INVESTIGATION OF BIOLOGICAL ACTIVITIES OF NEW DERIVATIVES AS ANTIOXIDANT DRUG: SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

New Derivatives have been synthesized using benzaldehyde with p-amino Phenol and Phenyl hydrazine through condensation reaction. Reaction progress has been monitored using TLC in solvent system i.e. ethyl acetate and hexane (1:1 Ratio). The synthesized product has been characterized by IR, UV-Visible and NMR spectroscopy. The antioxidant properties have also been carried out using DPPH free radical. The IC_{50} values of for the $\mathbf{R_1}$ and $\mathbf{R_2}$ were found to be **0.025 ± 0.09 mg/mL** and **0.041 ± 0.08 mg/mL,** respectively. Results revealed that $\mathbf{R_1}$ showed the greater rate of antioxidant activity than $\mathbf{R_2}$ against DPPH Free radical.

Keywords: Benzaldehyde; Antioxidant; Phenyl Hydrazine; DPPH.

INTRODUCTION

Cancer is caused by uncontrolled growth of abnormal cells. It is a major public health problem after cardiovascular disorder across the world which affected millions of lives. Although major advances have been made in reducing cancer incidence in United States, the numbers of cancer patients continue to increase (Kumar et al, 2015). Chemotherapy is one of the most effective approaches used for treating cancer patients, however lack of selectivity and development of drug-resistance reduce the efficacy of cancer chemotherapy (Lalehzari et al, 2008)). In the last few decades, enormous efforts have been made to develop potential anticancer drugs. The search for effective and safe anticancer drugs remains critically important for the researchers globally. Pyrazole is a five-membered heterocyclic compound with two adjacent nitrogen atoms. Pyrazole tethered heterocyclic compounds displayed diverse chemotherapeutic potentials (Jun et al, 2012; Shankarwar et al, 2015 and Ahmed et al, 2015). They represented variety of biological activities such as anti-inflammatory, anti-microbial, anti-malarial, anti-hypertensive, anti-tubercular, anti-viral, neuro-protective, anti-depressant, anti-cancer etc. Several pyrazole derivatives gained application on the clinical level and some of them have been proven as useful entity for the development of potent chemotherapeutic agents. The important pyrazole based drugs available in the market are Celecoxib, Apixaban, Fipronil, Betazole, Tepoxalin, Fezolamine, Pyrazomycin, Fomepizole, Mepirizole, Difenamizole, Lonazolac, Tolpiprazole, Deracoxib, Crizotinib, abolitionist and many

more. Some pyrazole based drugs are depicted in Figure 1. Pyrazofurin, a naturally occurring pyrazole tethered analogue, displayed broad spectrum antiviral activities. Moreover, a large numbers of pyrazole derivatives find application in agriculture, horticulture,

food industry and, in supramolecular and polymer chemistry. Some pyrazoles (**Figure 1**) have liquid crystal properties while some are used as dyes and UV stabilizers (Zoubi et al, 2013). Chalcones are α,β-unsaturated ketones that are widely distributed in plants. They are just precursors of flavonoids and isoflavonoids. In the last few decades, large numbers of interesting chalcone derivatives have been synthesized.

Various classes of natural and synthetic chalcones have been investigated owing to their diverse chemotherapeutic potentials. They showed diverse pharmacological activities such as anti-inflammatory, antileishmanial, anti-invasive, anti-malarial, antibacterial, antifungal, anti-protozoal and anticancer etc (Arif et al, 2020).

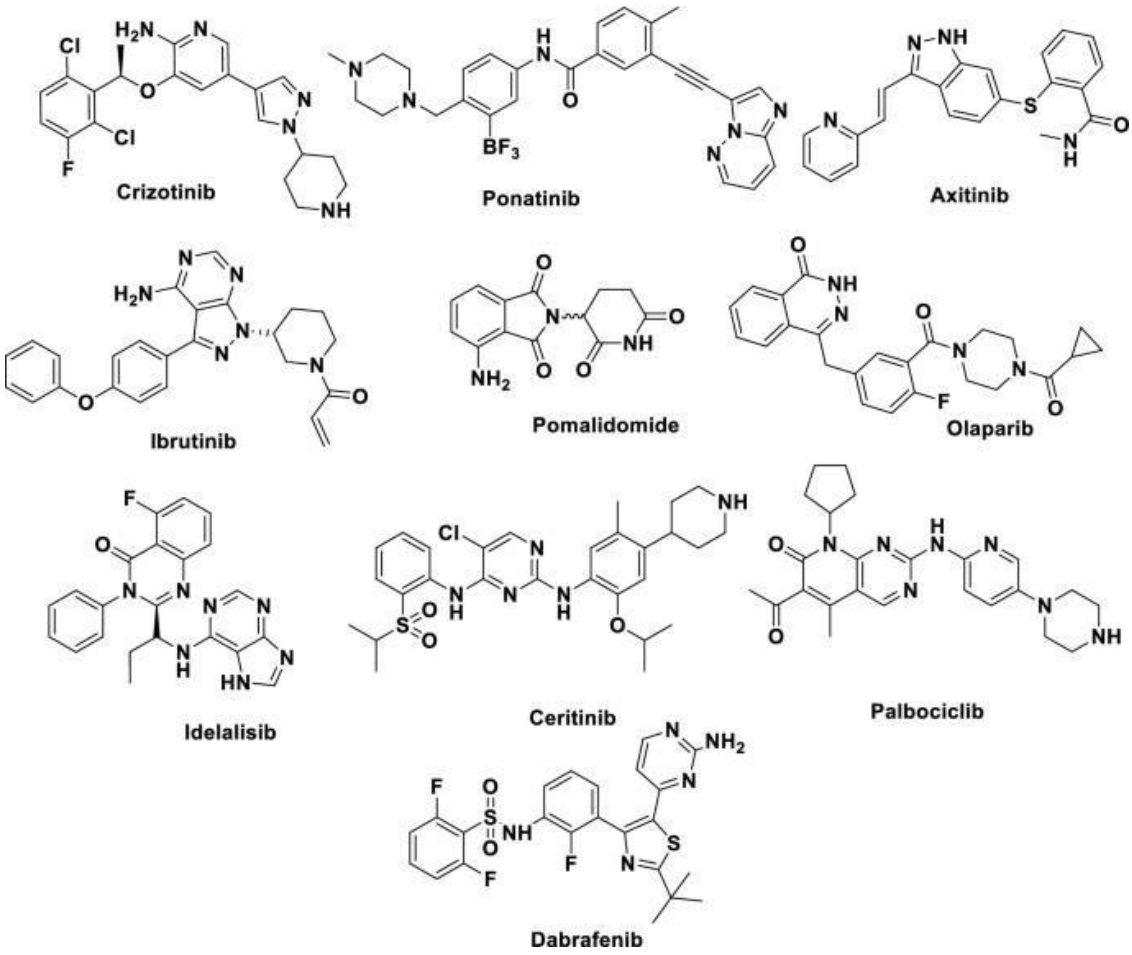


Figure 1: Some pyrazole based heterocyclic drugs

In previous year, three hydrazone derivatives have been synthesized using condensation reaction of 4- hydrazinyl benzoic acid with three aromatic aldehydes namely: thiophene-2-carbaldehyde, thiophene-3-carbaldehyde and 2-furaldehyde in ethanol at 78 °C reflux. The synthesized molecules have been characterized using spectroscopic and physicochemical methods including UV–Vis, IR, ^{1}H NMR, ^{13}C NMR, ^{15}N NMR and melting point determination. Optimized molecular structures, UV–Vis and IR spectra modeling, the reactivity, the stability and some quantum chemical parameters of the synthesized molecules were modeled utilizing density functional theory (DFT). The obtained theoretical results were found in good agreement with the experimental results.

EXPERIMENTAL WORK

Chemicals

p-hydroxy benzaldehyde, p-amino Phenol, Phenyl hydrazine, glacial acetic acid, Methanol were used . All solvents were of AR and LR grade which were used without further purification. Precoatedaluminium sheets (silica gel 60 F_{254},Merck Germany) were used for thin layer chromatography (TLC) and spots were visualized under UV light.

Instruments/Equipment

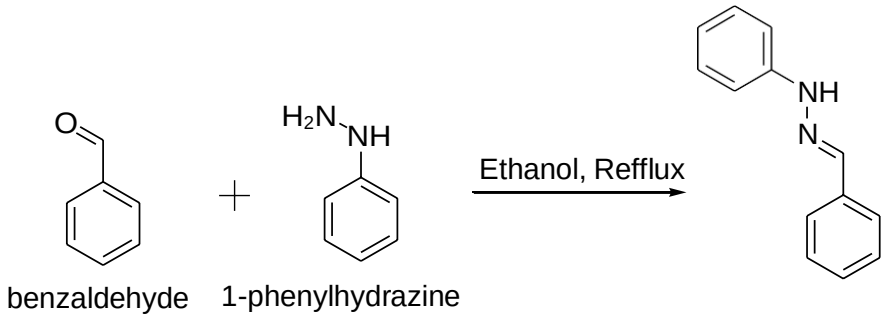
UV-Visible (Perkin Elmer Lambda 40), Infra red (Agilent technologies) and NMR spectrophotometer (Bruker DPX-300 NMR), UV light cabinet and Melting point apparatus (Veego Technology).

Synthesis of Compound (R_1)

An ethanolic solution of Phenyl hydrazine (0.114g, 5 mmol) was slowly added to the ethanol solution of benzaldehyde (0.106g, 5mmol) in 250 mL round bottom flask. The dark green colored solution was refluxed for 2 h with constant stirring and poured into cold water. The completion of the reaction was monitored by using TLC (ethyl acetate: hexane, 1:1). The blackish precipitate that obtained was filtered, washed with distilled water and ethyl acetate and finally dried under vacuum on fused $CaCl_2$ and recrystallized in ethanol. Yield 83%. The synthesis of Compound (**R_2**) is representated in **Scheme I**.

Synthesis of Compound (R_2)

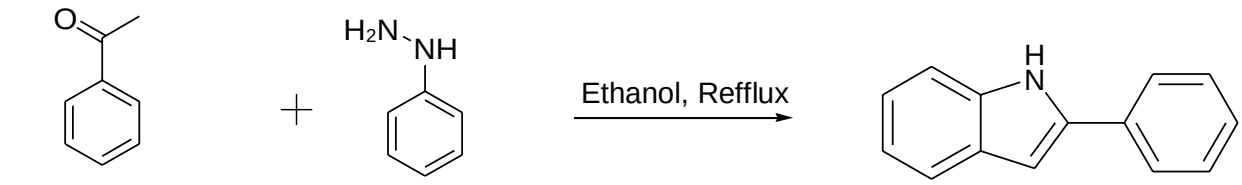
To the solution of compound (0.610g, 5mmol) in 10-20 ml of ethanol benzaldehyde (0.144g, 5mmol) phenyl hydrazine dissolved in 10-20 ml of methanol was added. The mixture was stirred for 15 minutes and kept overnight. The yellow precipitate was filtered, washed with ethyl acetate and dried in vacuum dessicator. Yield is 81%. Thesynthesis of Compound (**R_1**) is represented in **Scheme II.**



benzaldehyde 1-phenylhydrazine (*E*)-1-benzylidene-2-phenylhydrazine

(R1)

Scheme I. Synthesis of Compound (R_1).



acetophenone 1- phenylhydrazine 2- phenyl-1*H*-indole

(R2)

Scheme II. Synthesis of Compound (R_2)

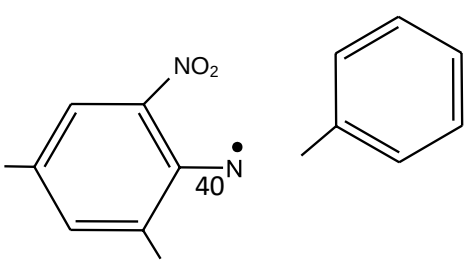
ANTIOXIDANT STUDIES

DPPH Radicals Scavenging Activity

Scavenging activity of antioxidants compounds was measured by the DPPH (2,2-diphenyl-1-picryl-hydrazyl) (**Figure 2**) free radical assay which was the best method based on electron-transfer. DPPH free radical scavenging activity of the compound was measured by the method. Test compound (1ml) in methanol and in each solution 0.5 ml 0.1 mM DPPH free radical in methanol was added. All test compounds were incubatedat 60^0C for 2 h and the decrease in absorbance was noted at 513 nm using UV-Vis. Spectrophotometer (Nayab et al, 2016). Absorbance of DPPH without compound was recorded at 513 nm as acontrol. For each of the test compound experiment was done in triplicate and antioxidant property of the compounds was measured by using the equation:

$$\% \text{ Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where $A_{control}$ = absorbance of DPPH free radical in methanol without an antioxidant and A_{sample} = absorbance of DPPH free radical in the presence of an antioxidant.



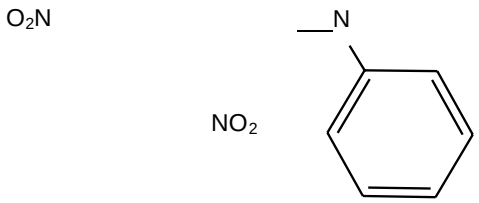


Figure 2. 2,2-diphenyl-picryl-hydrazyl (Free radical).

RESULTS AND DISCUSSION

All the synthesized compounds are stable in air. All the compounds are soluble in Methanol, DCM and acetic acid. The solubility of the compounds in different solvents is given in **Table 1.** The reaction was monitored by thin layer chromatography (TLC) in solvent system in ethyl acetate: hexane (1:1) for ligand. The molecular weight, analytical data, molar conductance, colour, % yield and melting point are presented in **Table 2.**

Table 1. Solubility of the compounds

Solvents	**Compound (R_1)**	**Compound (R_2)**
Methanol	√	√
Water	X	X
DCM	√	√
Ethyl acetate	√	√
Petroleum Ether	X	√
Chloroform	√	√
Hexane	X	√
Acetic acid	√	√

Table 2: Molecular weight, analytical data, molar conductance, colour, % yield and melting point.

Compounds (Mol. Wt.)	**Yield (%)**	**Colour**	**M.P. (°C)**	**Analytical data (% Calcd).**			
				C	**H**	**N**	**O**
$[C_{13}H_{11}N_2]$, (212)	83	Blackish Green	231	73.58	79.5	6.12	
$[C_{13}H_{11}N_2]$, (210)	81	Brown	132	80.7	4.76	13.33	

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[1]H NMR Spectra

The [1]H NMR spectra of all the compounds was exhibited well resolved signals. [1]H NMR spectrumshowed a signal at 12.46 ppm and 12.03 ppm as singlet due to -NH protons for R_1 and R_2, respectively. The signal appears at 8.93 ppm as singlet may be attributed to proton of azomethine group for the Compound (R_1). The signals as multiplet for the ligand appear at 7.39 –8.21 ppm due to protons of aromatic rings (**Figure 3**).

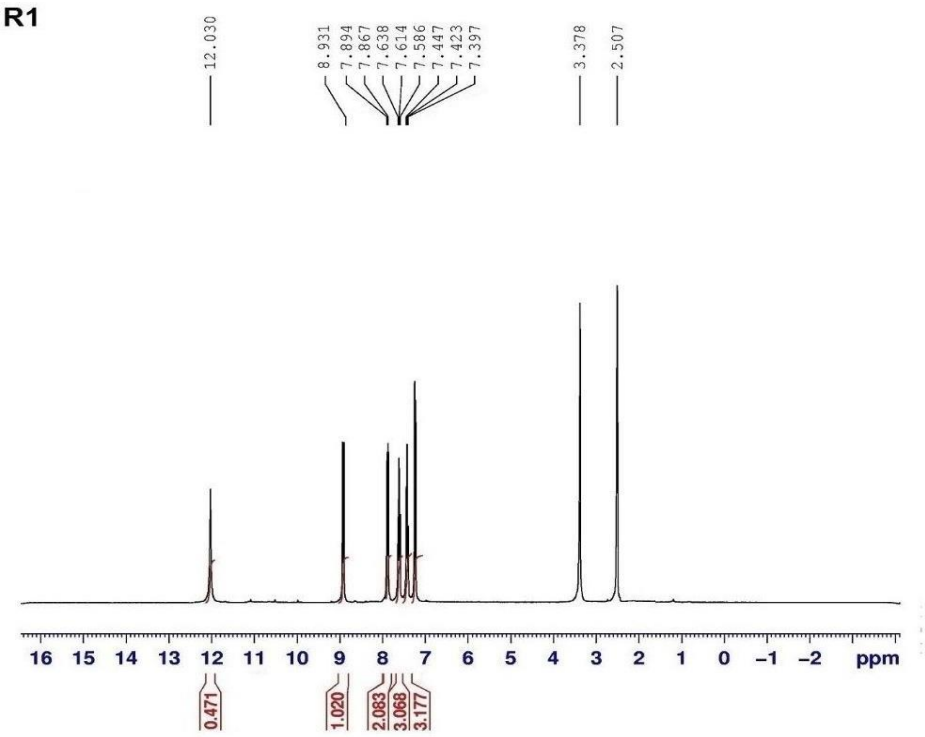

Figure 3 : [1]H NMR Spectra of Compounds R_1 and R_2

ANTIOXIDANT STUDIES

DPPH Radicals Scavenging Activity

DPPH is a stable free radical which can accept an electron or hydrogen radical and itself become reduced. Change in colour take place from purple to yellow after the incubation of sample which confirms the presence of antioxidant moiety in test compounds. Decrease in the absorbance at 511 nm was recorded and IC_{50} value (Minimum concentration of the compounds required for the 50% inhibition) was calculated from the graph (**Figure 4**). Lower the IC_{50} values, stronger the ability of compounds to scavenge DPPH free radical (Thalamuthu et al. 2014; Ansari et al. 2016). The IC_{50} values of for the **R_1** and **R_2** were found to be **0.025 ± 0.09 mg/mL** and **0.041 ± 0.08 mg/Ml ± 0.011,** respectively which reveals that scavenging activity of **R_1** is greater than **R_2**.

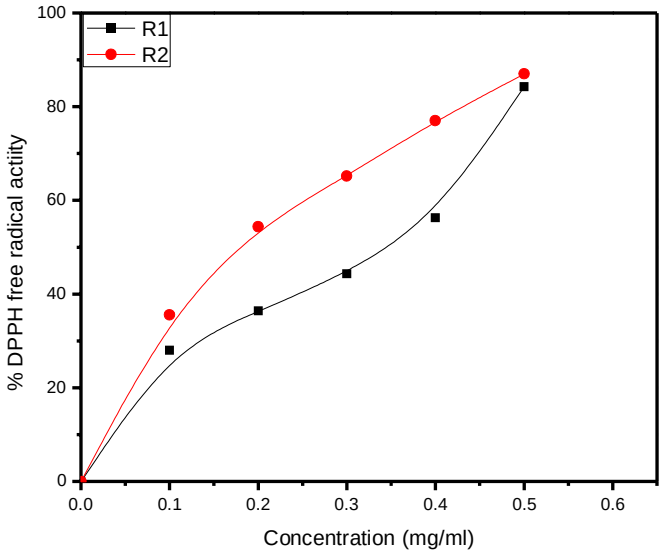

Figure 4. DPPH Free Radical Activity of Compounds **R_1** and **R_2**.

CONCLUSION

In current research work, new derivatives were synthesized and structurallycharacterized by UV–Vis., IR and ^{1}H NMR spectral analysis and Antioxidant properties. All synthesized compounds are stable in air. These derivatives are known to have a wide spectrum of applications in the design and development of drugs for the treatment of various diseases caused by different microorganisms like bacterial, fungal, ameobal, viral and several other pathogenic diseases.

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