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Synthesis, Spectral Characterization and Biological Study of Heterocyclic Azo Dyes

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ABSTRACT

Series heterocyclic mono azo dyes based on 8-hydroxyquinoline were synthesized and characterized by UV–Vis, FT-IR, ^{1}H NMR, mass spectroscopic techniques and elemental analysis. These dyes are synthesized by diazotization of different 4-substituted anilines (aniline, 4-methyl aniline, 4-Nitro aniline and 4-chloroaniline)by coupling with 8-hydroxyquinoline. Further the synthesized compounds were screened for their biological activity. The results of these investigations revealed that the newly synthesized compounds are showed good antimicrobial activity. In addition, the anti-oxidant activity of the dyes was explored in detail.

KEYWORDS:8-hydroxyquinoline; diazotization; coupling; azo dyes; biological activity.

1. INTRODUCTION

Azo dyes are compounds that contain azo groups linked to methine or aromatic sp^2-hybridized carbon atoms. The formation of diazotizing reagent starts with protonation of nitrous acid under strongly acidic conditions and azo coupling occurs at low temperature in the presence of nucleophilic coupling components. The reactivity of a nucleophilic substrate increases with increase in phenolates and amines [1-4]. Azo dyes are the oldest and largest class of industriallysynthesized organic dyes, accounting forover 50% of all commercial dyes. The great majority of them aremonoazo compounds, which have the common structure unit ofthe azochromophore (–N=N–), linking two aromatic rings[5].Many azo dyes have been extensively used asdyes in various fields such as dyeing of textile fibers, coloredplastics, biologicalmedical studies and advanced applications in organic synthesis [6]. Recently, applications of such coloringmaterials to high technology have been attracting muchattention. Dyes are used in various fields such as printing, electronicphotography, color formers, liquid crystal displays,laser technology, data storage and solar energy conversion [7]. Also, some of such dyes have found use as non-linear optical(NLO) materials. Such compounds have potential use inoptical communications, information processing, frequencydoubling and integrated optics [8-9].Azo dyes possess potent biological activities such as anti inflammatory[10], antitumor agents[11-12], antihistamines[13], antibacterial[14], schistosomicidal agents[15], antituberculotic[16], insecticides[17], antiviral [18] and antifungal[19] etc.

In recent years, heterocyclic coupling compounds received a great deal of attention because of their excellent properties which are light fastness, good substantively, good migration and have a very brilliant shade.

Owing to the high number of positive applications of azo dyes in different fields and previously work carried out in our laboratory [20-22],the present work is focused on the synthesis, characterization and screening for antimicrobial activity of newly synthesized heterocyclic azo dyes which were synthesized by coupling reaction of 8-hydroxy quinoline with 4- substituted aniline derivatives as diazo components.

2. MATERIAL AND METHODS

Infrared spectra of azo dyes were recorded in the region of 4000 cm^{-1} – 400 cm^{-1} on a FT-IR 8400s SHIMADZU spectrometer in KBr pellets. The ^{1}H NMR spectra were recorded in DMSO-d_6, using Bruker 500 MHz instrument using TMS as a standard. The mass spectra were recorded with a LC-MSD – trap – XCT plus

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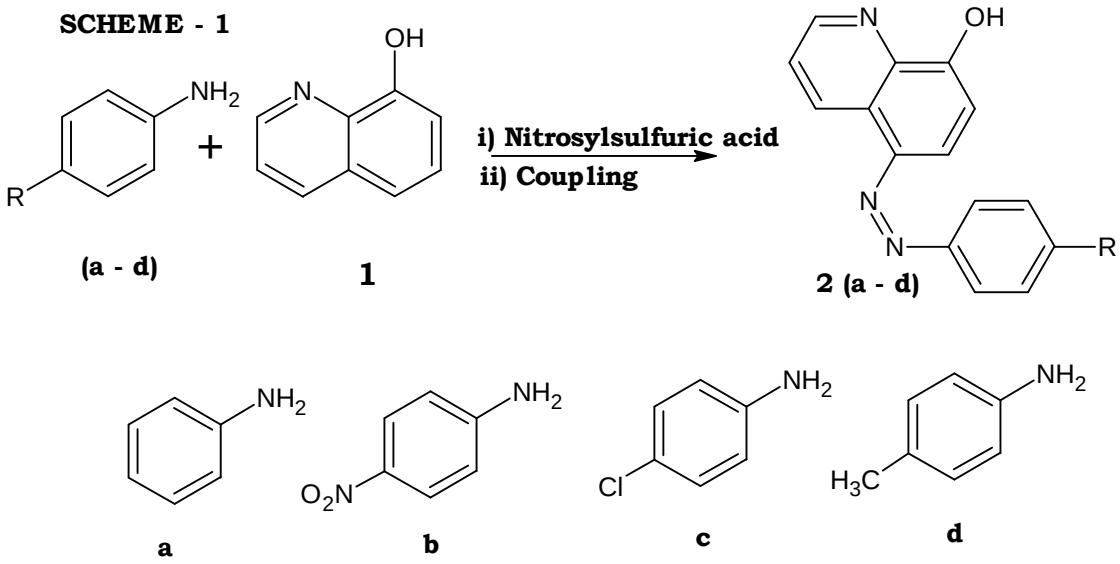
mass spectrometer. The UV-Visible absorption was recorded indifferent solvents using SHIMADZU UV-Visible 1650 spectrometer in a wavelength range 200-800 nm.All analytical grade chemicals were used directly. Melting point of the synthesized compounds was determined in scientific melting point apparatus and uncorrected. The progress of reaction was monitored by TLC using silica gel coated plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation.

2.1. Experimental procedure

2.1.1. Procedure for Synthesis of pyridine based azo dyes: 2(a-d)

Aniline (2.0×10^{-3}mol) was dissolved in glacial acetic acid/propionic acid mixture (2:1, 6.0 ml) and was rapidly cooled in an ice/salt bath to 0-5^{0}C. The liquor was then added in portions during 30 min to a cold solution of nitrosylsulphuric acid (prepared from sodium nitrite (0.15 g) and concentrated sulphuric acid (3 ml at 50 ^{0}C). The mixture was stirred for an additional 2 hours at 0 ^{0}C. The resulting diazonium salt was cooled in salt/ice mixture. After diazotization was complete the diazo liquor was slowly added to vigorously stirred solution of 8-hydroxy quinoline (2.0×10^{-3}mol) in potassium hydroxide (2.0×10^{-3}mol) and water (2 ml). The solution was stirred at 0 – 5 ^{0}C for 2 hours. After 2 hours, the pH of the reaction mixture was maintained at 4-6 by the simultaneous addition of saturated sodium carbonate solution. The mixture was stirred for 1 hour at room temperature. The resulting solid was filtered, washed with cold water and dried.



3. RESULTS AND DISCUSSION

As shown in Scheme 1, heterocyclic azo dyes 2(a-d)were prepared through the diazotization of 4-substituted anilines and coupled with 8-hydroxy quinoline as coupling component. The synthesized dyes were characterized by IR, UV-Vis, mass, ^{1}H NMR and elemental analysis.

Infrared spectra of synthesized dyes 2(a-d) (in KBr), a sharp signal at the range of 1486-1543cm^{-1} proved the stretching frequency of -N=N-, aromatic -C=N- stretching observed at 1186-1270cm^{-1} range. A broad peak appeared at the region 3400-3200 cm^{-1} which confirms the presence of hydroxyl group (-OH). The ^{1}H NMR spectra was recorded in DMSO-d_6 at room temperature showed 6.8 - 7.6 aromatic protons, in 2d a singlet peak at the range of 2.4 for methyl group.

Synthesized heterocyclic azo dyes were in good yield and absorption spectra of these azo dyes 2(a - d) were recorded in different solvents like ethanol, methanol, acetone, DMF and DMSO at a concentration of 10^{-5}- 10^{-6}molL^{-1}. Azo dyes showed an intense lowest energy charge-transfer absorptionband in the UV-visible region.As the dielectric constant of solvent increased, the band originated by π - π* electronic transitions

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shifted to higher wave length. The absorption maxima (λmax) of all the dyes falls in the range 445 – 498 nm and the values are given in Table-I.

3.1. Physical and Spectral data of synthesized compounds

3.1.1.Preparation of5-[(phenyl)diazenyl]quinolin-8-ol:[2a]

This dye was prepared from aniline and 8-hydroxy quinoline as wine red crystals (yield-57% , m.p:178).IR [(KBr) υ $_{max}$/cm^{-1}]:absorptions bands at 3313 cm^{-1}, (Ar-OH),3126 (aromatic C-H), 1562 (C=N), 1499 cm^{-1} (-N=N-).^{1}H NMR (DMSO-d_6): 7.2-8.5 (m, 6H), 9 (d, 1H), 8.7 (d, 1H);MS *m/z* =280 (M^+)Anal. calcd. for$C_{16}H_{13}N_3O_2$: C,68.81; H,4.69; N,15.05; Found: C, 68.78; H,4.65; N, 15.01%.

3.1.2. Preparation of 5-[(4-nitrophenyl)diazenyl]quinolin-8-ol:[2b]

This dye was prepared from 4- nitro aniline and 8-hydroxy quinolineas brown crystals (yield-61%, m.p:189).IR [(KBr) υ $_{max}$/cm^{-1}]:absorptions bands at 3338.24 cm^{-1},(Ar-OH), 1186.07cm^{-1},(C=N), 1527.67cm^{-1} (-N=N-).^{1}H NMR (DMSO-d_6): 8.5(s,2H), 8.05(d,1H), 7.8(d,1H), 6.3(d,1H), 7.5(d,1H).,MS *m/z* =295 (M^+), Anal. calcd. for$C_{15}H_{10}N_4O_3$: C,61.22; H,3.43; N,19.04; Found: C, 61.18; H,3.40; N, 19.01%.

3.1.3. Preparation of 5-[(4-chlorophenyl)diazenyl]quinolin-8-ol:[2c]

This dye was prepared from 4-chloro aniline and 8-hydroxy quinoline as brick red crystals (yield-68% , m.p:213).IR [(KBr) υ $_{max}$/cm^{-1}]:absorptions bands at 3351 cm^{-1}, (Ar-OH), 1667 cm^{-1},(C=N), 1489.20cm^{-1} (-N=N-);^{1}H NMR (DMSO-d_6): 6.8-7.1 (m, 4H), 7.9 (d, 1H), 6.4 (d, 1H).,MS *m/z* =284 (M^+)Anal. calcd. for$C_{15}H_{10}ClN_3O$: C,63.50; H,3.55; N,14.81; Found: C,63.46; H,3.51; N,14.79%.

3.1.4. Preparation of 5-[(4-methylphenyl)diazenyl]quinolin-8-ol:[2d]

This dye was prepared from 4-methyl aniline and 8-hydroxy quinoline as wine red crystals (yield-70% , m.p:201).IR [(KBr) υ $_{max}$/cm^{-1}]:absorptions bands at 3335.49cm^{-1} (Ar-OH), 1656 cm^{-1}(C=N), 1488.29 cm^{-1} (-N=N-);^{1}H NMR (DMSO-d_6): 2.4(s, 3H CH_3), 7.1-7.3 (m, 4H), 7.9(d,1H), 6.2(d,1H); MS *m/z* =264 (M^+), Anal. calcd. for$C_{16}H_{13}NO$; C,79.99; H,4.98; N,15.96; Found: C,79.95; H,4.95; N,15.93%.

Figure-1: Absorption spectra of dyes 2a in different solvents

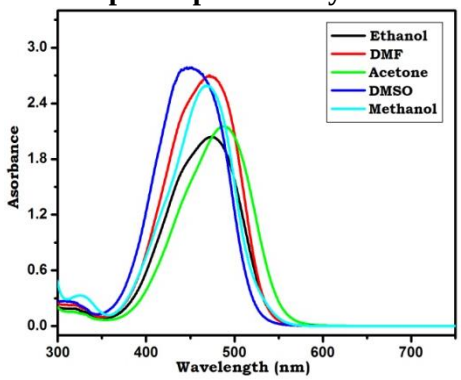

Figure-2: Absorption spectra of dyes 2b in different solvents

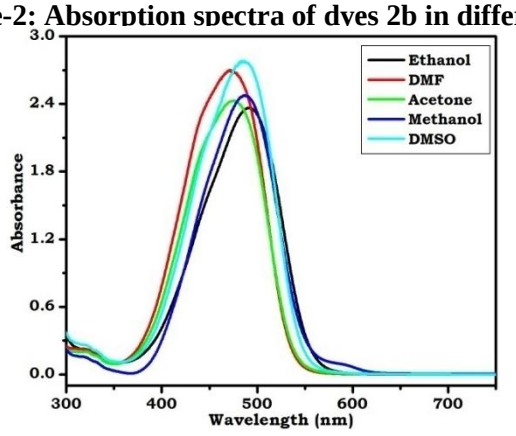

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Figure-3: Absorption spectra of dyes 2cin different solvents

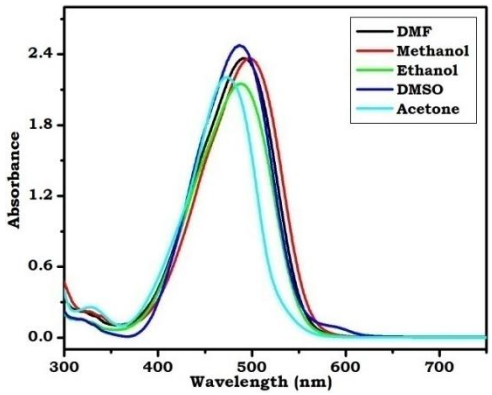

Figure-4: Absorption spectra of dyes 2din different solvents

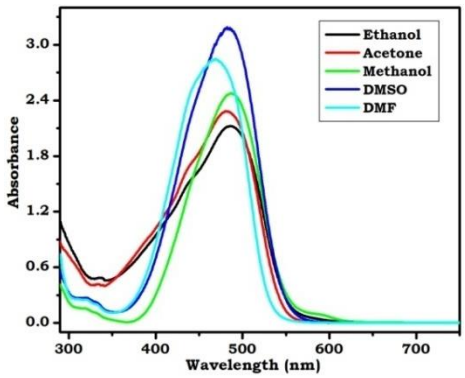

Table-1: Absorbance maxima of the Azo dyes 2 (a - d) in different solvents

Solvents	λ_{max}(nm)			
	2a	2b	2c	2d
Dimethyl sulfoxide (DMSO)	447.43	485.91	487.21	484.31
N,N-Dimethyl formamide (DMF)	472.72	472.62	489.91	470.54
Methanol	468.82	488.61	497.87	489.55
Acetone	488.61	475.32	472.62	482.80
Ethanol	474.02	491.21	488.61	486.79

3.2. Biological activity:

Bacterial and Fungal strains:

The following bacteria and fungi were used for the experiment. Bacteria: *K. pneumonia* ATCC 25923, *Escherichia coli* ATCC 25922, *S. typhimurium*ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37°C. Fungi: *Aspergillusflavus*,and *Candida albicans MTCC 227* are used in this study. These cultures are obtained from the Department of Microbiology, Kuvempu University. All fungi strains were maintained on potato dextrose agar (PDA) at ±25 °C.

3.2.1. Antibacterial activity:

The antimicrobial activity of newly synthesized compounds was evaluated using agar disc diffusion assay [23]. Briefly, a 24 and 48 hours old culture of selected bacteria was mixed with sterile physiological saline

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(0.9%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 (10^6 colony forming units (CFU) per ml). Petri plates containing 20 ml of Mueller Hinton Agar and Sabourauddextrose agar was used for antibacterial activity. The inoculum was spread on the surface of the solidified media and Whatmann filter paper No. 1 discs (5 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the plates. Streptomycin was used as positive control for bacteria. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 hour at 37° C. The diameters of zone of inhibition were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading.

Table-2: In vitro antibacterial activities of the compounds 2(a - d)

Sl No		***Escherichia Coli***		***Staphylococcus Aureus***		***Pseudomonas Aeruginosa***	
		Diameter of zone of inhibition (mm)					
	Conc. in mg/ml	1	0.5	1	0.5	1	0.5
1	**Control**	00		00		00	
2	**Standard *Streptomycin***	16±0.2	10±0.1	15±0.2	10±0.2	16±0.2	13±0.2
3	**2a**	02±0.2	01±0.1	02±0.1	01±0.1	03±0.2	01±0.2
4	**2b**	09±0.2	06±0.1	09±0.1	05±0.1	08±0.2	06±0.2
5	**2c**	05±0.2	02±0.1	05±0.1	03±0.1	04±0.2	02±0.2
6	**2d**	04±0.2	01±0.1	02±0.1	02±0.1	03±0.2	01±0.2

3.2.2. Antifungal activity:

Poisoned food technique was performed to investigate antifungal effect of test compounds against *Aspergillusniger*, and *Candida albicans.* All synthesized compounds were tested for their antifungal activity. The fungi employed for screening were *Aspergillusniger*and *Candida albicans.* The test organisms were sub-cultured using potato dextrose agar medium. The potato-dextrose-agar medium was sterilized by autoclave at 121°C (15 lb/sq. inch), for 15 minutes. The Petri-plates, tubes and flasks plugged with cotton were sterilized in autoclave at 121°C, for an hour. Into each sterilized Petri-plate (10 cm diameter), about 30 ml each of molten potato dextrose-agar medium inoculated with respective fungus (5 mm disc of the fungus grown) was transferred, aseptically. The petri dishes were incubated at 28° **C** temperature. The diameter of the zone of inhibition was read with help of an 'antibiotic zone reader'. The experiments were performed in triplicate in order to minimize the errors. The inhibition percentage of the *Aspergillusniger*, and *Candida albicans*mycelial growth was calculated.

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Table-3: In vitro antifungal activities of the compounds 2(a - d)

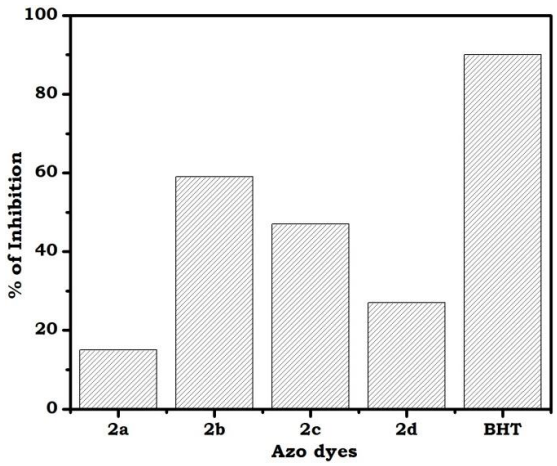
Sl No		Aspergillus Flavus		Chrysosporium Keratinophilum		Candida Albicans	
		Diameter of zone of inhibition (mm)					
	Conc. in mg/ml	1	0.5	1	0.5	1	0.5
1	**Control**	00		00		00	
2	**Standard Fluconazole**	13±0.2	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2
3	**2a**	05±0.5	03±0.4	07±0.3	04±0.8	09±0.5	07±0.3
4	**2b**	10±0.5	07±0.4	13±0.8	09±0.7	11±0.5	07±0.7
5	**2c**	09±0.5	06±0.4	08±0.3	05±0.8	09±0.5	07±0.3
6	**2d**	06±0.5	04±0.4	08±0.3	05±0.8	07±0.5	06±0.3

All the newly synthesized dyes 2(a - d) were evaluated for *in vitro* antibacterial activity against gram positive and gram negative bacteria and also evaluated for in vitro antifungal activity against human pathogens using conventional well plate method. The organic compounds 2b and 2c exhibited clinically appropriate antibacterial activity when compare to the remaining compounds. The compound 2d showing moderate antibacterial activity when compare to the standards ***(Streptomycin)***. The antifungal activity of the newly synthesized compounds was carried out using the fungal pathogens. The compound 2b and 2c showed good and comparable activity that of the standard on the other hand the compound 2a and 2d showed medium activity.

3.2.3. Antioxidant activity (DPPH Method):

Free radicalscavenging capacities of different compounds were determined according to the previously reported procedure [24], using the stable 2, 2-diphenyl-1-picryhydrazyl radical (DPPH). The stock solution of extracts were prepared (1 mg/mL) and DPPH (0.004%) using 95% of methanol. Freshly prepared DPPH solution was taken in test tubes and extracts were be added (100 µg) to every test tube so that the final volume was adjusted to 3mL and after 10min, the absorbance will be read at 517nm using a spectrophotometer UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). BHT has to be used as a reference standard and was dissolve in distilled water. Control sample prepared containing the same volume without any extract and reference ascorbic acid. 95 % methanol will serves as blank. The assay was carried out in triplicate and the percentage of inhibition was calculated.

Figure-5:DPPH radical scavenging activity



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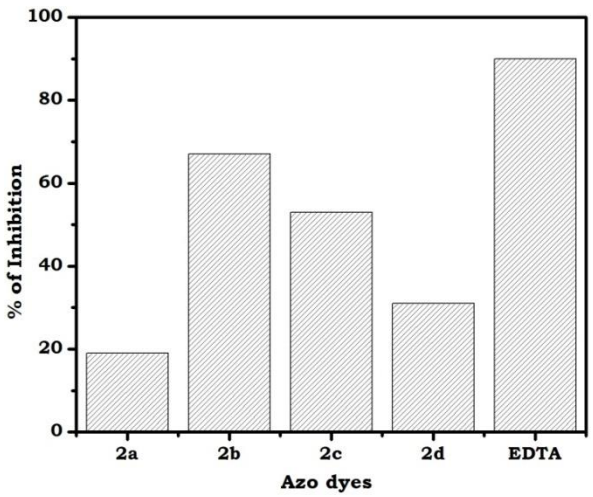
The kinetic behavior of the free radical scavenging compounds has been studied by allowing them to reach with a stable free radical, namely (DPPH radical). This assay was selected for this study because it measures the hydrogen donating ability of antioxidants in a relatively short time compared to other methods and spectrophotometric characterization is also possible. This method is widely followed by many researchers. The compound 2b and 2c having very potent DPPH activity when compare to the other synthesized compounds. This result indicates, the compounds 2b and 2c have ability to donate proton to the DPPH radicals. But, the remaining compounds have lower scavenging activity.

3.2.4. Metal ion Chelating assay:

The ferrous ion chelating potency of 1-14 SB, 1-3 TC and 1-3 AZ synthesized organic compounds was investigated according to the method [25] with little modification, wherein the Fe^{2+} chelating ability of synthesized compounds was monitored by absorbance of the ferrous ion ferrozine complex at 562 nm. Briefly, the reaction mixture, containing 100 µg concentration, $FeCl_2$(2 mM) and ferrozine (5 mM) was adjusted to a total volume of 3 ml with double distilled water, shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank. The ability of organic compounds to chelate ferrous ion was calculated.

Figure-6: Metal ion Chelating activity



The compound 2b, 2c showed good metal chelating activity and compound 2d showed moderate activity. Compound 2a showed lower metal ion chelating activity.

4. CONCLUSION

In this study, we have synthesized four heterocyclic azo dyes by linking 8-hydroxy quinoline with various 4-substituted anilines by diazotizing–coupling method. Their structures were confirmed by FT-IR, UV–Vis spectra, ^{1}H NMR, mass spectral date and elemental analysis. All the dyes exhibited antibacterial activity and the most active dye was 2b. Presence of electron withdrawing group in this dye is likely to increase their antibacterial activities. Further investigations arenecessary to determine the mechanism of activity.

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