Synthesis and Evaluation of Antimicrobial Activities of New 1,2,4-Triazole Derivatives

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Abstract

Extensive research is being carried out in medicinal chemistry, on 1,2 ,4- triazoles due to it's wide pharmacological features. It is the core structure of many natural and synthetic pharmaceuticals. This versatile 1,2,4-triazole moiety can be structurally modified to get newer analogues to overcome the résistance problem. The presented work describes synthesis of a new series of 1,2,4-triazole derivatives namely 3,5-diarylsubstituted-1,2,4-triazole derivatives via benzoxinones. The corresponding 5-substituted salicylamides, required as precursors were prepared as per the literature. The treatment of benzoxinones with hydrazine hydrate afforded the 3,5-diarylsubstituted-1,2,4-triazoles. The structure of each novel compound was confirmed on the basis of ^{1}H NMR spectral data and elemental analysis .Upon being tested for their antimicrobial activity against Gram positive, Gram negative bacteria, and fungi, some of the compounds exhibited moderate inhibitory effects against both Gram positive and negative bacteria and certain strains of fungi.

Keywords — *Antimicrobial activity, Antifungal activity, benzoxinones, 3,5-diarylsubstituted-1,2,4-triazole derivatives, Gram positive and Gram negative bacteria and fungi*

I. INTRODUCTION

One of the major problems of the pharmaceutical world today is the increasing drug resistance to the current chemotherapeutic agents. Microbial infections can be life-threatening and are difficult to cure because of their re-occurrence. Hence design of new compounds for resistant fungi & bacteria has become one of the important areas of the antimicrobial research to date.

Currently triazole based formulations containing fluconazole, tubeconazole and metaconazole are used frequently due to their broad spectrum activities. 1,2,4-triazole is not only an important class of five member heterocyclic ring possessing diverse therapeutic applications but also the core structure of many synthetic compounds having wide a pharmacological spectrum including activities such as antimicrobial [1],[2],[3],[4] anti-inflammatory, analgesic[5], [6,]and fungicidal [7],[8]. In addition to their pharmacological properties, their low toxicity gives them a variety of uses in medicinal chemistry. Apart from their wide applications in the pharmaceutical industry, 1,2,4-triazole derivatives like triadimefon, propiconazole, and flupoxam can also be used effectively as fungicides in crop protection. 1,2,4- triazoles can be functionalized by substitution at 1,3,5-positions to influence change in properties to make them even more applicable in the medicinal as well as agricultural industries.

In the proposed work, research was focused on synthesizing new derivatives of 1,2,4-triazoles by substituting phenyl groups at 3 and 5-position of the triazole ring. The phenyl ring having electron withdrawing or electron donating groups can alter the polarity which in turn will affect the efficacy. The antimicrobial activity of these new derivatives can be further evaluated to understand their properties.

II. MATERIALS AND METHODS

^{1}H-NMR spectra were recorded on Varian NMR spectrometer, operating at 600MHz in $CDCl_3$ for the substituted benzamides and in DMSO-d^6 for the triazoles. Elemental analysis data was obtained on Thermo-finnigan C,H,N analyser. The reaction course and purity of the final products, & monitoring the reaction course, was followed by TLC on Silica gel (Fluka F_{60}254 20 x 20; 0.2mm) using toluene: ethyl acetate as eluent. The melting points were determined by open capillary method and column chromatography was performed using silica gel.

III. RESULTS AND DISCUSSIONS

5-substitued-salicylicylic acids (1a-e) were converted to acid chlorides, followed by their conversion to corresponding salicylamides (2a-e) [9], Scheme-1. Salicylamide on reaction with benzoyl chloride gave benzoxazinone (3a-h) [10] ,[11], Scheme-2. Benzoxazinone on reaction with hydrazine hydrate gave the new compounds 3-(2-Hydroxyphenyl)-5-phenyl-1,2,4-triazoles (4a-e) [12], Scheme-2 .

A. General Synthetic Procedures

1) Synthesis of 5-Substituted salicylamides (2a-e): Preparation of 5- Chlorosalicyloyl chloride: To a 100ml 3-necked round bottom flask with magnetic bar, was added 5-Chlorosalicylic acid (8.63g, 0.05mol) and 50 ml chloroform. To this Thionyl chloride (4.56ml, 0.0625 mol) was added slowly at 50-55^{0}C, followed by 4-5 drops of dimethyl formamide. The reaction mixture was stirred for 2

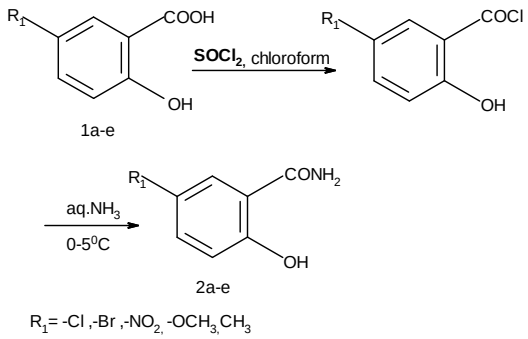
hours at reflux temperature. Completion of the reaction was confirmed by thin Layer chromatography. Excess of thionyl chloride and chloroform were distilled under vacuum to give 5-Chlorosalicyloyl chloride. (8.60g, 90%)

5-Chlorosalicyloyl chloride obtained as above, was dissolved in 50ml chloroform and treated cautiously with 170ml concentrated ammonia solution (d 0.88) maintaining the temperature 0-5^{0}C, and then stirred in the same ice-bath for 3-4 hours. The reaction mixture was then kept overnight at room temperature, next day solid separated out. This was filtered, washed with water & dried to isolate 5-Chlorosalicylamides. The crude solid was crystallised to get pure 5-Chlorosalicylamide (5.14g, 60%), MP = 230-232^{0}C.

The following 5-Substituted salicylamides were prepared as above.

5-Bromosalicylamide, MP = 240-243^{0}C,
5-Nitro-salicylamide, MP = 220-222^{0}C,
5-Methoxy-salicylamide, MP = 202^{0}C,
5-Methyl-salicylamide, MP = 182^{0}C

Scheme-1

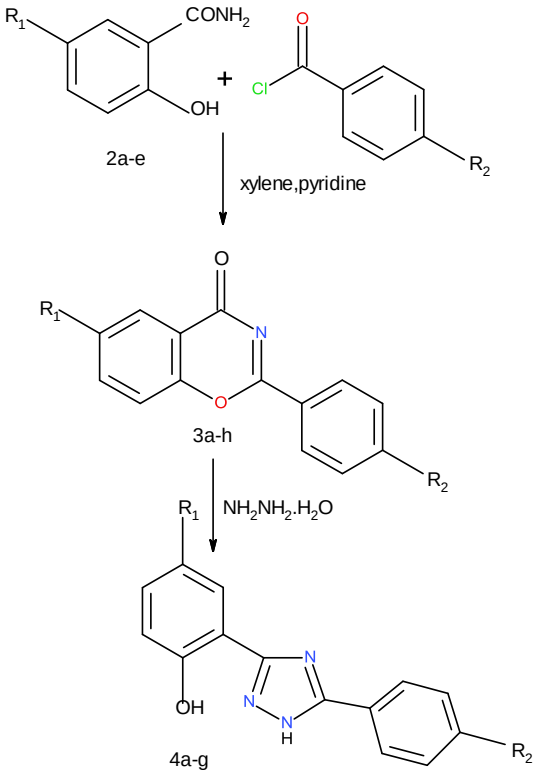


2) Synthesis of Aryl-1,3-benzoxazin-4-ones. Scheme-2:

2-(4'-Chlorophenyl)-4H-1,3-benzoxazin-4-one (3a): To the suspension of salicylamide (6.85g,0.5mol) in xylene (30ml) and pyridine (1ml) was added, 4-chlorobenzoyl chloride (10.15g,0.058mol) dissolved in 35ml xylene over a period of 30 minutes at 90-100^{0}C under nitrogen atmosphere. The assembly was fitted with Dean-stark apparatus to collect the water formed during the reaction at reflux temperature. After 2 hours, 25 ml of fresh xylene was added and distillation was continued for another 30 minutes. Xylene was distilled off under vacuum & product was crystallized from ethanol. Yield = 68%,
MP= 173-175^{0}C.

The following benzoxinones (3a-h) were prepared by similar procedure.

Scheme-2



Compsound	R_1	R_2
3a	H	-Cl
3b	H	-Br
3c	H	-OCH_3
3d	H	-NO_2
3e	H	-H
3f	-Br	-H
3g	-CH_3	-H
3h	-OCH_3	-H

Compound	R_1	R_2
4a	H	H
4b	H	-OCH_3
4c	H	-NO_2
4d	H	-Cl
4e	Br	-H
4f	-OCH_3	-H
4g	-CH_3	-H

2-(4'-Methoxyphenyl)-4H-1, 2-(4'-chlorophenyl)-4H-1,3-benzoxazin-4-one (3a): (Yield: 8.76 g,68%) MP= 173-175^{0}C (transparent crystals); IR (KBr) cm^{-1} 1735s,1675m, 1560s, 1495s, 1475m, 1340m,1290 ,1235m, 860m,770s. Calcd. for $C_{14}H_8ClNO_2$,: C, 65.2; H, 3.1; N, 5.3%. Found: C, 65.0; H, 3.3; N, 5.4%

2-(4'-Bromophenyl)-4H-1,3-benzoxazin-4-one (3b): (Yield: 9.82, 65%) MP= 140-142^{0}C (off white crystals); IR (KBr) cm^{-1} : 1745s, 1680m, 1580s, 1365m, 1290, 1245m, 860m,780s.
Calcd. for $C_{14}H_8BrNO_2$,: C, 55.28; H, 3.0 ;N ,4.75 Found: C, 55.63; H, 2.65;N,4.75%

2-(4'-Methoxyphenyl)-4H-1,3-benzoxazin-4-one (3c) (Yield: 3.0g, 60%) MP=183-185°C IR (KBr) cm^{-1} : 1750s, 1685s, 1600m, 1455m, 1380s, 1285m, 1245m, 780m, 760m. Calcd. for $C_{15}H_{11}NO_3$: C, 71.15;H, 4.35; N,5.53%. Found: C, 71.28; H, 4.26; N, 5.62.

2-(4'-Nitrophenyl)-4H-1,3-benzoxazin-4-one (3d) (Yield: 3.21g, 60%) MP= 173-175^{0}C (yellow needles);
IR (KBr) cm^{-1} : 1750, 1690, 1630, 1540 ,880, 720. Calcd. for $C_{14}H_8N_2O_4$: C, 62.7; H, 3.0; N, 10.4% Found: C, 62.8; H, 3.3; N,10.6

2-Phenyl-4H-1,3-benzoxazin-4-one (3e)
(Yield: 4.68g, 70%) MP = 98-100 ^{0}C (off white crystals), IR (KBr) cm^{-1}: 1730s, 1680, 1582, 1490, 1295, 1260, 1130 m , 890, 760, 725.
Calcd. for $C_{14}H_9NO_2$: C,75.34; H, 4.03; N, 6.28%. Found: C, 74.92; H, 3.9 ; N, 6.0

2-Phenyl-6-bromo-4H-1,3-benzoxazinone (3f)
(Yield 3.74, 62%) MP = 176-178^{0}C IR. (KBr): 1745, 1670, 1620m, 1550s, 1370, 1275, 960m, 780w, 740s cm-l.. Calcd. for $C_{14}H_8BrNO_2$: C, 55.63; H, 2.65; N,4.64% Found: C , 55.18 ; H,2.72 ; N,4.74

2-Phenyl-6-methyl-4H-1,3-benzoxazinone (3g)
(Yield 2.95g, 70%), MP = 117-119^{0}C IR. (KBr): 1735, 1680, 1610m, 1540s, 880m, 820, 740s cm-l, ,.
Calcd . for $C_{15}H_{11}NO_2$: C, 63.71; H, 4.64; N, 5.90%. Found: C63.40; H3.42; N5.62

2-Phenyl-6-methoxy-4H-1,3-benzoxazinone (3h)
(Yield 3.18, 63%.) MP =165-167^{0}C IR (KBr): 1740, 1685, 1630m, 1530s, 1275, 1240, 880, 820, 720 cm-l. Calcd . for $C_{15}H_{11}NO_3$: C, 71.15; H,4.35; N,5.53% Found: C, 71.0; H,4.5 ; N,5.62 %

3) Synthesis of 3,5-diarylsubstituted-1,2,4-triazle derivatives :

3-(2-Hydroxyphenyl)-5-phenyl-1,2,4-triazole.(4a):
To a stirred solution of 2-phenyl-4H-1,3-benzoxazin-4-one (2.50g,0.011mol) in methanol (20ml) and pyridine (4-5drops), hydrazine hydrate (1.2ml 98%) was cautiously added at 45-50^{0}C over 10 minutes under nitrogen atmosphere. The reaction mass was stirred at reflux for two hours and was then concentrated to distil off methanol on rota-vap.100ml cold water was added to the concentrated reaction mass to precipitate off dull white solid which was filtered and dried in oven to get 1.20g of the product. MP = 228 ^{0}C, yield 45%.

The following triazoles were prepared by similar procedure. **(4a-h)**

3-(2-Hydroxyphenyl) -5-phenyl-1,2,4-triazole (4a)
δ ppm. **:** 14.44 (1H, s , NH); 11.37(1H, s, OH); 7.55 (2H, t, m-Ph); 7.53 (1H , t, p-Ph); 8.09 (1H, d, o-Ph) 7.04 (1H, t, m-PhOx); 7.37 (1H, t, p-PhOx); 8.03 (1H,
d, m-PhOx); 7.00(1H ,d, o-PhOx).
Anal. calculated for $C_{14}H_{11}N_3O$: %Cal: C, 70.87; H, 4.67; N, 17.71. %Found: C, 70.86 , H,4.71; N,17.75.

3-(2-Hydroxyphenyl)-5-(4'-methoxyphenyl)-1,2,4-triazole (4b)
δ ppm.**:** 14.50 (1H, s, NH); 11.40 (1H, s, OH); 8.20 (3H, d, o-PhOx, m-PhOMe); 7.35(1 H , t, p-PhOx); 7.1(1H, d, o-PhOMe) ;7.03 (1H ,d ,o-PhOMe); 6.80 (2H, t, m-PhOx); 3.8(3H ,s, OMe)
Anal. Calcd. for $C_{15}H_{13}N_3O_2$ %Cal: C,67.42 ; H, 4.87; N, 15.73. % Found: C, 65.73; H, 4.58; N, 16.20.

3-(2-Hydroxyphenyl)-5-(4'-nitrophenyl)-1,2,4-triazole (4c)
δ ppm.**:** 14.50 (1H, s ,NH); 11.25 (1H, s, OH) ; 8.35 (4H, d, o-PhNO$_2$, o-PhOx, m-PhOx); 8.03(1H, d, m-PhNO$_2$), 7.38 (1H, t, p-PhOx); 7.05(1H ,d, m-PhNO$_2$); 7.00 (1H , t, m-PhOx)
Anal. calculated for $C_{14}H_{10}N_4O_3$: %Cal: C, 59.57; H,
3.55 ; N,19.86. %Found: C , 58.99; H, 3.34; N,20.77.

3-(2-Hydroxyphenyl)-5-(4'-chlorophenyl)-1,2,4-triazole (4d)
δ ppm. **:** 14.40 (1H, s , NH); 11.25 (1H, s, OH); 8.1(2H ,d ,o-PhCl); 8.0 (1H , d, m-PhCl), 7.6(2H,s) 7.4 (1H ,t ,o-PhOX), 7.0 (2H, m , PhOx)
Calculated for $C_{14}H_{10}N_3OCl$. %Cal : C, 61.87%; H: 3.68%; N: 15.46%. %Found: C, 62.08%,
H: 3.91%; N: 14.86.

3-(2'-Hydroxy-5'-bromophenyl)-5-phenyl-1,2,4-triazole (4e)
δ ppm.**:** 14.40 (1H, s ,NH); 11.50 (1H, s ,OH); 8.2 (1H, s); 8.08 (2H ,d); 7.50 (4H ,m); 7.0 (1H, d)
Calculated for $C_{14}H_{10}NOBr$. %Cal- C: 53.16; H,:3.16; N, 13.29. %Found- C: 53.31, H: 3.06, N: 13.40.

3-(2'-Hydroxy-5'-methoxyphenyl)-5-phenyl-1,2,4-triazole (4f)
δ ppm. : 14.40(1H, s,NH); 11.00(1H, s, OH); 8.07 (2H ,d); 7.52-7.57 (3H ,m); 7.50 (1H, m); 6.97 (2H,d), 3.80 (3H ,s)
Calculated for $C_{15}H_{13}N_3O_2$.%Cal- C: 67.41; H:4.86; N: 15.73. %Found C: 67.79, H: 4.83; N: 15.94.

3-(2'-Hydroxy-5'-methylphenyl)-5-phenyl-1,2,4-triazole (4g)
δ ppm. : 14.40 (1H, s , NH); 11.50 (1H, s, OH) ; 8.07 (2H, d); 7.82 (1H , s); 7.54 (3H ,m); 7.2 (1H ,d); 6.9 (1H ,d), 2.30 (3H , s) Calculated for $C_{15}H_{13}N_3O$,
% Cal- C: 71.71; H: 5.17, N:16.73 %Found- C: 71.75, H:5.02, N:16.92.

B. Antimicrobial activity

All the triazoles prepared above were tested for their antibacterial activity[13] against Gram positive Bacteria *Bacillus Subtilis*, *Staphylococcus Aureus*, and Gram negative bacteria *Escherichia Coli*, *Pseudomonas Aeruginosa.* Antifungal activity was screened against fungal species of *Candida Albicans* and Aspergillus *Brasilense.* All the solutions were prepared in dimethyl sulhpoxide (DMSO). A simple susceptibility screening using filter paper disc method was performed in duplicate using fresh

Mueller Hinton agar medium. This agar medium was treated with inoculum containing 10^6 colony forming unit per ml of each bacterial culture. Filter paper discs of 8mm diameter were cut from the agar wells and 200µg/100µl of the triazole solutions was added to the wells. The plates were incubated in the upright position at 37^0C for 24 h. Wells containing the same volume of DMSO were

Table1: Antibacterial & Antifungal Activities of the Synthesied 1,2,4-Traizoles, concentration (10mg/ml)

Compound	Microorganisms and Inhibition Zone (mm)					
	Bacillus Subtilis	***Staphylococcus Aureus***	***Escherichia Coli***	***Pseudomonas Aeruginosa***	***Candida Albicans***	***Aspergillus Brasilense.***
4a	8	8	8	8	8	8
4b	8	8	8	8	8	8
4c	8	8	8	8	10	10
4d	8	10	10	8	8	8
4e	8	10	10	8	8	8
4f	8	8	8	8	8	8
4g	8	8	8	8	8	8
DMSO	8	8	8	8	8	8
Amoxicillin	28	25	25	18	16	17
Fluconazole	26	22	21	21	25	25

Results are compared with the diameter of the inhibition zone**:** 8mm**:** No antimicrobial activity **;** >8mm: Antimicrobial activity positive.

prepared as negative control. Standard antimicrobial discs of amoxicillin 25µg/100µl (250mg) and fluconazole 20µg/100µl (200mg) were use as positive control. Three replicates were carried out for each triazole against the test organism. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (in mm).

From the above results , it is seen that 1,2,4-triazoles having hydroxyl group along with chloro or bromo substituents in the same benzene ring (4d & 4e) show higher antibacterial activity against *Bacillus Subtilis and Staphylococcus Aureus* while compound (4c) having hydroxyl and nitro group in the same benzene ring showed higher antifungal activity against *Candida Albicans and Aspergillus Brasilense*.

However the activities exhibited by standards are higher that the compounds under study at much lower concentration.

IV. CONCLUSION

Seven new 3,5-(substituted)-diphenyl1,2,4-triazole derivatives were synthesised using simple synthetic route and their structures were characterized by IR, ^{1}H NMR and elemental analysis. They were tested for antimicrobial and antifungal activity and effect of electron withdrawing & electron donating substituents in the phenyl ring was studied. It was observed that triazoles containing chloro, bromo groups in the phenyl ring had significant antimicrobial activity and nitro group had better antifungal activity over other tested compounds.

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