SYNTHESIS CHARACTERIZATION ANTIOXIDANT ACTIVITY OF PARADIMETHYLAMINO BENZALDEHYDE DERIVATIVES

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ABSTRACT: A new series of p-dimethyl amino benzaldehyde heterocyclic Schiff base derivatives (compound 1 to compound 3) were synthesized by the condensation reaction of p-dimethyl amino benzaldehyde and substituted amines by conventional synthesis method (cyclocondensation) and tested for antimicrobial activity. The structures of new pyrazoline derivatives were characterized by IR spectroscopy, NMR spectroscopy and mass spectrometry. The synthesized compounds were tested for their in vitro antioxidant activity by DPPH scavenging method and hydrogen peroxide scavenging method. The synthesized compounds were also studied for their DNA binding activity. The results showed that compounds compound1, compound2, and compound 3 all are exhibited lowest IC50 value 0.84±0.007, 1.17±0.003 and 1.29±0.012 mg/ml respectively for DPPH method and 0.86±0.86, 1.16±0.005 and 1.29±0.05 mg/ml respectively in hydrogen peroxide scavenging method and the results are accordance with the results obtained by DPPH free radical method.

Key words: Schiff base, Benzaldehyde, Antioxidant, DNA binding

I. INTRODUCTION

Heterocyclic compounds occur widely in nature and in a variety of naturally occurring compounds. A large number of heterocyclic compounds are essential to life. Various compounds such as antibiotics, alkaloids, essential amino acids, the vitamins, hemoglobin, the hormones and many synthetic drugs and dyes contain heterocyclic ring system. The knowledge of the heterocyclic chemistry is useful in biosynthesis, drug designing and metabolism. There are a large number of synthetic heterocyclic compounds with additional important applications and many are valuable intermediates in synthesis.

Heterocyclic compounds play an important role in biological systems. Biomacromolecules like nucleic acids (DNA and RNA) contain pyrimidine and purine heterocyclic ring structures[1] [55]. The essential amino acids proline, histidine and tryptophan; the vitamins B12 and E families; the coenzyme precursor riboflavin (vitamin B2), pyridoxine, folic acid and biotin, the chlorophyll; the haemoglobin; the harmones like kinetin, serotonin, histamine; the bile pigments, all contain heterocyclic units. Heterocyclic compounds interact with the biological molecules causing physiological effect. Some heterocyclic compounds of natural origin producing physiological effect are morphine, codeine, papaverine, coniine, theophylline, theobromine, atropine, morpholine and quinine etc.. Morpholine and quinine were used as active ingredient in many natural remedies before the development of modern chemistry.

Heterocyclic systems containing nitrogen, sulphur and oxygen atom constitutes a large class of compounds of biological and medicinal interest[2] [56]. Many heterocyclic systems (mainly five and six membered compounds) have been successfully incorporated into novel drug leads and therapeutics. The synthetic drugs such as fluconazole (antifungal), ciprofloxacin (antibiotic), AZT/Zidovudine (antiviral), ritonavir (anti-HIV), imatinib (anticancer), paroxetine (anti-depressant), diazepam (antianxiety), rofecoxib (anti-inflammatory), mitiglinide (anti-diabetic) etc. are heterocycles.

In the present scenario, there is a tremendous demand for biologically active molecules in the market and superior drug in shorter time is always a challenging task for medicinal chemists. But at the same time the synthesis of new patentable molecules with high activity and selectivity, drug-likeness and good pharmacokinetic properties is equally fascinating[3] [57]. To achieve this, sometimes many heterocyclic ring structures are designed in such a way that their binding efficiency with the receptor increases after carrying certain structural modifications[4] [58]. This is a boon to the medicinal chemists and provides long-term advancement in the medical field[5] [59].

Para-dimethylamino benzaldehyde plays a vital role in many fields generally obtained from the reaction of Chitosan with monochloroacetic acid and in alkaline condition[6] [1]. Para-dimethylamino benzaldehyde has higher moisture absorption and retention and better biological, chelating and sorption properties[7-9] [2-4]. Moreover, Para-

dimethylamino benzaldehyde derivatives exhibit low toxicity, biocompatibility, biodegradable, antibacterial property and apoptosis inhibitory activity[10-12] [5-7]. So, it could promote growth in skin keratinocytes and skin fibroblast, and wound healing to make as a good wound dressing material[13] [8].

Free radicals are atoms, molecules or ions that possess an unpaired electron in orbit[14] [1]. They are continually formed in the body and can become toxic when obtained in high concentration or in the presence of a deficiency in the natural antioxidant defenses. At high concentration free radicals can damage lipids, proteins, and DNA within cells and tissues. The human body does have essential defense pathways to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. However, the unbalances between the formation and detoxification of free radical species results in the development of oxidative stress. This leads to the development of severe diseases, such as cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, and neurodegenerative disorders[14] [2]. Antioxidants are compounds that can interact with free radicals in a safe way, terminate the reaction, and convert them to a harmless molecule by offering an electron. Antioxidants therefore reduce the oxidative stress and thus protecting the cells from oxidative damage[15] [3].

The design of new antioxidant compounds has become an important therapeutic matter given the wide-ranging damage to cellular macromolecules caused by reactive oxygen species (ROS). Therefore, extensive research has been focused to identify new antioxidants to prevent radical-induced damage. In this study, we concentrated on design, synthesis, characterization, and evaluation of the antioxidant activity of newly synthesized Para-dimethylamino benzaldehyde Schiff base. The structures of the various synthesized compounds were verified based on elemental analysis, along with infrared (IR) and 1D-NMR spectral data.

II. EXPERIMENTAL

2.1 Material

All chemicals and reagents were used are of analytical grade. Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. binding of potent Schiff base derivatives to Ct-DNA was investigated by fluorescence quenching, absorption spectroscopy, circular dichroism, and viscosity measurements.

2.2. Synthesis

2.2.1 General procedure for preparation of para-dimethylamino benzaldehyde Schiff base derivatives

Heterocyclic Schiff base derivatives (**compound 1 to compound 3**) were synthesized by the condensation reaction of *p*-dimethylaminobenzaldehyde and substituted amines in 1:1 molar ratio. All the derivatives were prepared in good yields with characteristic colour. The compounds were soluble in chloroform and DMSO and insoluble in water. Melting points were in the range of 180–290 oC. The progress of the reaction was monitored by TLC in ethyl acetate: hexane (1:1).

An appropriate amount of corresponding amines (10 mmol) in 20 ml ethanol was slowly added to a solution of 10 mmol 4-(dimethylamino)benzaldehyde in 20 ml ethanol. Few drops of conc. sulphuric acid were added to the mixture solution. The coloured solutions were refluxed with constant stirring for 8 h at 80 ^{o}C. After completion of reaction mixture was poured into ice-cold water. The coloured precipitates were filtered off by buchner funnel and finally dried in vacuum dessicator on fused $CaCl_2$ and recrystallized in ethanol.

N-(4-(dimethylamino) benzylidene)-2,6-dichloropyrimidin-4-amine (Compound 1)

^{1}H NMR (300 MHz, DMSO-d6) δ in ppm: 7.95-8.04 (m, 4H, Ar-H), 6.25 (s, 1H, Ar-H), 9.14 (s, 1H, -CH=N), 3.061 (s, 6H, -N-CH3).

^{13}C NMR (100 MHz, DMSO-d6) δ in ppm: 62.59, 155.10, 148.29, 142.90, 133.04, 131.98, 127.62, 125.59, 124.97, 111.51, 40.79.

5-(4-(Dimethylamino) benzylideneamino) quinolin-8-ol (Compound 2)

^{1}H NMR (300 MHz, DMSO-d6) δ in ppm: 8.05-8.10 (m, 4H, Ar-H), 7.86-7.95 (m, 2H, Ar-H), 7.65-7.70 (m, 2H, Ar-H), 6.76 (d, 1H, Ar-H), 8.95 (s, 1H, -CH=N), 3.03 (s, 6H, -N-CH3), 11.45 (s, 1H, -OH).

^{13}C NMR (100 MHz, DMSO-d6) δ in ppm: 160.25, 151.21, 148.49, 138.72, 134.77, 133.18, 129.81, 128.36, 123.86, 122.71, 115.84, 40.82.

5-(4-(Dimethylamino) benzylideneamino)-2H-1,2,4-triazole-3-thiol (Compound 3)

^{1}H NMR (300 MHz, DMSO-d6) δ in ppm: 8.05-8.10 (m, 4H, Ar-H), 11.52 (s, 1H, -NH), 13.96 (s, 1H, -SH), 9.23 (s, 1H, -CH=N), 3.23(s, 6H, -N-CH3).
^{13}C NMR (100 MHz, DMSO-d6) δ in ppm: 163.21, 158.75, 148.29, 142.85, 133.04, 131.98, 127.62, 119.02, 40.79.

2.3. Antioxidant Assay

2.3.1 DPPH Radical Scavenging Activity

2,2-Diphenyl-1-picryl-hydrazyl is a stable free radical which was used for the estimation of antioxidant activity. The free radical scavenging ability of the test compounds was studied using the DPPH assay[16] [1]. The test compounds in ethanol were prepared separately and added to the ethanol solution containing DPPH (0.01 mmol) within the range of 0.5–2.5 mg/ml and adjusted to a final volume of 3 mL using ethanol as solvent. The scavenging ability of the test compounds was monitored spectrophotometrically by measuring the absorbance at 517 nm after 20 min. The % inhibition was obtained using equation. The 50 % decline in absorbance of the DPPH solution was derived from the graph with the concentration (μM) plotted against the absorbance. These concentration values were used to determine the IC_{50} values in μM.

$$\%\ of\ inhibition = \frac{ABScontrol - ABSsample}{ABScontrol} \times 100$$

2.3.2 Hydrogen Peroxide Scavenging Activity

The ability of the synthesized compounds to scavenge hydrogen peroxide was measured by using standard H_2O_2 scavenging assay method[17] [2]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (10x, pH 7.4). A series of various concentrated solution of each of the synthesized coumarin compounds (0.5 – 2.5 mg/ml) were prepared in ethanol (95%) and added (1 ml) to the hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution. Ascorbic acid was used as standard. All the experiments were carried out in triplicates in dark condition. The percentage of scavenged hydrogen peroxide was calculated by using the following equation,

Percentage of scavenged $H_2O_2 = [(A_i - A_t) / A_i] \times 100$

Where A_i is the absorbance of control and A_t is the absorbance of test.

H_2O_2 scavenging activity of the Coumarins was expressed in terms of IC_{50} value.

III. RESULT AND DISCUSSION

3.1 Synthesis

New Para dimethyl amino benzaldehyde derivatives (compound 1 to compound 3) were synthesized by the condensation reaction of *p*-dimethylaminobenzaldehyde and substituted amines in 1:1 molar ratio. All the derivatives were prepared in good yields. The synthesized compounds were analysed by ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a **Bruker DPX-400 MHz** spectrometer. The physiochemical properties like melting point and percentage of yield were determined. (Table 1)

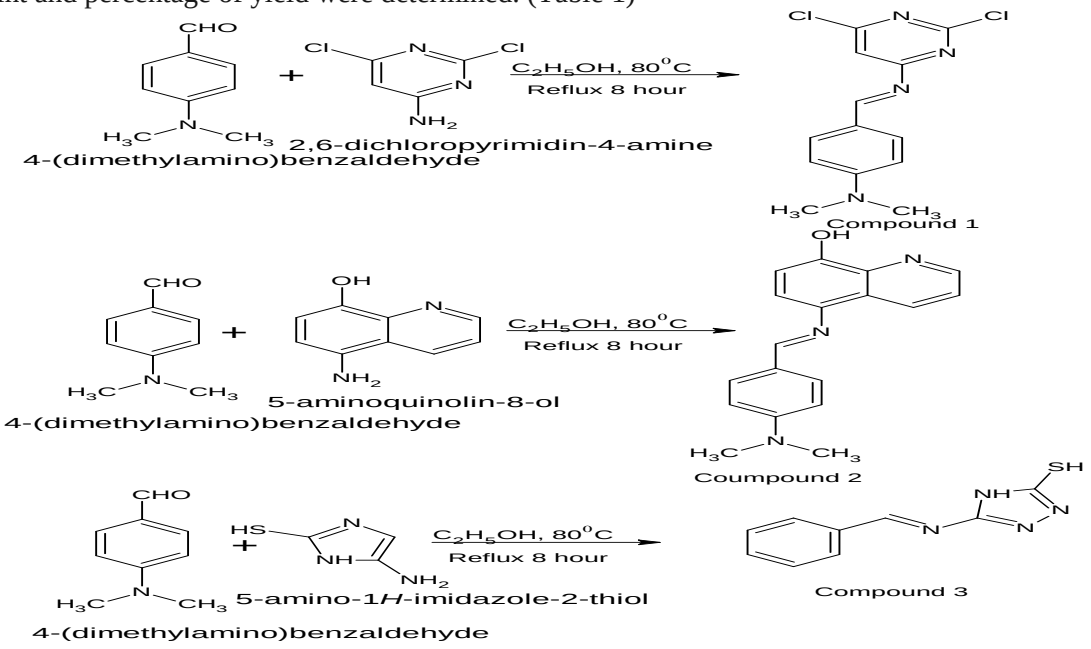

Table 1: Physiochemical character of synthesized compounds

Sl. No.	Compound	Molecular formula	Molecular weight	Melting point (°C)	% of yield	Nature
1	**Compound 1**	C13H12Cl2N4	295.17	280	69	Yellow solid
2	**Compound 2**	C18H17N3O	291.35	**208**	69	Brown solid
3	**Compound 3**	C11H13N5S	247.33	270	74	Orange solid

3.2 Antioxidant activity

3.2.1 *DPPH Radical Scavenging Activity*

2,2-Diphenyl-1-picryl-hydrazyl is a stable free radical which was used for the estimation of antioxidant activity. The absorbance at 517 nm was measured and IC50 value was calculated from the graph given. Higher the ability of compound to scavenge the DPPH refers to lower IC50 value. It was observed that increase in concentration there is increase in % of antioxidant activity The results showed that compounds **compound1, compound2,** and **compound 3** all are exhibited lowest IC50 value 0.84±0.007, 1.17±0.003 and 1.29±0.012 mg/ml respectively. (Table 2)

Table 2: DPPH Radical scavenging activity of synthesized compounds

Concentration (mg/ml)	% Antioxidant activity		
	Compound 1	Compound 2	Compound 3
0	0	0	0
0.5	42	42	36
1	51	43	40
1.5	65	54	52
2	70	60	55
2.5	72	75	67

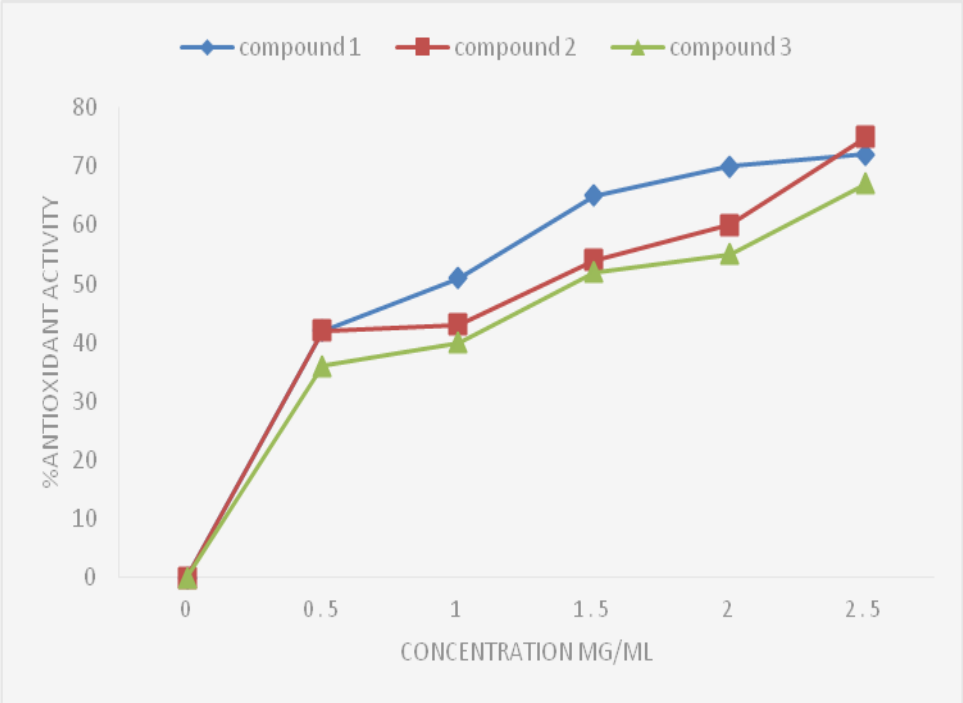

Fig.1. Graph for *DPPH Radical Scavenging Activity*

3.2.2 Hydrogen Peroxide Scavenging Activity

For the investigation of antioxidant activity hydrogen peroxide was used to monitor the ability of the target compounds. The IC50 values of the compounds **compound 1, compound 2 and compound 3** found to be 0.86±0.86, 1.16±0.005 and 1.29±0.05 mg/ml respectively (**figure 4.10**) and the results are accordance with the results obtained by DPPH free radical method. (Table 3)

Table 3: Hydrogen peroxide scavenging activity of synthesized compounds

Concentration (mg/ml)	% Antioxidant activity		
	Compound 1	Compound 2	Compound 3
0	0	0	0
0.5	46	41	35
1	52	45	45
1.5	58	52	50
2	62	56	58
2.5	70	71	67

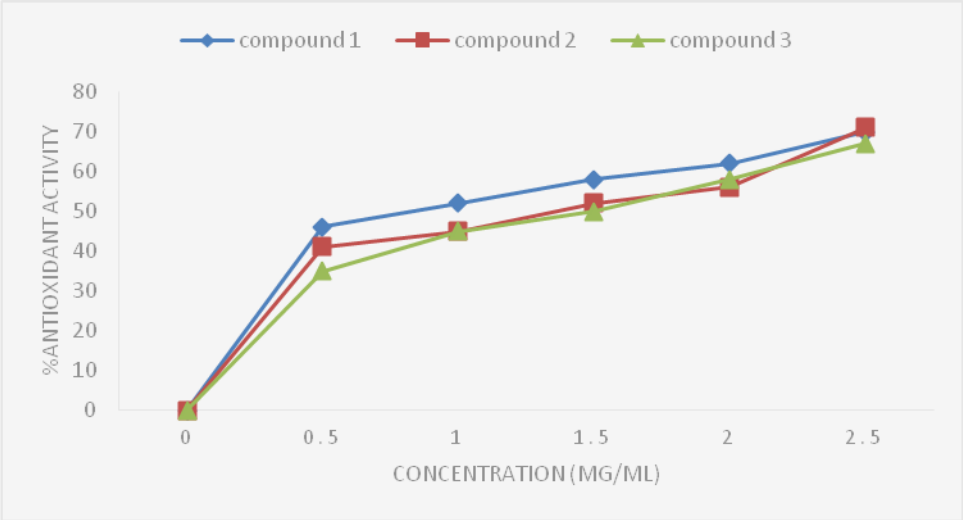

Fig.2. Graph for Hydrogen peroxide scavenging activity

IV.CONCLUSION

Three new compounds of **para-dimethylamino benzaldehyde Schiff base** were successfully synthesized with a good yield of 69–74 %. The characterisation of compounds were identified using different spectral studies. The synthesized compounds exhibited a wide-ranging of possibly promising antioxidant activities. All compounds displayed antioxidant activities using the DPPH and hydrogen peroxide scavenging method. Compounds **1** showed the highest antioxidant activity in both the method. This was due to the fact that all these compounds could generate free radical centers, which were stabilized by the hyper-conjugative effect and the electron-withdrawing groups present in the structures. The mechanism of oxidation of phenolic compounds in a reaction with DPPH is considered as sequential proton-loss electron transfer (SPLET) based mechanism in protic solvent like EtOH.

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