BIOLOGICAL EVALUATION OF HETEROCYCLIC DERIVATIVE: SYNTHESIS AND CHARACTERIZATION

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ABSTRACT

In current research work, we have synthesized a series of heterocyclic derivative of Phthalic anhydride with substituted amines (2, 4-dinitrophenyl hydrazine, p-nitro amine and o-phenylenediamine through condensation reaction. The progress of reaction has been monitored by using TLC in ethyl acetate and hexane (1:1 Molar Ratio). The synthesized product has been characterized by IR, UV-Visible and NMR spectroscopy. The antioxidant properties have also been studied through DPPH free radical and hydrogen peroxide assay. IC_{50} value of the derivatives **A_1**, **A_2** and **A_3** was found to be **730±0.72 µg/mL, 0.514±0.516 µg/mL** and **0.512 ±0.510 µg/mL**, respectively against DPPH free radical and **0.407±0.39 µg/mL, 0.429±0.42 µg/mL** and **0.434 ± 0.43 µg/mL,** respectively against hydrogen peroxide. Results revealed that **A_2** showed the greater rate of antioxidant activity than other derivatives.

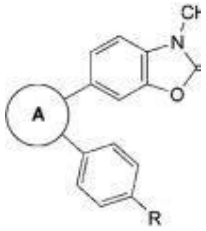
Keywords: Heterocycles; Antioxidant; Phthalic anhydride; DPPH.

INTRODUCTION

Heterocyclic compounds are a cyclic compound that has atoms of at least two different elements as a member of its rings. It is a branch of organic chemistry dealing with the synthesis, properties, and applications of these heterocycles (Zhu et al; 2003). Common hetero-atoms are nitrogen, oxygen and sulphur. Since rings can be of any size, from three-membered to 6-membered. This work has been devoted to organic heterocyclic compounds in which the rings contains at least one carbon atom; all atoms other than carbon is considered as hetero-atoms. The five-membered ring contains more than one or two hetero-atoms such as azole, pyrrole, thiazole, thiadiazole, oxadiazole, traizene. And when it comes to the applications of heterocyclic compounds, they are used in agrochemicals and pharmaceuticals industries and very important for medicinal chemistry by this it can expand the available drug like chemical space into more effective drug.. Heterocycles are the starting material in organic compounds, used in corrosion inhibitors , sanitizers, anti-ordinates & developers. Heterocyclic Chalcones have many pharmacological activities, such as anti microbial agents, antiviral, anti- inflammatory etc. Antifungal, anti-inflammatory, antibacterial, anticonvulsant, anti-allergic, herbicidal, and anticancer activity was shown as majority of active heterocycles (Darapourand and Shiri; 2023). Thaidiazole is a heterocyclic compound having two nitrogen atoms and one sulphur atoms in a cyclic ring and its related compounds are called 1,3,4 thiadiazole. Some of the examples of pharmaceutical drugs containing heterocyclic compounds are diazepam, isoniazid, choloropromazine, metronidazole, barbituric acid, captopril, chloroquinine, azidothymidine and antipyrine (Verma et al; 2022). There are numerous quinolines based heterocyclic derivatives

which were synthesized earlier such as thiadiazine, thiadiazoles, triazoles thiazines, oxazines,isoxazoles and pyrazoles. Thiazole shows a broad spectrum of pharmacological activities such as antifungal, antibacterial, anti-inflammatory and anticancer etc. They have been also used as reagents in organic synthesis as precursors, in polymers, dyestuffs, additives, pharmaceuticals, agrochemicals and veterinary product surfactants (Jasim et al; 2022, Sallam et al; 2023, Shankarwar et al; 2015). Schiff bases are synthetically accessible and structurally diverse compounds, typically obtained by facile condensation between an aldehyde, or a ketone with primary amines. Schiff bases contain an azomethine (-C=N-) linkage that stitches together two or more biologically active aromatic/heterocyclic scaffolds to form various molecular hybrids with interesting biological properties. Schiff bases are versatile metal complexing agents and have been known to coordinate all metals to form stable metal complexes with vast therapeutic applications (Jagadeesan et al; 2023, Benramdane et al; 2015). Mannich bases are the end products of Mannich reaction and are known as beta-amino ketone carrying compounds.

Many researchers have been synthesized diaryl heterocyclic derivatives of 2-oxo-5H- furan, 2-oxo-3H-1, 3-oxazole, and 1H- pyrazole moieties as the central heterocyclic ring and their inhibitory activities on COX-1 and COX-2 isoforms were evaluated by using a purified assay. The 2-oxo-5H-furan derivative was Identified as potent COX-inhibitor with (COX-1 IC_{50}=0.061 and COX-2 IC_{50}=0.325) and the selective index (SI=0.19). And for 1H-pyrazole derivative, **1** was found to be potent COX-2 inhibitor, about 38 times more potent than Rofecoxib (COX-2 with IC50= 0.011 and 0.398 respectively but they do not show selectivity for COX-2 isoform. Compound **1** show strong and selective COX-2 inhibitory activity (Serdarunlu, et al; 2010).



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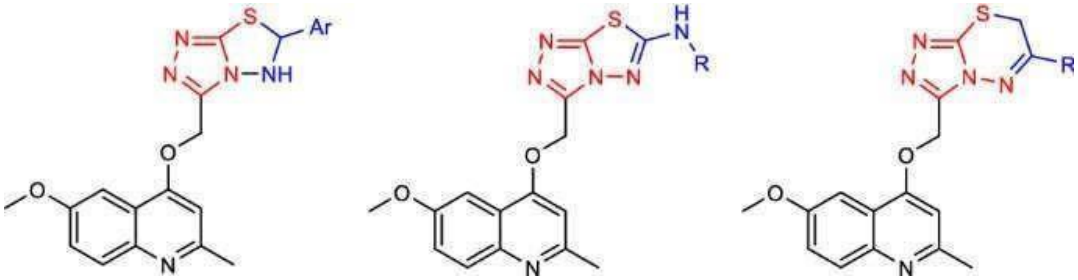
A series of quinoline based derivative (thiadiazines, thiadiazole and triazole) were synthesized and their antibacterial activity for Gram Positive and Gram Negative bacteria was evaluated. The synthesized derivative was characterized and their structure was seen by NMR, FT-IR and by mass spectrometery (Salman et al; 2022).



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The synthesis and biological evaluation of ethyl 5-amino-4-(3-pyridyl)-2-methyl- 6,7,8,9-tetrahydro-4*H*-pyrano[2,3-*b*] quinoline-3-carboxylates is also carried out by few researchers. They found that these compounds inhibit AChE with a mild potency, mitigatesthe $[Ca^{2+}]_c$ triggered by high K^+, and cause neuro-protection against Ca^{2+} overloading and free radical-induced neuronal death (Contelles et al; 2006).

The synthesis and antimycobacterial activity of a series of 27 different derivatives of 3-benzyl-6-bromo-2-methoxy-quinolines and amides of 2-[(6-bromo-2-methoxy-quinolin-3-yl)-phenyl-methyl]-malonic acid monomethyl ester is carried out in previous year. The antimycobacterial activity of these compounds was evaluated in vitro against *Mycobacterium tuberculosis* H37Rv for nine consecutive days upon a fixed concentration (6.25 μg/mL) at day one in Bactec assay and compared to untreated TB cell culture as well as one with isoniazide treated counter part, under identical experimental conditions. The compounds have shown 92–100% growth inhibition of mycobacterial activity, with minimum inhibitory concentration (MIC) of 6.25 μg/mL. Based on our molecular modelling and docking studies on well-known diarylquinoline antitubercular drug R207910, the presence of phenyl, naphthyl and halogen moieties seem critical. Comparison of docking studies on different stereo isomers of R207910 as well as compounds from our data set, suggests importance of electrostatic interactions. Further structural analysis of docking studies on our compounds suggests attractive starting point to find new lead compounds with potential improvements (Upadhayaya et al; 2009). A new series of 4-(4-substituted-anilino) quinolines derivatives was synthesized from amine derivatives via Gould–Jacobs reaction. All synthesized compounds were evaluated for their cytotoxic activity against two human cancer cell lines; breast carcinoma (MCF-7) and non-small cell lung cancer (A549). The tested compounds showed a broad range of activities (IC_{50}=3.42–23.32 and 5.97–22.01μM) in comparison with doxorubicin (IC_{50}=2.07and0.02μM) and erlotinib (IC_{50}=1.14 and 19.26μM) for MCF-7 and A549 respectively. In addition, molecular docking studies were performed and the results were in agreement with the in vitro cytotoxic data (Abdellatif et al; 2017).

As the development of most potential cholinesterase pharmaco-therapeutics, new class of thirty analogs oxindole-based chalcone were synthesized by reacting nitro-substituted oxindole with various substituted benzaldehyde in the presence of a base in ethanol under reflux conditions. The synthesized compounds were characterized through different spectroscopic and spectrometric techniques such as ^{1}H NMR, ^{13}C NMR, HREI-MS and tested for their ability to inhibit acetylcholinesterase and butyrylcholinesterase that exhibited a variable degree of inhibitory potential with IC_{50} values ranging from 0.20±0.010 to 11.20 ± 0.30 μM for acetylcholinesterase and 0.30 ± 0.010 μM to 13.20 ± 0.30 μM for butyrylcholinesterase as compared to the standard drug donepezil (IC_{50} value 2.16 ± 0.12and4.5 ± 0.11 μM respectively). Structure-activity relationship of this series has been established which is mainly based on the position and nature of

the substituent on the phenyl ring. Molecular docking study was also carried out to discover the binding affinity of active derivatives with enzymes (Taha et al; 2023).

Inhibition of histone deacetylase (HDAC) has been regarded as a potential therapeutic approach for treatment of multiple diseases including cancer. Based on pharmacophore model of HDAC inhibitors, a series of quinoline-based N-hydroxy cinnamamides and N- hydroxyl benzamides were designed and synthesized as potent HDAC inhibitors.

EXPERIMENTALWORK

Chemicals

Phthalic anhydride, 2-4 dinitrophenyl hydrazine, ethyl acetate, acetic acid, p- nitroaniline, o-phenyldiamine, ethanol, and methanol were used. Precoatedaluminium sheets were used for thin layer chromatography.

Instruments/Equipment

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

Synthesis of Heterocyclic Derivative (A_1)

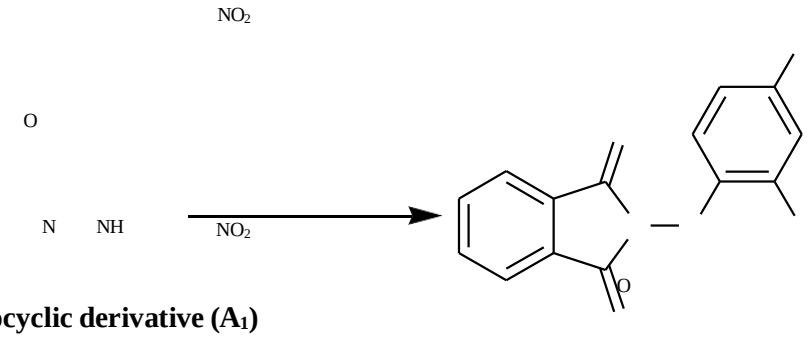
A solution of 01.48 gm of phthalic anhydride in10-15 ml of ethyl acetate in round bottom flask was added to 0.198 gm of 2,4-dinitrophenylhydrazinein15 ml ethyl acetate and put the mixture on magnetic stirrer. The bright orange coloured solution was refluxed for 2h with constant stirring and poured into cold water. The yellow coloured precipitate obtained was filtered, washed with ethanol and finally dried. Yield: 19%.The synthesis of heterocyclic derivative is represented in **Scheme I.**

Synthesis of heterocyclic derivative (A_2)

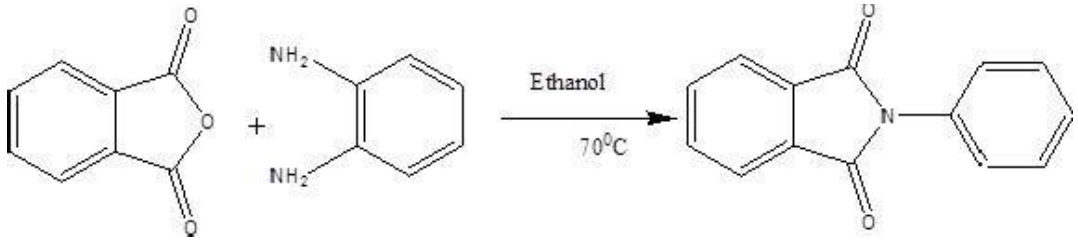
To the solution of 0.148gm of phthalic anhydride in 10-15 ml of acetic acid in round bottomed flask, a solution of 0.198 gm of 2,4-dinitrophenylhydrazine in 15 ml acetic acid was added. The transparent solution was refluxed for 2h with constant stirring and poured into cold water. The creamy yellow coloured precipitate obtained was filtered, washed with ethanol and finally dried. Yield : 13%. The synthesis of heterocyclic derivative is represented in **Scheme II.**

Synthesis of heterocyclic derivative (A_3)

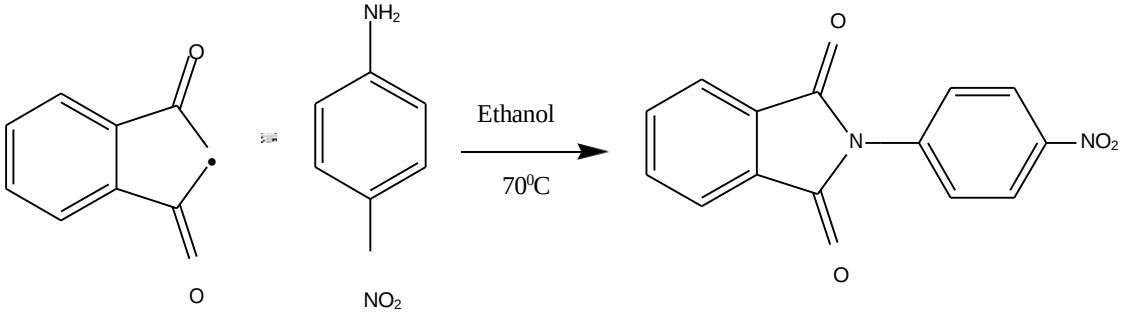
To the solution of0.690gm (5mmol) of p-nitroaniline in 10 ml of ethanol in round bottomed flask, 0.740gm (5mmol) of phthalic anhydride in 15ml ethanol was added. Mixture was refluxed until the solution became clear. The orange solution was refluxed for 2h with constant stirring and poured into cold water. The solution obtained was fully evaporated and the orange jelly precipitate was observed and the product was finally dried. Yield is 17%. The synthesis of heterocyclic derivative is represented in **Scheme III.**



Scheme I. Synthesis of heterocyclic derivative (A_1)



Scheme II. Synthesis of heterocyclic derivative (A_2)

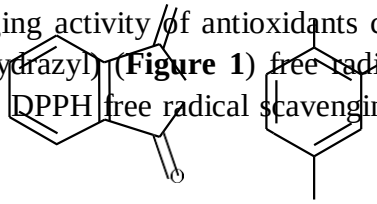


Scheme III. Synthesis of Heterocyclic derivative (A_3)

ANTIOXIDANT PROPERTIES

DPPH Radical Scavenging Activity

Scavenging activity of antioxidants compounds was measured by the DPPH (2,2- diphenyl-1-picryl-hydrazyl) (**Figure 1**) 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



(Alizadeh et al; 2015, Azza et al; 2015)). 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 incubated at 60^0C for 2 h and the decrease in absorbance was noted at 511 nm using UV-Vis. spectrophotometer. Absorbance of DPPH without compound was recorded at 513 nm as a control. For each of the test compound experiment was done in triplicate and antioxidant property of the compounds was measured by using the equation:

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

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.

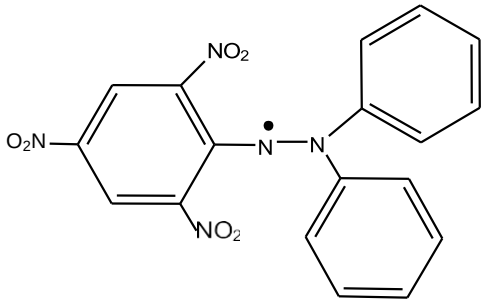


Figure 1. 2,2-diphenyl-picryl-hydrazyl(Free radical).

Hydrogen Peroxide Scavenging Activity

The scavenging ability of the compound to hydrogen peroxide was determined by using standard method (Patel; 2005, Ramadan et al; 2014). Hydrogen peroxide solution (2mM) was prepared in phosphate buffer (50mM, pH7.4). 3ml of test compounds, 1.8 ml of H_2O_2 solution was added and absorbance was recorded after incubating 10 minutes against phosphate buffer as a blank by UV-Vis spectrophotometer. The absorbance of test samples was noted at 240 nm and compared with hydrogen peroxide concentration which was taken as a control. The scavenging ability of hydrogen peroxide was calculated using following equation:

$$\%\text{Inhibition} = \frac{A_B - A_T}{A_B} \times 100$$

Where, A_B was the absorbance of blank (without compounds) and A_T was the absorbance of tested samples.

RESULTS AND DISCUSSION

All the synthesized compounds are stable in air. The ligand and its complexes are soluble in ethanol, methanol and DMSO. The solubility of the ligand and metal complexes 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.**

Table1. Solubility of the Heterocyclic Derivatives

Solvents	Compound(A_1)	Compound(A_2)	Compound(A_3)
Methanol	√	√	√
Water	√	√	X
DMF	√	√	√
Ethylacetate	√	√	X
Chloroform	X	X	X
Ethanol	√	√	X
Aceticacid	√	√	X

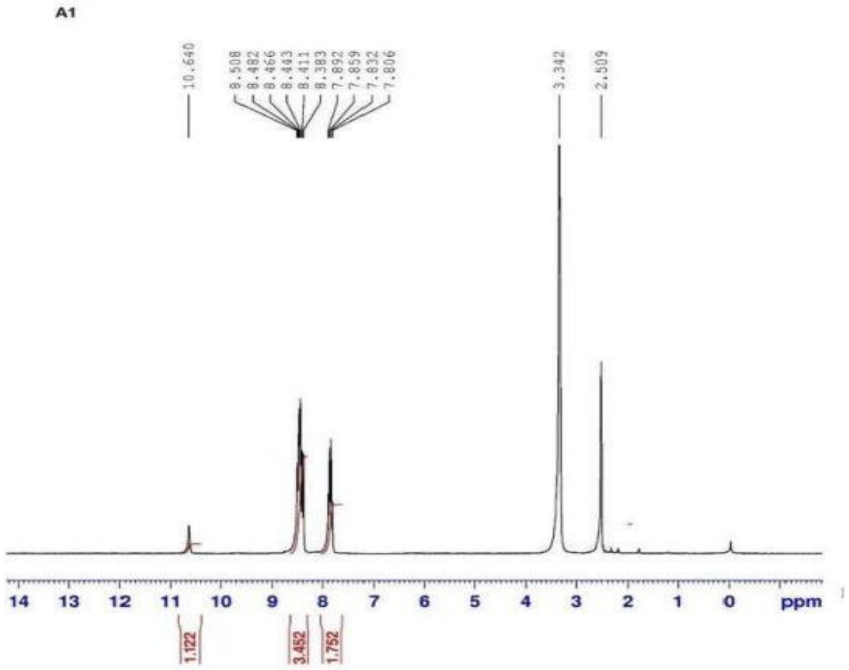
Table 2: Molecular weight, analytical data, molar conductance, colour, % yield and melting point.

Compounds (Mol. Wt.)	Yield (%)	Colour	M.P. (^{0}C)	Analytical data (% Calcd.)				
				C	H	N	O	
[$C_{14}H_{10}O_2N_2$], (A_1)(328)	19	Yellow	259^{0}C	51	2.42	17	29	
[$C_{14}H_{10}O_2N_2$], (A_2) (238)	13	Creamy Yellow	264^{0}C	70.5	4	11.5	13	
[$C_{14}H_8O_4N_2$] (A_3) (268)	17	Orange	221^{0}C	62.5	2.75	10.5	23.7	

^{1}HNMR Spectra

The ^{1}H NMR spectra of ligand was exhibited resolved signals. 1H NMR spectrum of heterocyclic derivatives **A_1** and **A_2** showed a signal at 10.64 ppm and 8.9 ppm as a singlet due to two phenolic protons, respectively (**Figure 2**). The signals as multiplet for the ligand appear at

7.22–8.86 ppm due to protons of aromatic rings.



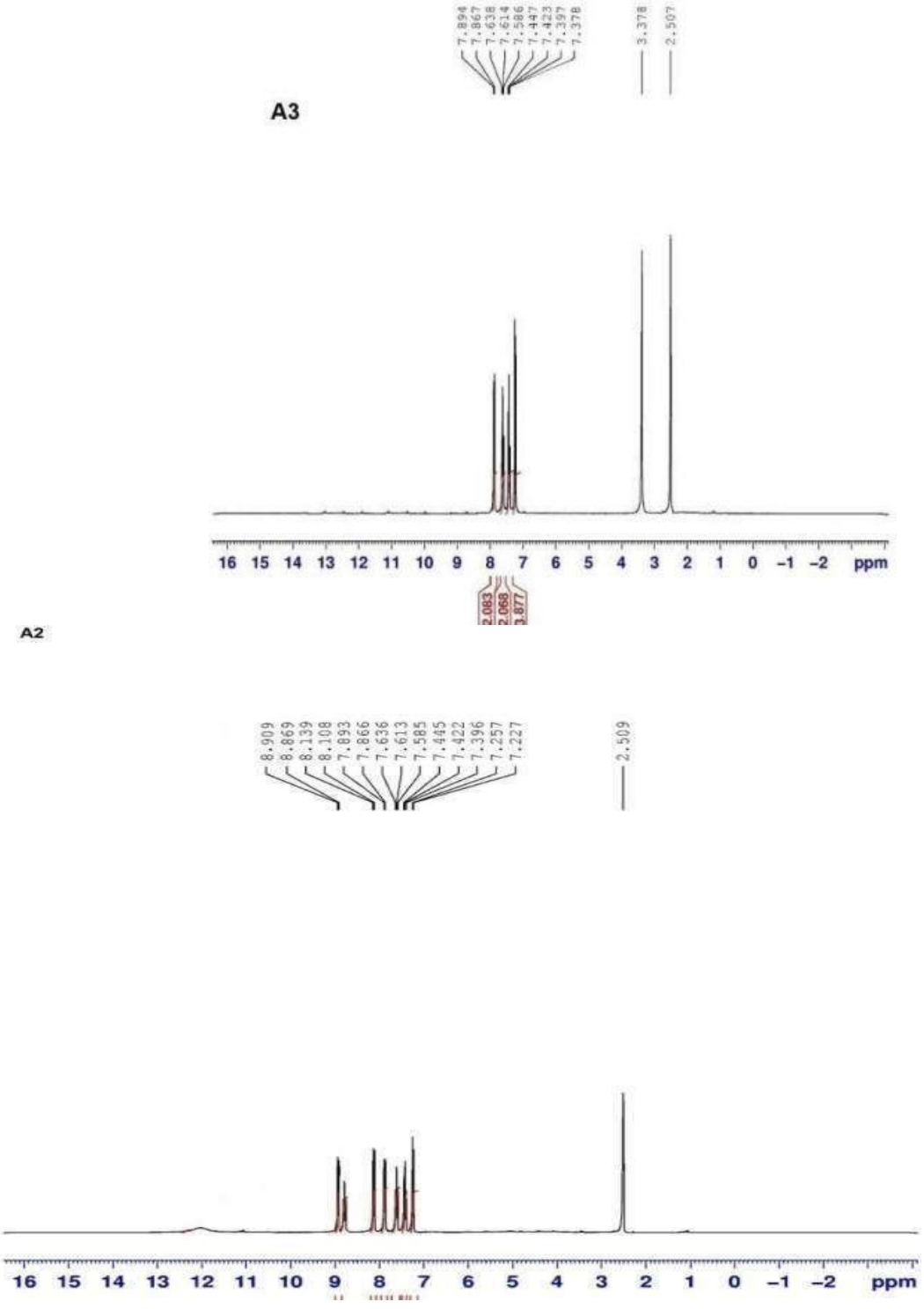


Figure 2. [1]HNMR Spectra of Hetero-cyclic Derivatives (A_1, A_2 and A_3)

ANTIOXIDANT STUDIES

DPPH Free Radical Scavenging Activity

The DPPH Free radical assay is a very significant and appropriate method which provided an easy and rapid way for the estimation of the antioxidant activity of the test compounds (**Figure 3**). It is a stable free radical that can accept hydrogen ion or electron on reaction with antioxidant compound and become reduced. IC_{50} value of the derivatives $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ was found to be **0.407±0.39, 0.429±0.42** and **0.434 ± 0.43,** respectively. Results revealed that heterocyclic derivative $\mathbf{A_2}$ showed significant activity as compared to other derivatives.

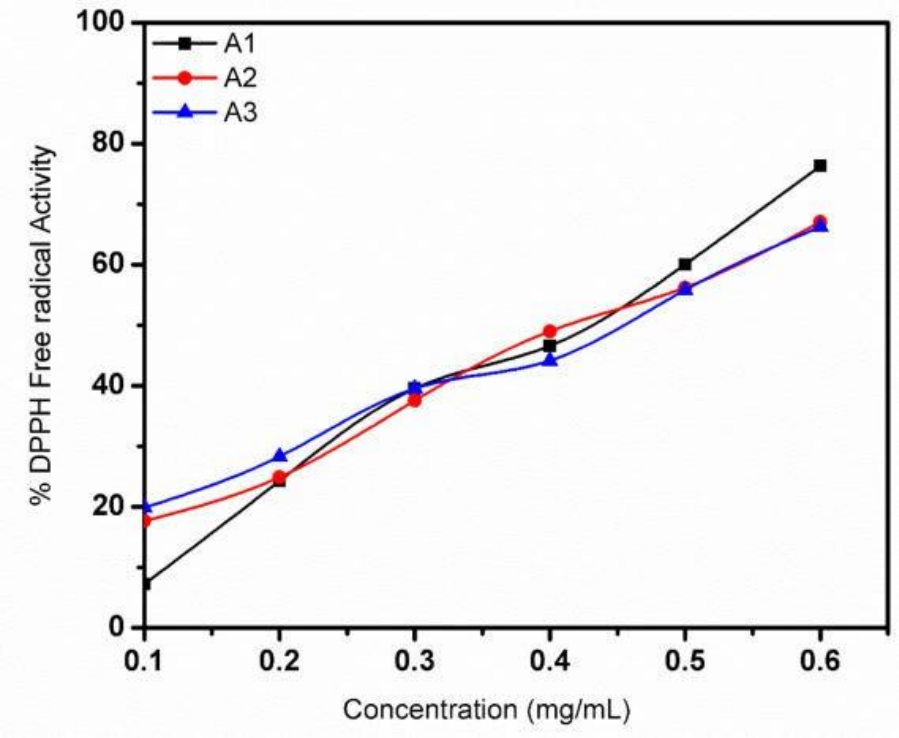


Figure 3. DPPH Free Radical Activity of Heterocyclic Derivatives (**A_1, A_2 andA_3)**

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide radical is very reactive species among all the oxygen containing compound used for the estimation of antioxidant activity of compounds (**Figure 4**).Therefore, the antioxidant activity of our compounds also estimated by hydrogen

peroxide radical. The capabilities of target compounds to scavenge the hydrogen peroxide radicals were monitored using UV-vis. Spectrophotometer (**Figure 4**). IC_{50} value of the derivatives **A_1, A_2** and **A_3** was found to be **0.730±0.72, 0.514±0.516** and **0.512 ± 0.510**, respectively. The investigation of antioxidant assay demonstrates that derivative **A_2** showed the greater rate of H_2O_2 scavenging activity than other derivatives and the results are accordance with the results obtained by DPPH free radical method.

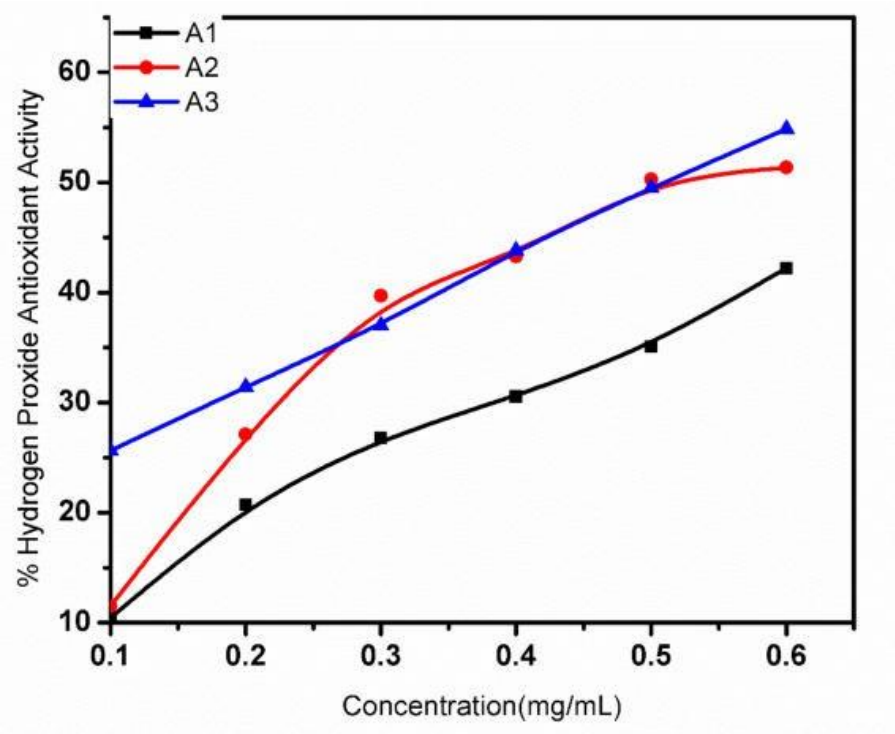


Figure 4. Hydrogen Peroxide Assay of Heterocyclic Derivatives (**A_1, A_2 and A_3**).

CONCLUSION

In this current research, heterocyclic derivatives were synthesized and structurally characterized by UV and ^{1}H NMR spectral analysis and Antioxidant properties. All synthesized compounds are stable in air. Heterocyclic 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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