesised compounds.

ACKNOWLEDGEMENT

Authors are thankful to the management of Vijaya College of Pharmacy for providing the laboratory facilities to carry out the experiment. Authors are thankful to NIPER-Hyderabad for providing analytical facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

BIBLIOGRAPHY

1. Kathiravan M K, Salake A B, Chothe A S, Dudhe P B, Watode R P, Mukta M S, Gadhwe S. The biology and chemistry of antifungal agents: a review, *Bioorganic and medicinal chemistry,* 20(19), 2012, 5678-5698.
2. Singhal N, Sharma P, Dudhe R, Kumar N. Recent advancement of triazole derivatives and their biological significance, *J. Chem. Pharm. Res,* 3(2), 2011, 126-133.
3. Dinakaran V S, Bomma B, Srinivasan K K. Fused pyrimidines: The heterocycle of diverse biological and pharmacological significance, *Der Pharma Chemica,* 4(1), 2012, 255-265.
4. N. A Agent, *Pelagia Research Library.*
5. Kokil G R, Rewatkar P V, Gosain S, Aggarwal S, Verma A, Kalra A, Thareja S. Synthesis and *in vitro* evaluation of novel 1, 2, 4-triazole derivatives as antifungal agents, *Letters in Drug Design and Discovery,* 7(1), 2010, 46-49.
6. Wahi A K, Singh A. Triazole: Recent Development and Biological Activitie, *Asian Journal of Biochemical and Pharmaceutical Research,* 1(2), 2011, 193-205.
7. Upmanyu N, Mishra P, Gupta B N, Dolly A, Garg G. Triazoles: As A Promising Medicinal Agents, *Pharmaceutical Reviews,* 4(3), 2006, 1-5.
8. Ghattas A E-B A, Moustafa H M, Hassanein E A, Hussein B R. Synthesis and Antibacterial Activity of Some New S-Triazole Derivatives, *Phosphorus, Sulfur, and Silicon and the Related Elements,* 187(12), 2012, 1469-1481.
9. Boechat N, Pinheiro L C, Silva T S, Aguiar A C, Carvalho A S, Bastos M M, Costa C C, Pinheiro S, Pinto A C, Mendonca J S. New Trifluoromethyl Triazolopyrimidines as Anti-Plasmodium falciparum Agents, *Molecules,* 17(7), 2012, 8285-8302.
10. Kumar H, Javed S A, Khan S A, Amir M. 1, 3, 4-Oxadiazole/thiadiazole and 1, 2, 4-triazole derivatives of biphenyl-4-yloxy acetic acid: Synthesis and preliminary evaluation of biological properties, *European journal of medicinal chemistry,* 43(12), 2008, 2688-2698.
11. Zhao X L, Zhao Y F, Guo S C, Song H S, Wang D, Gong P. Synthesis and Anti-tumor Activities of Novel [1, 2, 4] triazolo [1, 5-a] pyrimidines, *Molecules,* 12(5), 2007, 1136-1146.
12. Ostrowski S. Synthesis of Fused Pyrimidines from Nitroarenes via ortho-Nitroaromatic Carboximes, *Jordan Journal of Chemistry,* 3(4), 2008, 349-355.
13. Ruan Y, Jin L, Song B. Synthesis and antifungal activity of new 1-(2, 4-dichloro phenyl)-3-aryl-2-(1H-1, 2, 4-triazol-1-yl) prop-2-en-1-one derivatives, *Afr J Pharm Pharmaco,* 5(5), 2011, 602-607.

Please cite this article in press as: Sudheer Kumar Dokuparthi *et al.* Synthesis and *invitro* antifungal activity of chalcone derivatives linked with 1, 2, 4 - triazole, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 5(1), 2017, 60-64.