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Synthesis and Biological Evaluation of Novel 6-(3-(4, 5-Dihydro-1,5-diphenyl-1*H*-pyrazol-3-yl)phenylamino) Pyridazin-3(2H)-one Derivatives

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ABSTRACT

Pyrazoles are important nitrogen containing 5-membered heterocyclic compounds. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. Several 1,3,5-Triphenyl-1*H*-pyrazole containing 6-aminopyridazin-3(2H)-one derivatives has been synthesized. These new compounds were characterized using IR, ^{1}H-NMR and Mass spectra and Elemental analysis. They possess some potent biological activities. Therefore biological screening of novel compounds has been also done.

Keywords: Antibacterial Activity; Antifungal Activity; Efficient Synthesis

1. Introduction

Nitrogen heterocycles are of synthetic interest because they constitute an important class of natural and synthetic products, many of which exhibited useful biological activities [1]. An interest in five member systems with two adjacent nitrogen atoms occurs from saturated and partially saturated pyrazoles in biologically active compounds and natural products [2,3].

Substituted pyrazolines and their derivatives embedded with variety of functional groups are important biological agents. Thus, a significant amount of research activity has been directed towards this class. In particular, they are used as antitumor, antibacterial, antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents. In recent years, a significant portion of research in heterocyclic chemistry has been devoted to 2-pyrazolines containing different aryl groups as substituents, as evident in literature. Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial [4], antifungal [5], anti-inflammatory [6], and antidepressant actevities [7]. The pyrazoline function is quite stable, and has inspired chemists to utilize this stable fragment in bioactive moieties to synthesize new compounds possessing biological activities.

Several methods are employed in the synthesis of pyrazolines, including the condensation of chalcones with hydrazine, phenyl hydrazine [8-12], and condensation of chalcones with thiosemicarbazide in ethanol under strong basic or acidic conditions [13]. The desired chalcones [14] were synthesized by reacting 6-(3-acetyl-phenylamino) pyridazin-3(2H)-one with substituted aromatic aldehydes in presence of alkali. In a typical case, equimolar quantities of chalcones and phenyl hydrazine hydrochloride in presence of acetic acid and few crystals of sodium acetate, led the formation of pyrazolines **3a-l** (**Scheme 1**); continuing our investigations on the application of above mentioned an efficient and practical procedure for the synthesis of 1,3,5-Triaryl-2-pyrimidines with chalcones and phenylhydrazine hydrochloride in sodium acetate-acetic acid aqueous solution has been reported.

2. Results and Discussion

A variety of methods were reported for the preparation of this class of compounds. After pioneering work of Fischer and Knoevenagel in 19th century, the reaction of α,β-unsaturated aldehydes and ketones with phenylhydrazine in acetic acid under reflux condition became the most popular method of the preparation of pyrazolines [14]. In 1998, Powers *et al*. [15] reported the reaction of chalcones and phenylhydrazine hydrochloride in the presence of sodium hydroxide which was carried out in absolute ethanol at 70˚C, but; there are disadvantages such as longer the reaction time (8 h) etc. In 2005, the synthesis of 3,5-diaryl-2-pyra-zolines by the reaction of chloro

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chalcones with phenylhydrazine in acetic acid under reflux condition was reported for three hours. Effect of the reaction conditions on chalcones and phenyl hydrazine hydrochloride was summarized in **Table 1**. When the molar ratio of chalcones **3** and phenyl hydrazine hydrochloride was 1:1, the yield of 1,3,5-triphenyl pyrazoline obtained was very less. But by increasing the molar ratio to 1:2 and 1:3 the yield of products were also increased. It may be that sodium acetate is in favour the release of phenylhydrazine from phenylhy-drazine hydrochloride [14]. So reaction condition we chose were the molar ratio of chalcone:phenylhydrazine: sodium acetate was 1:3:0.15. We have performed the reaction of chalcone with phenylhydrazine hydrochloride by refluxing at 110˚C the yield of pyrazoline was 48% - 71% (**Table 1**).

From the results, the optimum reaction condition was chosen: Chalcone (3 mmol), phenylhydrazine hydrochloride (6 mmol), Sodium acetate (0.3 mmol).

Under this reaction system, a series of experiments for synthesis of pyridazin-3(2H)-one derivative were performed.

The following sequence of reaction appears to afford a satisfactory explanation of the mode of formation of the products (**Scheme 2**). This reaction involves the initial formation of an arylhydrazone with subsequent attack of nitrogen upon the carbon-carbon double bond.

3. Antibacterial and Antifungal Activity

All of the novel synthesized compounds were screened for their antifungal and antibacterial activity against the Gram – ve bacteria *Escherichia coli* (ATCC 8739) and Gram + ve bacteria *Staphylococcus aureus* (ATCC 6538), in addition to their antifungal activity against *Aspergillus niger* (ATCC 16404) *Candida albicans* (ATCC 10231) using ager diffusion method [16,17] at a concentration 20 mg/mL. DMSO used as a solvent. Compound **3f** shows highly efficient antibacterial activity against *S. aureus* (ATCC 6538) more than Penicillin standard. Compound **3b** show was also found to be efficient equivalent to standard penicillin.

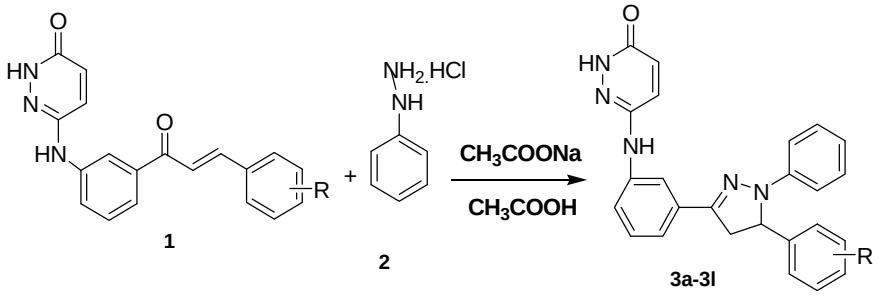
Standard Grysofulvin showed antifungal against *A. Niger* (ATCC 16404) zone of inhibition is 24 mm and *B. albicans* (ATCC 10231) shows zone of inhibition is 23 mm, where as compounds **3e** & **3b** are found to be highly potent against *A. niger* (ATCC 16404) and compound **3f** are highly efficient against *B. albicans* (ATCC 10231). From the result it can be conclude that compound 4f has antibacterial and antifungal activity more efficient than the standards used in this research work. The results, recorded as average diameter of inhibition zone in mm, are given in **Table 2**.

4. Experimental

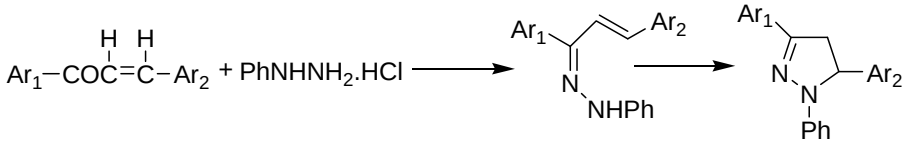
General Procedures: Melting points were determined in open capillary tubes and are uncorrected. The purities of the compounds were checked on silica-gel-coated Al plates. IR spectra were recorded in KBr on a Perkin Elmer Spectrum BX series FT-IR spectrometer. ^{1}HNMR spectra were recorded on Bruker DRX 300 MHz NMR spectrometer using TMS as internal standard and mass spectra on a Jeol D-300 spectrometer.

We synthesized novel chalcones using conventional method in the literature and used in this research work.

Pyridazin-3(2H)-one(3a-3l): Chalcone(6-(3-((E)-4-phenylbut-3-enoyl)phenylamino)pyridazzin-3(2H)one (1 mmol), phenyl hydrazine hydrochloride (2 mmol), sodium acetate (0.3 mmol) in 2 - 3 drops of acetic acid was reflux for 3 - 4 hrs at 110˚C temp. The solid was collected and recrystalized from alcohol to give corresponding 2-pyrazolines (**Scheme 1**).

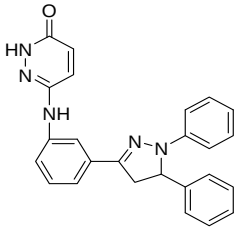
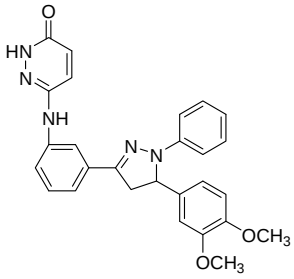
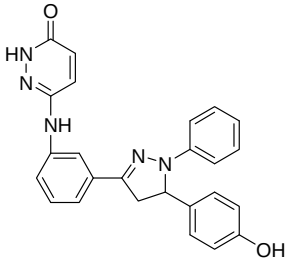
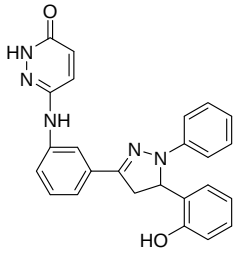
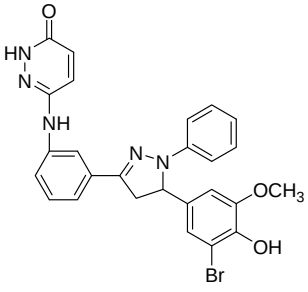
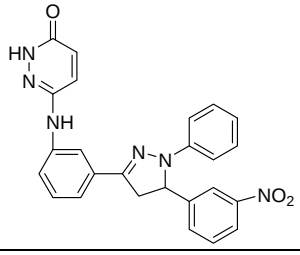


Scheme 1. Formation of pyrazolines (3a-l).



Scheme 2. The possible mechanism for the formation of products.

Table 1. Synthesis of pyridazin-3(2H)-one derivatives in sodium acetate and acetic acid.

Sr. No.	Products (3)	Yield (%)	Time (Hrs)	M.p. (˚C)
a		55	4	182
b		68	4	158 - 166
c		56	3.5	195
d		48	3.5	180
e		58	4	162
f		66	4	188

Continued

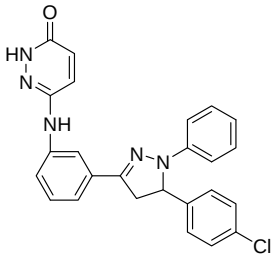
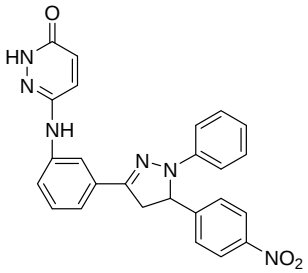
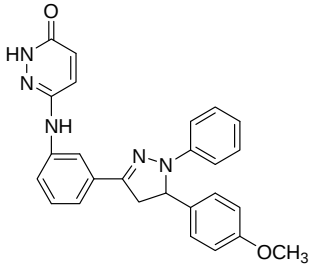
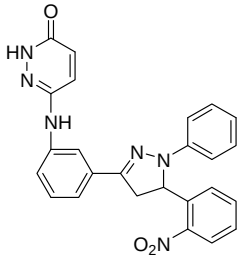
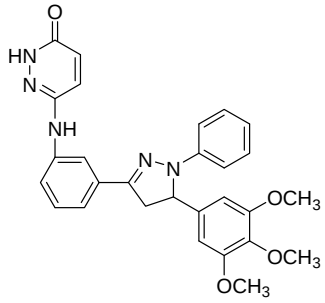
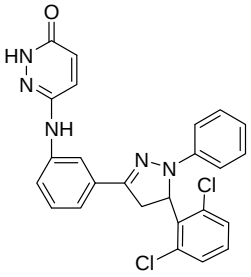
g		70	4	190 - 193
h		48	3.5	181
i		68	3.5	161
j		71	4	185 - 190
k		54	4	203
l		64	3.5	178

Table 2. Antifungal and antibacterial screening results of the synthesized 1,3,5-triaryl-2-pyrazolines derivatives.

Comp. Codes	Antibacterial Activity		Antifungal Activity	
	***E. coli* (ATCC 8739)**	***S. aureus* (ATCC 6538)**	***A. niger* (ATCC 16404)**	***B. albicans* (ATCC 10231)**
3a	15	15	13.	22.5
3b	17	20	25.5	13
3c	19	16	20.5	18
3d	16	15	13.5	16
3e	19	12	29.5	16.5
3f	12	21	-	24.5
3g	15	19	-	18
3h	15	15	16.5	17
3i	13	13	14.5	17.5
3j	16	11	-	13
3k	14	13	-	15.5
3l	16	11	13.5	15.5
Penicillin	22	20	-	-
Grysofulvin	-	-	24	23

*Zone of inhibition in mm.

5. Spectral Analysis

Compound (3a): Yield 55%, M. P. 182˚C; IR (KBr): 3240 (Ar-C=C Str.), 3250 (N-H Str.), 1675 (C=O); ^{1}HNMR (DMSO-d_6): δppm 1.9 (dd, 2H), 3.7 (d, 1H), 4.1 (brs, 1H, -NH), 5.9 (dd, 1H), 6.4 (dd, 1H, Ar-H), 6.7 (dd, 1H), 6.8 (dd, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 7.1 (brs, 1H, -NH), 6.40 - 7.05 (s, 5H, Ar-H), 7.1 (t, 1H, Ar-H), 7.05 - 7.25 (s, 5H, Ar-H); Mass; (m/z), 407; Elemental analysis (% for) $C_{25}H_{21}N_5O$, Calcd. C, 73.69; H, 5.19; N, 17.19; O, 03.95; found. C, 74.20; H, 5.24; N, 17.44; O, 4.00.

Compound (3b): ^{1}HNMR (DMSO-d_6): δppm 1.8 (dd, 2H), 3.5 (d, 1H), 3.8 (s, 6H, -OCH_3), 4.1 (brs, 1H, -NH), 5.9 (dd, 1H), 6.4 (dd, 1H, Ar-H), 6.45 (dd, 1H, Ar-H), 6.50 (s, 1H, Ar-H), 6.60 (dd, 1H, Ar-H), 6.7 (dd, 1H), 6.8 (dd, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 6.41 - 7.07 (s, 5H, Ar-H), 7.1 (brs, 1H, -NH), 7.1 (t, 1H, Ar-H). Mass; (m/z), 465; Elemental analysis (% for) $C_{27}H_{25}N_5O_3$ Calcd. C, 69.36; H, 5.39; N, 14.98; O, 10.27; found. C, 70.16; H, 5.42; N, 14.98; O, 10.30.

Compound (3d): Yield 48%, M. P. 180˚C; IR (KBr): 3268 (Ar-C=C Str.), 3220 (N-H Str.), 1677 (C=O); 3260 (-OH); ^{1}HNMR (DMSO-d_6): δppm 1.7 (dd, 2H), 3.7 (d, 1H), 4.1 (brs, 1H, -NH), 5.1 (brs, 1H, -OH), 5.9 (dd, 1H), 6.4 (dd, 1H, Ar-H), 6.7 (dd, 1H), 6.8 (dd, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 6.41 - 7.02 (s, 5H, Ar-H), 7.1 (brs, 1H, -NH), 6.45 (dd, 1H, Ar-H), 6.65 (t, 1H, Ar-H), 6.69 (dd, 1H, Ar-H), 6.90 (t, 1H, Ar-H), 7.1 (t, 1H, Ar-H). Mass; (m/z), 465; Elemental analysis (% for) $C_{25}H_{21}N_5O_2$ Calcd. C, 70.91; H, 5.00; N, 16.54; O, 7.56; found. C, 80.06; H, 5.00; N, 16.78; O, 7.70.

Compound (3f): Yield 66%, M. P. 188˚C; IR (KBr): 3253 (Ar-C=C Str.), 3230 (N-H Str.), 1677 (C=O), 1368 (NO_2); ^{1}HNMR (DMSO-d_6): δppm 1.7 (dd, 2H), 3.7 (d, 1H), 4.1 (brs, 1H, -NH), 5.9 (dd, 1H), 6.4 (dd, 1H, Ar-H), 6.7 (dd, 1H), 6.8 (dd, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 7.1 (brs, 1H, -NH), 7.1 (t, 1H, Ar-H), 6.40 - 7.05 (s, 5H, Ar-H), 7.45 (t, 1H, Ar-H), 7.50 (dd, 1H, Ar-H), 8.05 (dd, 2H, Ar-H), Mass; (m/z), 452; Elemental analysis (% for) $C_{25}H_{20}N_6O_3$ Calcd. C, 66.36; H, 4.46; N, 18.54; O, 10.61; found. C, 67.06; H, 4.57; N, 18.57; O, 10.70.

Compound (3l): Yield 48%, M.P.180˚C; IR (KBr): 3268 (Ar-C=C Str.), 3220 (N-H Str.), 1677 (C=O), 762 (-Cl); ^{1}HNMR (DMSO-d_6): δppm 1.9 (dd, 2H), 3.6 (d, 1H), 4.2 (brs, 1H, -NH), 5.9 (dd, 1H), 6.4 (dd, 1H, Ar-H), 6.7 (dd, 1H), 6.8 (dd, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 6.95 (t, 1H, Ar-H), 7.1 (brs, 1H, -NH), 7.1 (t, 1H, Ar-H), 6.41 - 7.06 (s, 5H, Ar-H), 7.15 (dd, 2H, Ar-H). Mass; (m/z), 475; Elemental analysis (% for) $C_{25}H_{19}Cl_2N_5O$ Calcd. C, 63.03; H, 4.02; Cl, 14.49; N, 14.74; O, 3.36; found. C, 62.93; H, 4.12; Cl, 14.50; N, 14.78; O, 3.40.

6. Conclusion

This methodology is maintaining environmental friendly approach for the synthesis of pyridazin-3(2H)-one de-

rivatives using sodium acetate and acetic acid. But by increasing the molar ratio to 1:2 and 1:3 the yields of increased, it may be that sodium acetate is in favour of release of phenylhydrazine from phenylhydrazine hydrochloride. So reaction condition we chose were the molar ratio of chalcone:phenylhydrazine:sodium acetate was 1.0:3.0:0.15. We have performed the reaction of chalcones with phenylhydrazine hydrochloride by refluxing at 110˚C, the moderate to good % yield of pyrazoline derivatives is observed. The synthesized compounds were further subjected for biological screening. Some pyrazoline derivatives are found to be more potent.

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