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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SOME NOVEL FORMAZANS

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ABSTRACT

Various substituted formazan derivatives received considerable importance during last decade as they are covered with wide variety of biological and pharmacological activities and have a wide range of therapeutic importance. Based on this a series of new formazan derivatives has been synthesized. Phenyl hydrazine was added drop wise to mixture of various substituted Aromatic aldehyde in dilute acetic acid forms various substituted Phenyl hydrazones. The solution of substituted Phenyl hydrazones in pyridine was added to diazonium salt solution such as hetero aryl amine (2-amino pyridine, 2-amino pyrazine) forms a novel various coloured formazan derivatives. The synthesized compounds were characterized by physical studies, like solubility, melting point, TLC and subjected to spectral studies like IR, ^{1}H-NMR and mass spectroscopy. All the synthesized compounds were screened for *In-vitro* antioxidant activity was performed by the DPPH scavenging method by using Ascorbic acid as reference standard. Results suggested that electron withdrawing groups like nitro, chloro containing compounds shown potent antioxidant. Rest of the compounds showed mild to moderate activity.

KEYWORDS

Formazans, Hetero aryl amine and Antioxidant activity.

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INTRODUCTION

Formazans are characterized by intense colours, ranging from cherry red to a deep purplish black and contain the characteristic chain of atoms -N=N-C=N-NH-. Formazans are generally solids of relatively low melting points in spite of large the size of the molecules[1]. It is obtained from reduction of tetrazolium salts. Tetrazolium salts are colourless or faintly yellow compounds and they are reduced to deeply coloured compounds known as

formazans. The formazan moiety is substituted with three phenyl groups at R, R', R" which is called 1, 3, 5-triphenyl formazan. They are often particularly soluble in chloroform and acetone; in water the solubility appears to be negligible, the solvent being colored.

MATERIAL AND METHODS

All chemicals were used of analytical grade from Sigma Aldrich and S.D. Fine Chem. Limited, and the solvents used were purified by standard methods. The synthesized compounds were evaluated for antioxidant activity by free radical scavenging DPPH method here ascorbic acid was used as reference standard.

Experimental Procedure

The schematic representation of synthesis of formazan derivatives was as follows,

Step 1

0.01 moles of phenyl hydrazine was added drop wise to a well-stirred mixture of 0.01 moles Aromatic aldehyde in dilute acetic acid (2ml in 10ml water) in a 100 ml conical flask at room temperature. The reaction mixture was further stirred for 1 hour and kept at room temperature for 30 minutes. The precipitated yellow crystalline mass was filtered and dried in an oven at 60^0C. The crude product was recrystallized from rectified spirit with charcoal treatment. Benzaldehyde Phenylhydrazone was obtained as fine colorless needles, with melting point 156^0C, and % yield was 80%.

Step 2

Preparation of Formazan derivatives

Synthesis of 1-phenyl-3-phenyl-5 (aryl/hetero aryl) formazan derivatives

0.01mol of substituted aryl amine (2-amino pyridine) was dissolved in a mixture of 5ml concentrated hydrochloric acid and 5ml water taken in a100ml conical flask, with constant stirring. The reaction mixture was in ice bath until the temperature fell below 50^0C separately, 1.6 g of sodium nitrite was dissolved in 7.5ml of water and chilled in ice bath below 50^0C. The sodium nitrite solution was filtered to obtain a clear solution and then added drop wise to the aniline mixture with vigorous shaking and the temperature was not allowed to rise above 100^0C. This diazonium salt solution of aryl and heteroaryl amine was filtered to obtain a clear solution and then added drop wise with continuous stirring to a solution of benzaldehyde phenyl hydrazone (0.01mol) in pyridine (20 ml), maintaining the temperature below 100^0C. The reaction mixture was allowed to stand for about 4 hours and was then poured in to 250 ml of ice-cold water with continuous stirring. The dark colored solid which separated out was filtered, washed successively with cold water, followed by hot water, finally with methanol and dried in air as well. The formazans thus synthesized were recrystallized from the mixture of chloroform and petroleum ether.

Step 3

Preparation of formazan Derivatives

Synthesis of 1-phenyl-3-phenyl-5(aryl/hetero aryl) formazan derivatives

0.01mol of substituted aryl amine (2-amino pyrazine) was dissolved in a mixture of 5ml concentrated hydrochloric acid and 5ml water taken in a100ml conical flask, with constant stirring. The reaction mixture was in ice bath until the temperature fell below 50^0C separately, 1.6 g of sodium nitrite was dissolved in 7.5ml of water and chilled in ice bath below 50^0C. The sodium nitrite solution was filtered to obtain a clear solution and then added drop wise to the aniline mixture with vigorous shaking and the temperature was not allowed to rise above 100^0C. This diazonium salt solution of aryl and heteroaryl amine was filtered to obtain a clear solution and then added drop wise with continuous stirring to a solution of benzaldehyde phenyl hydrazone (0.01mol) in pyridine (20 ml), maintaining the temperature below 100^0C. The reaction mixture was allowed to stand for about 4 hours and was then poured in to 250 ml of ice-cold water with continuous stirring. The dark colored solid which separated out was filtered, washed successively with cold water followed by hot water, finally with methanol and dried in air as well. The formazans thus synthesized were recrystallized from the mixture of chloroform and petroleum ether.

***In-vitro* antioxidant activity screening**

Antioxidant activity was performed by DPPH free radical scavenging assay method using ascorbic acid as reference standard. All the title compounds (FZ_1-FZ_6) were evaluated for *in vitro* antioxidant activity. The effect of the synthesized titled compounds was tested with different concentrations (25, 50, 75, 100µg/ml) against free radicals produced by DPPH. All derivatives were able to inhibit free radical production.

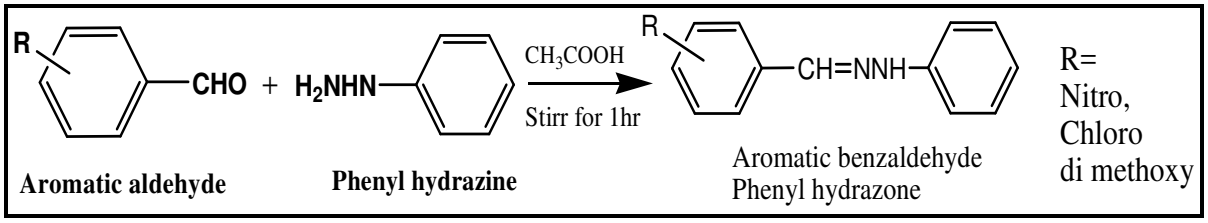
RESULTS AND DISCUSSION

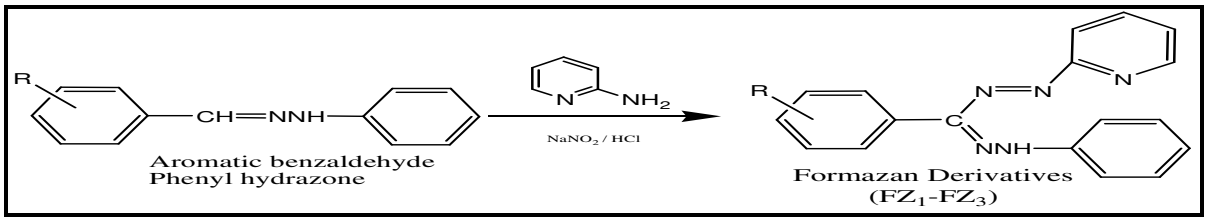
The antioxidant activities of the synthesized derivatives were evaluated by free radical scavenging DPPH method against the standard, Ascorbic acid. The results of this method were represented in the Table No.1.

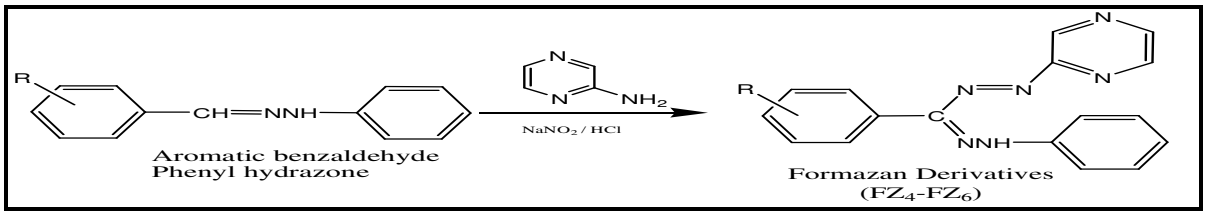
Antioxidant activity was performed by DPPH free radical scavenging assay method using ascorbic acid as reference standard. All derivatives were able to inhibit free radical production. The most effective one was FZ_5 (2Z, 4E)-3-(4-chlorophenyl) (phenyl)-5-(pyrazin-2-yl) formazan, showed potent activity due to the presence of chloro group at *para* position. The order of anti-oxidant activity of all synthesized compounds against free radicals as follows $FZ_{5>}$ $FZ_{6>}$ $FZ_{4>}$ $FZ_{2>}$ $FZ_{1>}$ $FZ_{3.}$

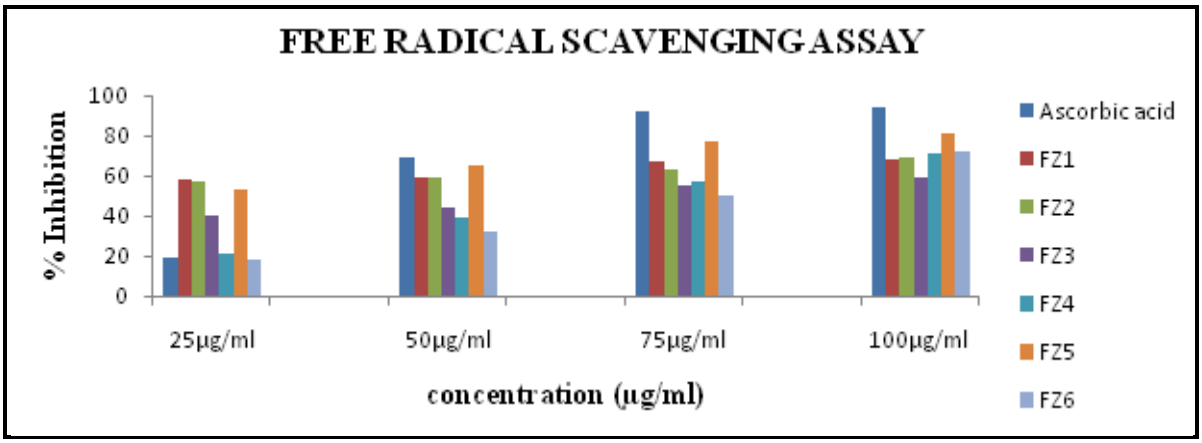
Table No.1: Anti-oxidant activity of test compounds by DPPH method

S.No	Con. µg/ml	FZ_1		FZ_2		FZ_3		FZ_4		FZ_5		FZ_6	
		Abs	% inh	Abs	% inh	Abs	% inh	Abs	% inh	Abs	% inh	Abs	% inh
1	25	0.085	57.5%	0.086	57%	0.121	39.5%	0.158	21%	0.089	53.5%	0.164	18%
2	50	0.082	59%	0.083	58.5%	0.112	44%	0.122	39%	0.070	65%	0.137	31.5%
3	75	0.067	66.5%	0.073	63.5%	0.090	55%	0.087	56.5%	0.046	77%	0.099	50.5%
4	100	0.064	68%	0.061	69.5%	0.082	59%	0.057	71.5%	0.038	81%	0.055	72.5%









Graph No.1: Anti-oxidant activity of ascorbic acid and synthesized derivatives

CONCLUSION

All the derivatives were evaluated for *in-vitro* antioxidant activity by DPPH method by using Ascorbic acid as internal standard. The electron withdrawing groups such as chloro at para position containing compounds(FZ_2, FZ_5) has shown the highest activity and the rest of compounds having electron donating groups (FZ_1, FZ_3 and FZ_4) shows mild to moderate activity. In brief electron withdrawing groups containing compounds possess potent activity than that of compounds containing electron donating groups.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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