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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

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°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 °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°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°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°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°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 °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

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BIOFERTILIZERS- THE GREEN WAY, TO SUSTAINABLE FARMING AND AGRICULTURAL PRACTISES.

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Abstract

Agriculture being one of the most important sector of Indian economy , there has been a constant focus on enhancing the economic, social, and environmental sustainability in it, which promotes the use of non-chemical fertilizers such bio-fertilizers. Use of microbial systems for nutrient mobilization, popularly known as bio-fertilizers are getting popular day by day as a result of which new systems are being introduced to meet our requirement of different crops and under different cropping systems. Water-soluble fertilizers provide an optimal solution to increasing agricultural yield owing to the host of advantages offered by them. Being water-soluble, these fertilizers are the ideal solution for feeding the necessary amounts of micro and macro nutrients to growing crops. These fertilizers have proven benefits in terms of raising agricultural productivity and are, therefore, expected to experience surging demand. Widespread adoption of bio-fertilizers will thus be one of the market trends in the forthcoming decade.This paper aims to focus the importance of bio-fertilizers as a promising tool for the sustainable development in agricultural sector.

Introduction

India is the second-most populous nation in the world and has 60.45% (as per World Bank 2016) of land used for agriculture. The accelerated rate of crop production exerts pressure on existing farm land forcing the use of chemical fertilizers. Prolonged & excessive use of various chemical and inorganic fertilizers can kill the micro fauna and flora, causing problems of soil fertility, loss of crops, pest and disease problems and pollution of the environment. As a result, farmers are gradually moving toward a more sustainable farming[1] option, that is, organic farming. Organic farming is the agricultural product management system that actively encourages & improves biodiversity, biological cycle and soil's microbial activity. This article explains the concept of bio-fertilizers, one of the important measures of organic farming.

What are Bio-fertilizers?

Bio-fertilizers are a mixture of living cells from yeast, moulds, bacteria and plants and enzymes to synthetic products that are easily degradable, which require less energy and create less waste during their production. Bio-fertilizers contribute plant nutrients through nitrogen fixation, phosphate solubilisation, and maintaining soil fertility. They also enhance the degradation of organic matter. Bio-fertilizers can be supplementary to chemical fertilizers[2,3]. Currently used bio-fertilizers are solid carrier based and have the drawback of low shelf life, inability to sustain in adverse conditions, and formation of clumps upon application which leads to a reduced inefficiency. To overcome these drawbacks, liquid bio-fertilizers have been developed[4]. Liquid bio-fertilizers are unique liquid formulations containing the desired beneficial microorganisms and their biological secretions, along with special cell substances that encourage the formation of dormant spores or cysts for longer shelf life and tolerance to adverse conditions.

- Enhance the beneficial effects of microorganisms in soil.
- Improve soil properties, sustain soil fertility.
- Improve humas production.
- Convert plant nutrients in available form.

Bio-fertilizerrequirement in India:

Based on crop area in India, the present requirement of bio-fertilizers is around 5,50,000 metric tonnes and there is an ample potential to increase it to 50,000-60,000 tons by 2020[7] ; however, the total production in our country is much less than requirement. State wise, Uttar Pradesh, Maharashtra & Tamil Nadu has 11-14 Bio-fertilizer units.

Precautions while using bio-fertilizer-

- Bio-fertilizers should be of good quality containing minimum 10^7 -10^8 viable microbial counts.
- They should be preserved from sunlight, heat & moisture, store at optimum temperature of 25-27^{0}C.
- Chemical and bio-fertilizers should not be used together, as the microorganisms could be killed.
- Packets must specify the expiry date, batch number, and the application dose.
- They should not be mixed with warm water

Benefits and Government Policies for Bio-fertilizers:

Incidentally, India hosts a staggering number of marginalized and smallholder farmers, almost eighty percent of them. Bio-fertilizers can play significant role in sustainable crop production under integrated nutrient management system. Due to low cost and consistent performers, the bio-fertilizers not only put less-burden on farm budget, but also provide nutrients slowly, which prevents their loss by lea hing under heavy rains. Bio-fertilizers are likely to be commercially popular in long run, once the information is available to the farmers & producers.

Government initiatives also support organic farming. Such initiatives will encourage farmers to adopt organic farming practises like the use of bio-fertilizers. [8] Currently the total requirement of various bio-fertilizers for seed, root treatment and soil is estimated to be about 0.426 million ton, based on net cultivated area. With increasing interest in the usage of bio-fertilizers, the government is also promoting it through various schemes like National Mission for Sustainable Agriculture (NMSA), Paramparagat Krishi Vikas Yojana (PKVY), Rashtriya Krishi Vikas Yojana (RKVY) and National Mission on Oilseeds and Oil Palm (NMOOP), National Food Security Mission (NFSM) and Indian Council of Agricultural Research (ICAR). ICAR, Pusa under Network project on Soil Biodiversity-Bio-fertilizer has developed improved and efficient strains of bio-fertilizer specific to different crops and soil types. Liquid bio-fertilizer technology with higher shelf life has also been developed. The ICAR also imparts training and organizes Front Line Demonstrations (FLDs) to educate farmers on all these aspect.

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