SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF 1, 2, 4-TRIAZOLE BENZOIC ACID DERIVATIVES

SARITA CHAURASIA*, CH DHANALAXMI, CHAWAN AWANIJA, MASEERA TAHREEM, SHAIKHA NAHID, M. DIVYA BHARATI

Department of Pharmaceutical Chemistry, Bojjam Narasimhulu Pharmacy College for Women, Vinaynagar, Saidabad. Hyderabad-500059.

ABSTRACT : A large number of 1,2,4-triazole-containing ring system have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, central nervous system stimulants, antianxiety, and antimicrobial agents. To overcome the rapid development of drug resistance, new agents should preferably have chemical characteristics that clearly differ from those of existing agents. Thus led to the design and synthesize the new antimicrobial agents. A novel series of 1,2,4-triazole benzoic acid scaffold was prepared by taking toluene as starting compound to get 4-hydrazinobenzoic acid them condense substituted benzoic to get the desired compound. The synthesized compounds were biologically screened for antifungal and antibacterial activity. The newly synthesized derivatives of triazole benzoic acid showed antifungal activity against fungal species, A.niger, C.albicans, C.neoformans and T.paradoxa and antibacterial activity against bacterial species, S.aureus, E. coli, B.subtilis and S.typhosa. It was observed that the compounds tested showed positive results for fungi and bacterial sttain on comparison to standard Miconazole and Ciprofloxacin.

Keywords: Antibacterial activity, antifungal activity, Ciprofloxacin, Miconazole, triazole.

I.INTRODUCTION

A large number of 1, 2, 4-triazole, a heterocyclic derivative exhibits important therapeutic activities such as antifungal, [1] anticonvulsant, [2] anti-tubercular, [3] antioxidant, [4] anti-inflammatory, [5] COX-2 inhibition, [6] anticancer, [7] and antimicrobial activity. [8] Furthermore, 1, 2, 4-triazole ring system has been incorporated into a wide variety of therapeutically interesting drug candidates like ribavirin (antiviral agent), rizatriptan (antimigraine agent) and fluconazole, itraconazole (an antifungal agent).[9] Thus, there is a need to explore these pharmacophores for the development of novel molecules with differentactivities.

Fungal and bacterial infections have become an important complication and major cause of mortality in immunocompromised individuals suffering from tuberculosis, cancer, AIDS, etc. [10] Amphotericin B is the most frequently used drug in the treatment of systemic mycoses in spite of its toxic effect on humans. Other antifungals like azole derivatives (fluconazole, an orally active triazole agent, and itraconazole), allylamines, thiocarbamates, fluoropyrimidines are some agents actually working in patients with impaired resistance such as those who have AIDS. While these new compounds are often used in treatment of fungal infections, resistance to these drugs is increasing, moreover many of currently available drugs have undesirable side effects, which clearly indicates an urgent need for development of new antimicrobial agents. [11,12]

Prompted by these observations, triazole derivatives may be the potential candidate to investigate as a safe antimicrobial agent, as these may not affect the host. All newly synthesized triazole derivatives were screened for their antifungal activity against fungi *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*, *Thielaviopsis paradoxa*. Also, these newly synthesized triazole derivatives were evaluated for antibacterial activity against bacterial strain *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhosa* .

II. MATERIALS AND METHODS

Reagents, starting materials and solvents were purchased from common commercial suppliers. The melting points of synthesized compounds were determined by an open capillary method on a Veego digital melting point apparatus. Mass spectral analysis was carried out using Applied Biosystem QTRAP 3200 MS/MS system in ESI mode. The infra-red spectra of the synthesized compounds were recorded on Fourier transformer infra-red spectrophotometer Model Schimadzu 8400S using potassium bromide pellets. ^{1}H NMR spectra were recorded on the Bruker NMR using DMSO-d6, tetramethylsilane as an internal standard.

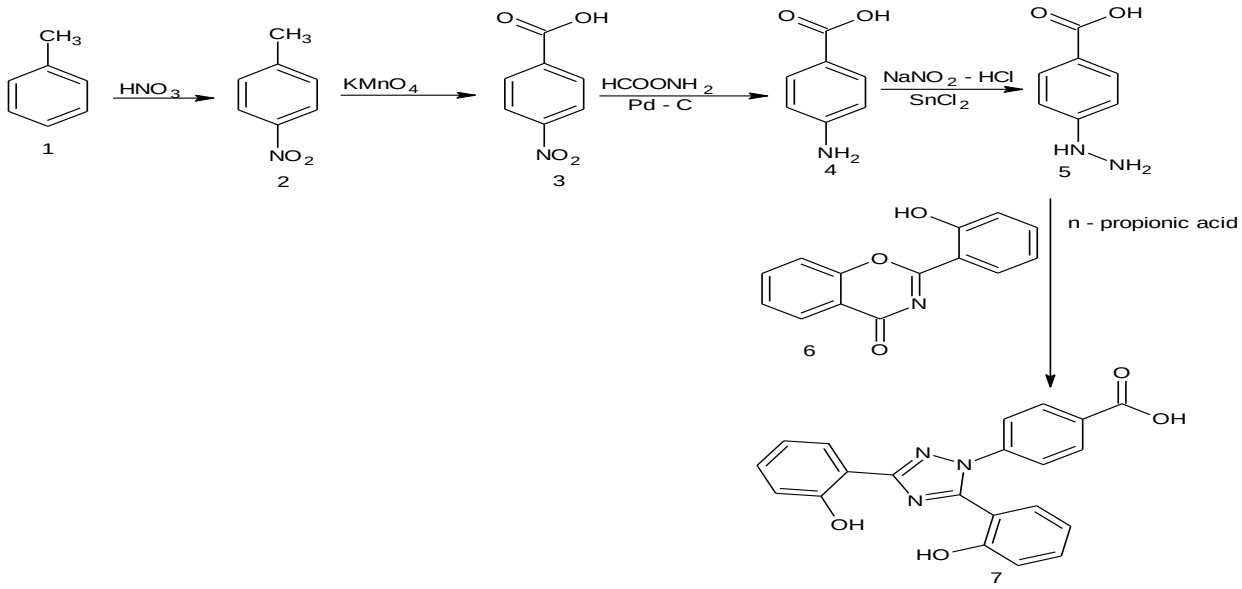

Fig.1. Reaction scheme

III.EXPERIMENTAL

3.1. Preparation of 4-nitrotoluene- (2):

Toluene **(1)** (20 g, 0.2 mol) was added to the round-bottom glass flask equipped with a magnetic stirring cooled in an ice bath. A pre-cooled mixture of 65% HNO_3 (20 cm^3) and 98% H_2SO_4 (20 cm^3) was added dropwise over 40 minutes to the vigorously stirred mixture at 5°C-10°C. The mixture was further stirred for 3 hours at 14°C. Stirring was continued for an additional hour at 25°C. The mixture was then poured into ice-water (300 cm^3) and extracted three times with ether (50 cm^3). The combined organic layers were successively washed with 5% $NaHCO_3$ solution (50 cm^3) and twice with water (50 cm^3). Removal of the solvent under vacuum yielded oil. The crude oil was crystallized with chilled n-hexane to obtain 4-nitrotoluene **(2).**

3.2. Preparation of 4-nitrobenzoic acid- (3):

4-Nitrotoluene **(2)** (14.4 g, 0.1 mol) and magnesium sulphate heptahydrate (29 g) in water (70 cm^3) was added to a round-bottom glass flask equipped with a magnetic stirring bar and reflux condenser. The mixture was stirred vigorously and heated at 85°C. To the stirred reaction mass, a solution of potassium permanganate (29 g, 0.18 mol) in (150 cm^3) boiling water was added slowly over 30 minutes at 85°C. The temperature of the reaction mixture was maintained at 85°C for 2 hours. The mixture was then allowed to cool to 25°C and the brown precipitate formed was filtered off Ethanol (20 cm^3) was added to the filtrate and the mixture was again heated for 45 minutes at 85°-90°C, then cooled to 10°C and the precipitate formed was filtered off the reaction mass was cooled to 10°C and the pH was adjusted to 3-4 using 20% H_2SO_4 (200 cm^3). The reaction mass was stirred for 1 hour at 10°C and filtered. The solid obtained was washed with water (4 cm^3) and dried in desiccator affording 4-nitrobenzoic acid **(3).**

3.3. Preparation of 4-aminobenzoic acid (4) :

4-Nitrobenzoic acid (3, 10 g, 0.06 mol) and methanol (100 cm^3) were added to a round-bottom glass flask equipped with a magnetic stirring bar and reflux condenser. Ammonium formate (10 g, 0.16 mol) and 10% Palladium on carbon 50% wet (1 g) were added to the solution. The reaction mass was reflux at 90° - 100°C for 3hour. After completion of reaction, the catalyst was filtered off using celite and the solvent was removed under vacuum. The product obtained was dried in a desiccator to afford 4-aminobenzoic acid (4).

3.4. Preparation of 4-hydrazinobenzoic acid (5):

4-Aminobenzoic acid **(4,** 6 g, 0.04 mol), water (30.0 cm^3) and concentrated HC1 (30.0 cm^3) were added to a round-bottom glass flask equipped with a magnetic stirring bar and cooled in an ice bath. 20% Aqueous $NaNO_2$ solution (25.0 cm^3) was added maintaining the temperature at 0*-10°C and the mixture was stirred for 0.5 hour. A mixture of $SnC1_2.2H_20$ (20.0 g, 0.105 mol) and concentrated HC1 (40 cm^3) were then added to the reaction mass maintaining the temperature at 0°C-10°C followed by stirring in an ice-bath for 0.5 hour. The solid was filtered and then dried. The white powder obtained was 4-hydrazinobenzoic acid **(5).**

3.5. Preparation of 4-[3, 5-bis (2-hydroxypheny1)-1H-1, 2, 4-triazol-1yl]-benzoic acid(7):

n-Propionic acid (30 cm^3) was added to a round-bottom glass flask equipped with a magnetic stirring bar and reflux condenser. 2-(2-Hydroxyphenyl)-4H1,3-benzoxazin-4-one **(6,** 6 g, 0.39 mol) and 4-hydrazinobenzoic acid **(5,** 4.5 g, 0.3 mol) were added to the reaction mass and reflux at temperature 100°C for 2hour. After completion of the reaction,

ethyl acetate (40 cm^3) was added to the reaction mass. The suspension was further stirred for 30 minutes maintaining the temperature at 15°-20°C. The resulting crystalline product was collected by filtration and dried to give 4-[3,5-bis(2-hydroxypheny1)-1H-1,2,4-triazol-1yl]-benzoic acid **(7).**

4-[3,5-bis(2-hydroxypheny1)-1H-1,2,4-triazol-1yl]-benzoic acid (7a)

77% yield - M.P. =142-144°C, IR (KBr) n/cm: 3109.04, 2935.46, 1504.37, 1446.51. ^{1}H NMR (DMSO-d_6 , 400 MHz): d 7.26-7.92 (m, 11H), 10.07 (s, 1H), 13.72 (s, 1H). MS-API: $[M + H]^+$ 360.02 (calculated 361) Anal. calculated for $C_{24}H_{22}N_4O_5$: C, 49.87; H, 3.63; N, 15.51; Found: C, 49.53; H, 3.23; N, 15.01;

4-[3,5-bis(2-nitropheny1)-1H-1,2,4-triazol-1yl]-benzoic acid (5b)

79% yield - M.P. =125-127°C, IR (KBr) n/cm: 3107.04, 2932.26, 1505.17, 1443.53. ^{1}H NMR (DMSO-d_6 , 400 MHz): d 7.33-7.88 (m, 10H), 10.42 (s, 1H), 13.86 (s, 1H). MS-API: $[M + H]^+$ 350.02 (calculated 351) Anal. calculated $C_{15}H_{12}Cl_2N_4S$: C, 51.29; H, 3.44; Cl, 20.19; N, 15.95; S, 9.13 Found: C, 51.30; H, 3.20;; N, 15.55

4-[3,5-bis(2-chloropheny1)-1H-1,2,4-triazol-1yl]-benzoic acid (7c)

81% yield - M.P. =167-169°C, IR (KBr) n/cm: 3108.02, 2934.44, 1501.20, 1445.45. ^{1}H NMR (DMSO-d_6 , 400 MHz): d 7.18-7.93 (m, 11H), 10.02 (s, 1H), 13.12 (s, 1H). MS-API: $[M + H]^+$ 300.08 (calculated 300) Anal. calculated: C, 59.98; H, 4.36; N, 18.65;.68 Found: C, 59.20; H, 4.25; N, 18.20.

3.5 Antimicrobial Activity Study

In view of developing new class of antimicrobial agents, synthesized novel compounds and were screened for their *in vitro* antimicrobial activities to determine zone of inhibition at 100 μg/mL against two gram +ve and two gram –ve bacteria Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhosa and four fungi Aspergillus niger, Candida albicans, Cryptococcus neoformans, Thielaviopsis paradoxa strains using cup plate method where inoculated Muller-Hilton agar for bacteria and Sabouraud dextrose agar for fungi was poured onto the sterilized petri dishes (25–30 mL each petri dish). The poured material was allowed to set (30 min) and thereafter the 'cups' (6mm diameter) was made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into these cups the test compound solution (0.1mL) was added with the help of a micro pipette. The plates were incubated at 37°C for 14h for bacteria and 30 h for fungi and the results were noted. The test solution was prepared by DMSO as solvent. Clinically antimicrobial drugs Ciprofloxacin and Miconazole were used as the positive control and DMSO was used for blank.

Antibacterial Activity

The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains by the serial plate dilution method.[13] Ciprofloxacin was used as the standard drag andthe antibacterial activity was determined by measuring the diameter of inhibition zone. The results of such studies are given in the Table 1.

Antifungal Activity

The newly synthesized compounds were also screened for their antifungal activity against four fungal strains by the serial plate dilution method.[13] Meconazole was used as the standard drug and the antifungal activity was determined by measuring the diameter of the inhibition zone. The results of such studies are given in Table 2.

Table 1: Antibacterial activity of synthesized compound

Compound	S.aureus		E. coli		B.subtilis		S.typhosa	
	2mg	5mg	2mg	5mg	2mg	5mg	2mg	5mg
7a	-	-	-	+	-	-	-	+
7b	-	+	-	++	+	+	+	++
7c	+	-	+	+	+	+	+	+
Ciprofloxacin	+	+	+	++	+	++	+	++

Inhibition zone diameter in mm: (+) 11-14mm, (++) 15-18mm

Table 2: Antifungal activity of synthesized compound

Compound	A.niger		C.albicans		C.neoformans		T.paradoxa	
	2mg	5mg	2mg	5mg	2mg	5mg	2mg	5mg
7a	-	+	-	+	+	++	+	+
7b	-	-	-	+	-	+	-	-
7c	+	+	-	+	-	+	+	+
Miconazole	-	+	+	++	+	++	-	++

Inhibition zone diameter in mm: (+) 11-14mm, (++) 15-18mm

IV.RESULT AND DISCUSSION

All the synthesized compounds underwent antifungal evaluation against fungal species: *Aspergillus niger, Candida albicans, Cryptococcus neoformans, Thielaviopsis paradoxa* and antibacterial evaluation against bacterial strain *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhosa*. The results

obtained from the evaluation study for antibacterial and antifungal activity are provided in Table 1 and Table 2. The results so obtained indicated that compound 7a antifungal activity near to Meconazole while other synthesized compound showed less potent antifungal activity than Meconazole
Compound 7b showed antibacterial activity near to ciprofloxacin.

V. CONCLUSION

A novel series of 1,2,4-triazole benzoic acid was successfully synthesized and tested for antifungal activity against four fungal strains and antibacterial activity against four bacterial strains. The results of the biological studies revealed that among the four fungal strains, C.albicans and C.neoformans were found to be more sensitive to the all the compound at a concentration of 5mg and compound 7a shows better activity against all testes fungi. From the study, it was concluded that the 1, 2, 4-triazole benzoic acid derivatives at a concentration of 5mg showed antibacterial activity against bacterial species, E. coli *and S. aureus*. Amongst 3 compounds synthesized, 7c showed antibacterial effect nearr to clinical candidate Cephalosporin.

REFERENCES

[1] Tsukuda T, Shiratori Y, Watanabe M, Ontsuka H, Hattori K, Shirai M, *et al.* Modeling, synthesis and biological activity of novel antifungal agents (1). Bioorg Med Chem Lett 1998; 8:1819-24.

[2] Küçükgüzel I, Güniz Küçükgüzel S, Rollas S, Otük-Sanis G, Ozdemir O, Bayrak I, *et al.* Synthesis of some 3-(arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4H-1,2,4-triazole derivatives and their anticonvulsant activity. Farmaco 2004; 59:893-901.

[3] Husain MI, Amir M. Synthesis of some new substituted thiosemicarbazides and triazoles as possible anticonvulsants. J Indian Chem Soc 1986; 63:317-9..

[4] Valentina P, Ilango K, Deepthi M, Harusha P, Pavani G, Sindhura KL, *et al*. Antioxidant activity of some substituted 1,2,4-triazo-5-thione Schiff base. J Pharm Sci Res 2009; 1:74-7.

[5] Sahin G, Palaska E, Kelicen P, Demirdamar R, Altinok G. Synthesis of some new 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones and their anti-inflammatory activities. Arzneimittelforschung 2001; 51:478-84.

[6] Navidpour L, Shafaroodi H, Abdi K, Amini M, Ghahremani MH, Dehpour AR, *et al*. Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4H-1,2,4-triazoles as selective COX-2 inhibitors. Bioorg Med Chem 2006; 14:2507-17.

[7] Demirbas N, Ugurluoglu R, Demirbas A. Synthesis of 3-alkyl (aryl)-4-alkylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-ones and 3-alkyl-4-alkylamino-4,5-dihydro-1H-1,2,4-triazol-5-ones as antitumor agents. Bioorg Med Chem 2002; 10:3717-23.

[8] Holla BS, Sarojini BK, Rao BS, Akberali PM, Kumari NS, Shetty V. Synthesis of some halogen-containing 1,2,4-triazolo-1,3,4-thiadiazines and their antibacterial and anticancer screening studies - Part I, II. Farmaco 2001; 56:565-70.

[9] Shivarama Holla B, Sooryanarayana Rao B, Sarojini BK, Akberali PM, Suchetha Kumari N. Synthesis and studies on some new fluorine containing triazolothiadiazines as possible antibacterial, antifungal and anticancer agents. Eur J Med Chem 2006; 41:657-63.

[10] Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev 1996; 9:499-511.

[11] Alexander BD, Perfect JR. Antifungal resistance trends towards the year 2000. Implications for therapy and new approaches. Drugs 1997; 54:657-78.

[12] Sauleau A, Sauleau J. Phosphorus Sulfur Silicon 2000;167: 219.

[13] Silvia N, Lo´pez V, Marý´a A, Castelli Susana, Zacchino N, Domý´nguez, *et al* . Bioorganic and Medicinal chemistry, 2001, 9,1999-2013.