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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF HETEROCYCLIC AZO DYES DERIVED FROM 2-AMINOBENZOTHIOZOLE

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ABSTRACT

As a part of systematic investigation of synthesis and biologically active compound 2-amino benzothiazole, several new azo dyes were synthesized by diazotization of 2-amino benzothiazole and coupled with different coupling compounds such as naphthol derivatives, 8-hydroxy quinoline and N, N-dimethyl aniline. The structures of the dyes were confirmed by UV-Vis, IR and ^{1}H NMR spectroscopic techniques. New compounds were screened for their antimicrobial activity by well plate method (zone of inhibition). Antioxidant studies of the synthesized compounds were also performed by measuring the DPPH radical scavenging assay and metal chelating method. Results revealed that compounds 2a, 2d and 2g showed much better antibacterial activity. The compounds 2c, 2d and 2f are having good antioxidant property. Compound 2f have not shown any activity towards both bacterial and fungal strains.

Keywords: 2-aminobenzothiozole, Naphthol derivatives, 8-hydroxy quinoline, N, N-dimethyl aniline, Biological activities.

INTRODUCTION

Azo-dyes are the most important and versatile class of synthetic organic compounds, with an enormous variety of applications [1]. These can be obtained easily and inexpensively using a wide variety of diazo and coupling components. They have high dyeing and good fastness properties and wide applications in areas such as dyeing of textile fibers, plastics, leather, paper and bio-medical studies [2].

In recent, years heterocyclic based azo dyes, have found great success due to their higher tinctorial strength, brighter dyeing and excellent light, washing and sublimation fastness, and chromophoric strength in relation to diazo dyes based uniquely on azobenzene derivatives [3]. Heterocyclic azo dyes have wide applications as high level-dying agents in the dyestuff industries [4]. The increasing usage of these dyes in electronic industry, such as colorimetric sensors, nonlinear optical (NLO) devices and liquid crystalline displays (LCDs) have been investigated as potential sensitizers for photodynamic therapy (PDT) and has attracted much attention [5].

Benzothiazoles are bicyclic ring system with multiple applications. Benzothiazole-based azo dyes are considered to be the first example of the successful commercial exploitation of heterocyclic amines, by using the 2-aminobenzothiazole nucleus as diazonium component in the production of red dyes. Due to their cheapness, brightness, and dyeing performance, this type of dyes has become economically important [6]. Benzothiazole derivatives are potent biological agents [7] and they possess potent biological activities such as antihistamines [8], antitumor [9-11], anti-inflammatory [12], schistosomicidalagents [13], antibacterial [14], antituberculotic [15], insecticides [16] and production of drugs in chemotherapy [17]. Benzothiazoles also show significant effects against cancer. Similarly azo compounds also exhibit microbial activities such as antibacterial [18], antiviral [19], antifungal [20], etc.

With this object in view and also work carried out in our lab on above class of components [21-22], we now focused on the synthesis and screen the antimicrobial activity of some benzothiazole derivatives containing azo group in their structure. 2-aminobenzothiazole was prepared by the method reported in the literature [23]. The compound was converted into corresponding diazonium salt by diazotization reaction and it was further coupled with various coupling agents (Naphthol derivatives, 8-hydroxy quinolone and N, N-dimethyl aniline). These compounds were characterized and screened for their antimicrobial activity against Escherichia Coli, Staphylococcus Aureus, Pseudomonas Aeruginosa and Aspergillus flavus, Chrysosporium keratinophilum Candida albicans and Antioxidant activity

MATERIALS AND METHODS

All the solvents and chemicals were purchased from S.D.Fine Chemicals (India) and were used after further purification. Melting points were measured using standard melting point apparatus from Sunder Industrial Products (India) and are uncorrected. The UV-Visible absorption spectra were recorded in Acetone with a SHCIMADZU UV-1800 spectrometer at concentration range of 10^{-4} M. IR spectra were recorded in the region of 4000cm^{-1} to 400cm^{-1} on a FTIR-ALPHA BRUKER IR spectrometer in KBr pellets. The proton nuclear magnetic resonance (1H-NMR) spectra were recorded in DMSO d_6 at 400MHz using BRUKER-400 spectrometer using tetra methyl silane as an internal standard.

Synthesis of 2 amino benzothiazole [1]

A mixture of aniline (0.01 *M*) and potassium thiocyanate (0.01 *M*) in glacial acetic acid (20 ml) was cooled and stirred in round bottom flask. To this solution bromine (0.01 *M*) was added from dropping funnel at such a rate that the temperature does not rise beyond 0^{0}C. After all the bromine has been added, the solution was stirred for about 2hrs at 0^{o}C. It was allowed to stand for overnight during the period an orange precipitate was settled at the bottom. To the precipitate water (6 ml) was added quickly slurry was heated at 85^{o}C on steam bath and filtered under hot condition. The orange residue was placed in a reaction flask and treated with 10 ml of glacial acetic acid, heated again to 85^{o}C and filtered in hot. The filtrate was cooled and neutralized with concentrated ammonia solution to pH 6 when dark yellow precipitate was appeared and recrystallized from benzene to obtain the 2 amino-benzothiazole. The completion of the reaction was monitored on TLC by using silica gel-G coated plates by using ethyl acetate and petroleum ether (7:3) as the eluent and observed in UV light. Yield 74%; IR [(KBr) υ_{max}/cm^{-1}]: 3490 cm^{-1} (NH), 1580 cm^{-1} (C=N); ^{1}H NMR DMSO-d_6): (d, ppm) 6.12 (s, 2H, NH_2), 6.79-7.13 (m, 3H, Ar-H).

Preparation of 3-[1,3-benzothiazol-2-yldiazenyl]naphthalen-2-ol [2a]

2-Aminobenzothiazole (2.0 mmol,) was dissolved in glacial acetic acid: propionic acid mixture (2:1, 6.0 ml) and was quickly cooled in an ice/salt bath to 0-5^{0}C. A cold solution of nitrosylsulphuric acid (prepared from sodium nitrite (2.2 mmol,) and concentrated sulphuric acid (3 ml at 50^{0}C). The mixture was stirred for an additional 3 hrs at the same temperature. Excess nitrous acid was destroyed by the addition of urea. Coupling compound (a-e) (2.2 mmol) was wetted with Tween-80 (1% solution, few drops) [24]. To this mixture, hot water (25 ml) and sodium hydroxide solution (7 ml, 10%w/v) were added slowly and the mixture was heated until a

clear solution was obtained. The solution was cooled to 0–5 ºC in an ice bath. Freshly prepared diazo liquor was added to this solution drop by drop, over a period of an hour keeping the temperature below 0- 5 ºC. The reaction mixture was further stirred for two hrs at 0–5 ºC maintaining pH 8.0 by adding the required amount of sodium carbonate solution (10 % w/v). The product was filtered, washed several times with hot water whereby a dark red solid was obtained. The solid was dissolved in DMF and precipitated by adding chloroform. Yield 86%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3053.15cm^{-1}(Ar-CH),1527.19cm^{-1}(-N=N-), 1608cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-d6): 10.9(s, 1H,NH), 7.912(d 1H Ar H), 7.834(d 1H Ar H), 7.725(d 1H Ar H), 7.607(t, 1H Ar H), 7.481(S 1H, Ar H), 7.373(t, 2H,Ar H), 7.268(s 2H, Ar H), 6.778(d, 1H, Ar H); Anal. calcd. for $C_{17}H_{11}N_3OS$: C,66.87; H,3.63; N,13.76; Found: C, 66.81; H, 3.59; N, 13.74%.

Preparation of 4-[1,3-benzothiazol-2-yldiazenyl]-3-hydroxy-*N*-phenylnaphthalene-2-carboxamide [2b]

This dye was obtained from 2-amino benzothiozole and naphthol-AS as red crystals. Yield 57%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3069.21cm^{-1}(Ar-CH), 1541.26cm^{-1}(-N=N-), 1641cm^{-1}(C=N); ^{1}H NMR (400 MHz, DMSO-d6): 11.1(s, 1H,NH), 7.83(d,2H, Ar H), 7.73(d, 2H, Ar H), 7.67(d, 2H, Ar H), 7.53(t, 2H, Ar H), 7.46(t,2H,Ar H), 7.37(s, 1H, Ar H), 7.26(t, 3H, Ar H); Anal. calcd. for $C_{24}H_{16}N_4O_2S$: C, 67.91;H, 3.80;N, 13.20; Found: C, 67.87; H, 3.77; N, 13.17%.

Preparation of 4-[1,3-benzothiazol-2-yldiazenyl]-*N*-(4-chloro-2-methylphenyl)-3-hydroxynaphthalene-2-carboxamide [2c]

This dye was obtained from 2-amino benzothiozole and naphthol-ASTR as dark red crystals. Yield 74%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3046.85cm-1(Ar-CH),1556.31cm-1(-N=N-), 1624.51cm-1(C=N); 1H NMR (400 MHz, DMSO-d6): 11.3 (s,1H,NH),8.58 (s, 1H, Ar-H), 8.19(d,2H, Ar-H), 8.13(d,2H, Ar-H), 7.90(d,3H, Ar-H), 7.62(d, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.32 (t, 1H, Ar-H),7.27 (t, 1H, Ar-H). Anal. calcd. for $C_{24}H_{15}N_5O_4S$:C, 55.92; H, 2.93; N, 16.30; Found: C, 55.89; H, 2.90; N, 16.27%.

Preparation of 4-[1,3-benzothiazol-2-yldiazenyl]-2-methyoxyphenyl-3-hydroxy naphthalene-2-carboxamide [2d]

This dye was obtained from 2-amino benzothiozole and naphthol-AS OL as red crystals. Yield 66%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3055.48cm^{-1}(Ar-CH),1549.31cm^{-1}(-N=N-), 1631.51cm^{-1}(C=N); ^{1}H NMR (400 MHz, DMSO-d6): 11.3 (s,1H, NH), 8.59 (s, 1H, Ar-H), 8.23(d,2H, Ar-H), 7.84(s, 1H, Ar-H), 7.62((d,1H.Ar-H), 7.58(d, 1H, Ar-H), 7.38(t,1H, Ar-H), 7.28 (t, 1H, Ar-H),2.83(s,1H,-OCH_3). Anal. calcd. for $C_{25}H_{18}N_4O_3S$: C, 66.07; H, 3.99; N, 12.33; Found: C, 67.87; H, 3.77; N, 13.17%.

Preparation of 4-[1,3-benzothiazol-2-yldiazenyl]-3-hydroxy-*N*-(4-nitrophenyl) naphthalene-2-carboxamide [2e]

This dye was obtained from 2-amino benzothiozole and naphthol-AS BS as red crystals. Yield 64%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3059.24cm^{-1}(Ar-CH), 1542.26cm^{-1}(-N=N-), 1621cm^{-1}(C=N); ^{1}H NMR (400 MHz, DMSO-d6): (dmso-d6)ppm: 10.9 (s,1H, NH), 8.09(s,1H,Ar H), 7.87(t, 2H, Ar H), 7.70(d, 1H, Ar H), 7.51(t,2H, Ar H) 7.36(d, 3h, Ar H), 7.26(s, 3H, -CH_3), 7.13(d,1H, ArH), 6.90(d, 2H, Ar H); Anal. calcd. for $C_{25}H_{17}ClN_4O_2S$; C, 63.49; H, 3.62; N, 11.85; Found: C, 63.47; H, 3.59; N, 11.83.

Preparation of 5-[1,3-benzothiazol-2-yldiazenyl]quinolin-8-ol [2f]

2-Aminobenzothiazole (2.0x10^{-3}mol) was dissolved in glacial acetic acid and propionic acid mixture (2:1, 6.0 ml) and was quickly cooled in an ice/salt bath to 0-5^{0}C. The liquor was then added in portions during 15 min to a cold solution of nitrosylsulphuric acid (prepared from sodium nitrite (2.2 mmol) and concentrated sulphuric acid (3 ml at 50^{0}C). The mixture was stirred for an additional three hrs at the same temperature. Excess nitrous acid was destroyed by the addition of urea. After diazotization was complete, the diazo liquor was slowly added to vigorously stirred solution of 8-hydroxyquinoline (2.0x10^{-3}mol) in Potassium hydroxide (2.0x10^{-3}mol) and water (25 ml). The solution was stirred at 0-5^{0}C for two hrs and the pH of the reaction mixture was maintained at 10-11 by the simultaneous addition of 2.5% sodium hydroxide solution. In the end of procedure, the pH of reaction mixture was regulated at 4-5 by addition of 10% hydrochloric acid solution. After 30 min., the resulting solid was filtered, washed with cold ethanol and dried [25]. Recrystallization from DMF-H_2O gave dark red crystalline (2-benzothiazolylazo)-8-hydroxy quinoline. The completion of the reaction was monitored on TLC by using silica gel-G coated plates by using ethyl acetate and petroleum ether (6:4) as the eluent and observed in UV light. Yield 62%;**):** IR (KBr, ν max cm^{-1}): 3436-3243(-OH), 1565(N=N), 1626(C=N);^{1}H NMR (400 MHz, DMSO-d6): 8.76 (s, 1H, Ar-H), 8.63(d,1H, Ar-H), 8.24(d,2H, Ar-H), 7.93((d,1H.Ar-H), 7.86(d, 1H, Ar-H), 7.29 (d, 1H, Ar-H),7.18(t, 1H, Ar-H). Anal. calcd. for $C_{16}H_{10}N_4OS$; C, 62.73; H, 3.29; N, 18.29; Found: C, 62.70; H, 3.27; N, 18.26.

Preparation of 4-[1,3-benzothiazol-2-yldiazenyl]-*N*,*N*-dimethylaniline [2g]

This dye was obtained from 2-amino benzothiozole and *N*,*N*-dimethylaniline as dark red crystals. Yield 87%; IR [(KBr) υ $_{max}$/cm^{-1}]: 3062.21cm^{-1}(Ar-CH), 1528.99cm^{-1}(-N=N-), 1630.05 cm^{-1}(C=N); ^{1}H NMR (400 MHz, DMSO-d6): 8.10(d, 1H, Ar H), 8.023(d, 2H, Ar H), 7.87(d, 1H, Ar H), 7.49(t, 1H,Ar H), 7.374(t, 1H, Ar H), 7.26(s, 6H,N(CH_3)$_2$), 6.79(d, 2H Ar H); Anal. calcd. for $C_{15}H_{14}N_4S$; C, 63.80; H, 5.00; N, 19.84; Found: C, 63.78; H, 4.98; N, 19.81.

Biological Activity

Bacterial and Fungal strains

The following bacteria and fungi were used for the experiment. Bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. All bacterial strains were maintained on nutrient agar medium at ±37°C. Fungi: *Aspergillus flavus, Chrysosporium keratinophilum* 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.

Antibacterial and Antifungal activity

The antimicrobial activity of newly synthesized compounds was evaluated using agar disc diffusion assay. Briefly, a 24 and 48 hours old culture of selected bacteria and fungi was mixed with sterile physiological saline (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 and antifungal activity. The inoculum was spread on the surface of the solidified media and What man No. 1 filter paper discs (5 mm in diameter) impregnated with the test compound (20 µl/disc) were placed on the plates. Streptomycin (5 mg/disc) and Fluconazole (5 mg/disc) was used as positive control for bacteria and fungui. 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 and the fungal culture was incubated for 72 h at 25° C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading [26-29].

Antioxidant activity

DPPH activity

Free radical-scavenging capacities of different compounds were determined according to the previously reported procedure, using the stable 2, 2-diphenyl-1-picryhydrazyl radical (DPPH)[30]. Prepare the stock solution of extracts (1 mg/mL) and DPPH (0.004%) using 95% of methanol. Freshly prepared DPPH solution were taken in test tubes and extracts are to be added (100 µg) to every test tube so that the final volume will be 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 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 using the following formula.

% inhibition = (AB-AA/AA) X100

Where, AB= absorption of blank and AA = absorption of test

Metal ion Chelating assay

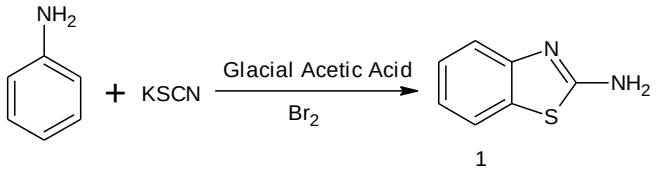
The ferrous ion chelating potency of 1-14 SB, 1-3 TC and 1-3 AZ synthesized organic compounds was investigated according to the method of Dinis et al., [31] with little modification, wherein the Fe^{2+} chelating ability of synthesized compounds was monitored by absorbance of the ferrous iron ferrozine 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 [32]. The absorbance of the mixture was measured at 562 nm against balnk. The ability of organic compounds to chelate ferrous ion was calculated using the following equation:

Chelating activity (%) = [(Abs of control-Abs of sample)/Abs of control] X 100

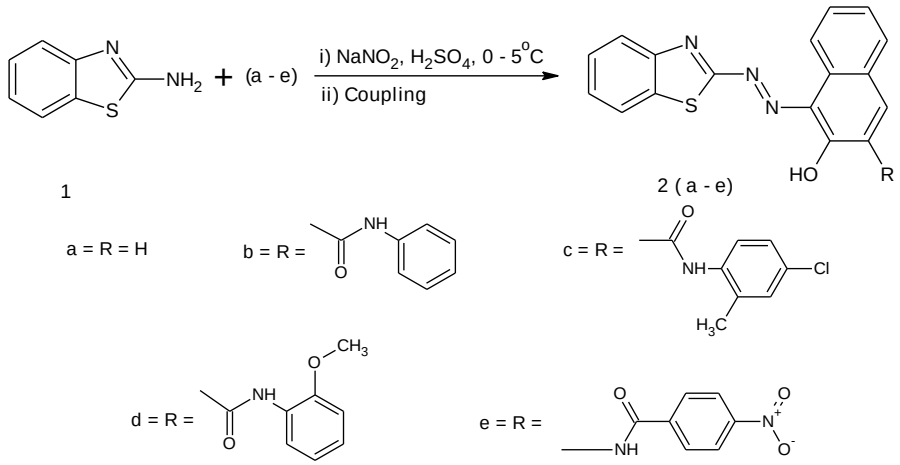
RESULTS AND DISCUSSION

As depicted in the scheme 1. 2-amino benzothiozole azo dyes were synthesized by a multi-step reaction sequence. 2-amino benzothiozole were prepared by reacting mixture of aniline and potassium thiocyanate in glacial acetic acid and bromine in a good yield. The amine group was diazotized and coupled with naphthol derivatives, 8-hydroxy quinolone and N, N-dimethyl aniline to obtain 2-amino benzothiozole substituted azo dye 2 (a-g). The compounds were recrystallized from different solvents. The purity of the compounds was checked by TLC. Spectral data, UV-Vis, IR and ^{1}H NMR of all synthesized compounds were recorded and found in full agreement with the proposed structures. The elemental analysis results were within ± 0.4% of the theoretical values.

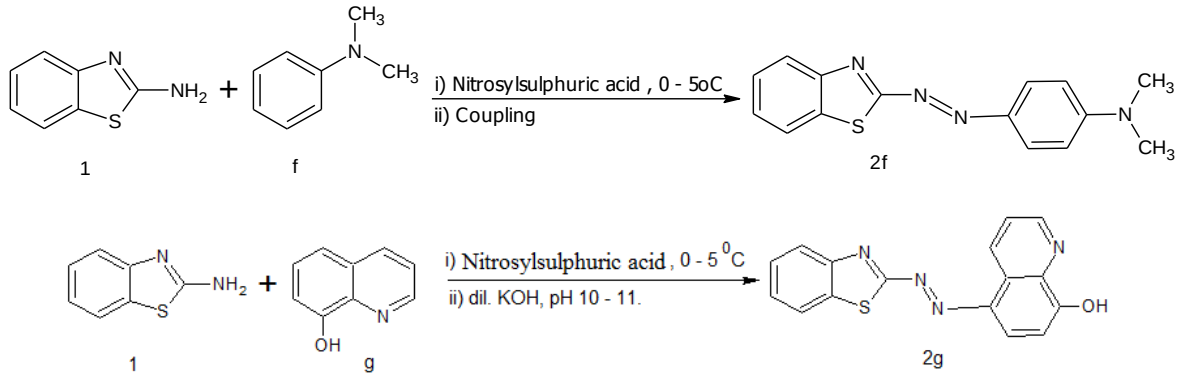
The IR spectra of compound 1 showed absorption peak at 3490 cm^{-1} for (NH),1580 cm^{-1} for (C=N);. The IR spectrum of the dye 2a showed absorption peak at 1561.4 cm^{-1} due to C=N stretching vibration, absorption peak at 1527.19cm^{-1} due to (-N=N-), and 1608cm^{-1} for (C=N). The ^{1}H NMR spectrum revealed a singlet at δ 11due to -NH protons and δ 6-8 due to aromatic proton. The IR spectrum of compound 2(a-f) showed absorption at 3300-3400 cm-1 attributed to –OH, 1500-1600 cm^{-1} due to N=N, absorption at 900-1050 cm^{-1} assigned to stretching absorptions of C-S groups. The absorption spectra of hetarylazo dyes 2(a-g) were measured in DMSO at a concentration of 10^{-4}M. Yield, melting point, λmax, molar absorptivity (ε), molecular formula and solubility of the heteralazo dyes are given in table 1. The UV-Vis, IR and ^{1}H NMR data was found in good agreement with the newly synthesized compounds.



Scheme 1: Synthesis of 2- amino benzothiazole



Scheme 2: Synthesis Naphthol based azodyes (2a-e)



Scheme 3: Synthesis of azodyes (2f-g)

Table 1: Yield, melting point, $\lambda_{max,}$ molar absorptivity (ε), molecular formula and solubility data of dye 2(a-g).

Dye	Yield (%)	M.P(^{0}C)	λ_{max} in nm	Log ε	Molecular formula	Mol.wt	Solubility
2a	86%	210-212	490	4.2	$C_{17}H_{11}N_3OS$	305.35	Acetone/Ethanol/ DMF/DMSO
2b	57%	192-196	485	4.51	$C_{24}H_{16}N_4O_2S$	424.47	Acetone/Ethanol/ DMF/DMSO
2c	74%	200-202	512	4.02	$C_{24}H_{15}N_5O_4S$	469.47	Acetone/Ethanol/ DMF/DMSO
2d	66%	204-205	529	4.58	$C_{25}H_{18}N_4O_3S$	454.5	Acetone/Ethanol/ DMF/DMSO
2e	64%	208-210	527	4.47	$C_{25}H_{17}ClN_4O_2S$	472.94	Acetone/Ethanol/ DMF/DMSO
2f	62%	238-240	547	4.29	$C_{16}H_{10}N_4OS$	306.34	Acetone/Methanol/ DMF/DMSO
2g	87%	165-168	513	4.68	$C_{15}H_{14}N_4S$	282.36	Acetone/Methanol/ DMF/DMSO

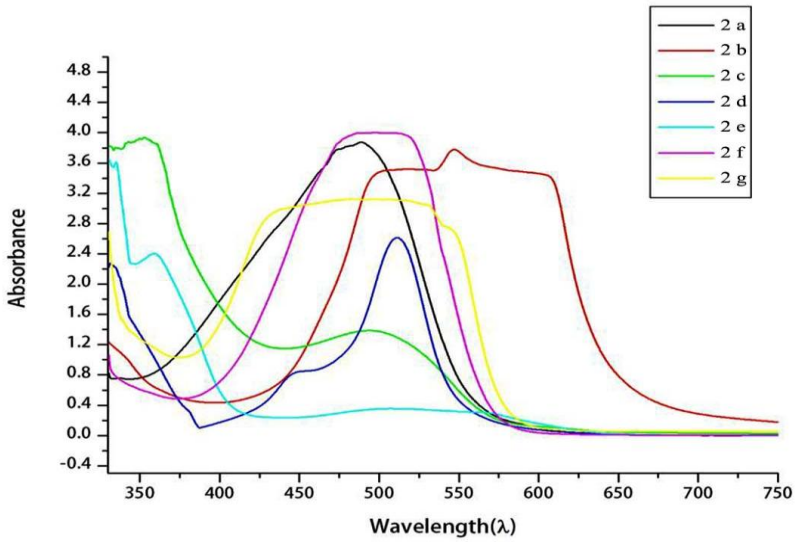


Fig. 1: Absorption spectra of dyes 2(a-g) in DMSO

Biological activity

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

S. 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**	06±0.2	04±0.1	07±0.1	05±0.1	08±0.2	04±0.2
4	**2b**	03±0.2	01±0.1	05±0.1	03±0.1	04±0.2	02±0.2
5	**2c**	04±0.2	01±0.1	02±0.1	00	03±0.2	01±0.2
6	**2d**	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2
7	**2e**	02±0.2	01±0.1	03±0.1	02±0.1	03±0.2	02±0.2
8	**2f**	-	-	-	-	-	-
9	**2g**	06±0.3	04±0.7	05±0.3	03±0.5	03±0.3	02±0.1

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

S. 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**	04±0.1	02±0.2	05±0.2	03±0.1	03±0.1	02±0.1
4	**2b**	-	-	-	-	-	-
5	**2c**	03±0.2	01±0.1	04±0.1	03±0.1	04±0.1	03±0.1
6	**2d**	05±0.1	03±0.1	04±0.2	03±0.1	05±0.1	02±0.1
7	**2e**	01±0.1	01±0.1	02±0.1	03±0.1	02±0.1	02±0.1
8	**2f**	-	-	-	-	-	-
9	**2g**	06±0.1	05±0.2	04±0.1	03±0.1	05±0.1	04±0.1

Synthesized organic compounds evaluated for the antimicrobial activity with standard drugs (***Streptomycin and* Fluconazole).** The closer look into the biological studies of these organic compounds revealed that compound 2a, 2d and 2g showed much better activities when compare to the other compounds. The results from the antimicrobial activity of synthesized organic compounds were (Table 2) prompted us to investigate their antifungal activity against important pathogens like Aspergillus Flavus, Chrysosporium Keratinophilum and Candida Albicans, but these compounds not have good activity towards the fungus when compare to the clinically important bacteria like E. coli, S. aureus and P. aeruginosa. Compound 2f have not shown any activity towards both bacterial and fungal strains.

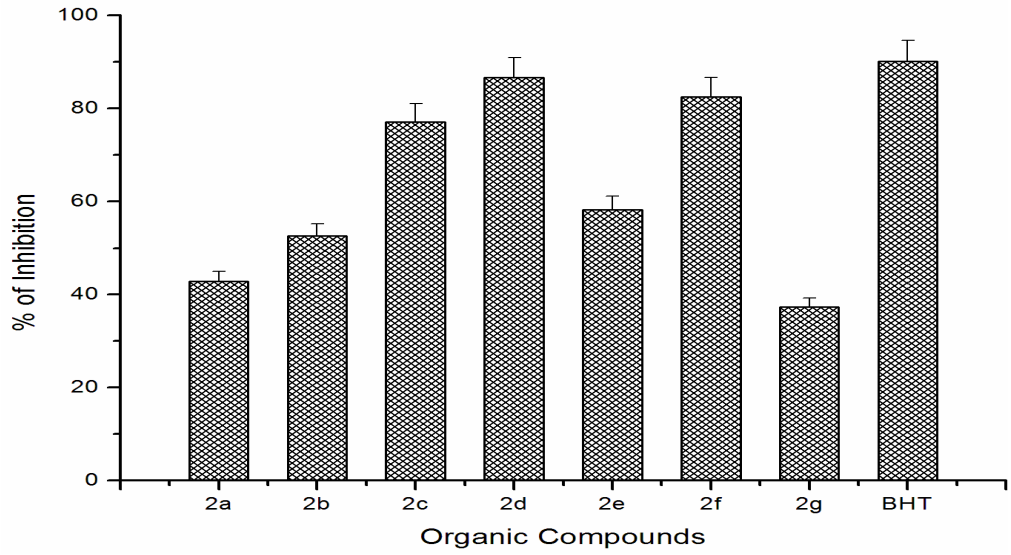


Fig. 2: DPPH radical scavenging activity

DPPH scavenging activity

The DPPH test provided strong information about the antioxidant property of the newly synthesized compounds with stable free radical. The DPPH radical showed a strong absorption band at 517 nm in visible region. As this free electron is scavenged by a free radical scavenger, the absorption decreases and resulting in the discoloration of DPPH takes place with respect to decrease in the free radicals present in the solution. The compounds 2c, 2d and 2f are having good and comparable antioxidant property with standard. The 2d compound has high IC50 value of 58.92µg when compare to the other two compounds 2c and 2f (51.44 and 55.68 µg).

Metal ion Chelating assay

Transition metals such as ions can able to stimulate lipid per oxidation by generating hydroxyl radicals through Fenton reaction and accelerate lipid per oxidation into peroxyl and alkoxyl radicals which lead to drive the chain reaction of lipid per oxidation.

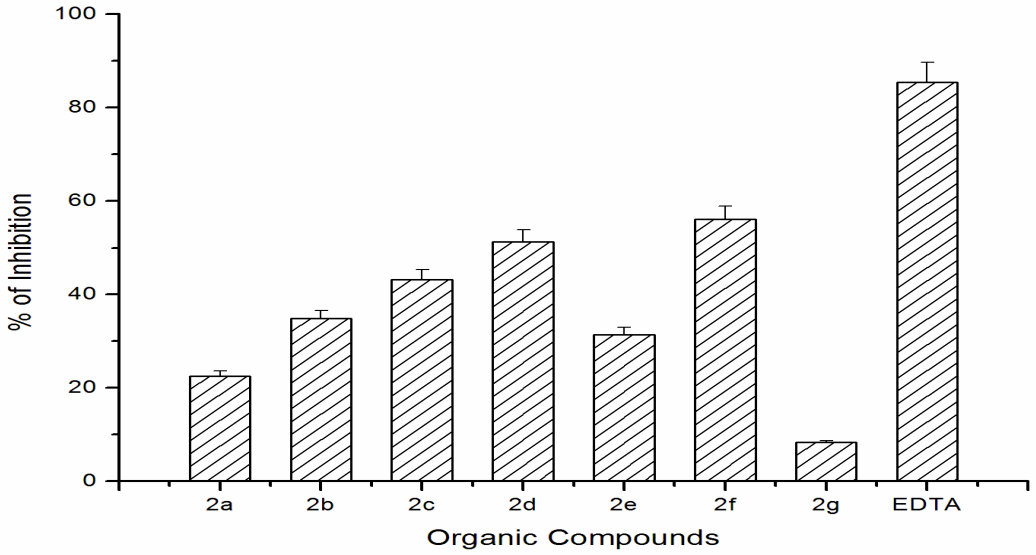


Fig. 3: Metal ion Chelating activity

Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. In addition, some phenolic and organic compounds exhibit antioxidant activity through chelation of metal ions. These compounds may be permitting that bond to metal ions due to their chemical structures. The chelating activity of organic compounds was evaluated against Fe^{2+}ion to estimate the potential antioxidant activities of the different newly synthesized compounds. The compounds 2c, 2d and 2f having a good metal chelating property when compare to rest of the compounds.

CONCLUSION

This investigation proposes a convenient, economical and useful method for the synthesis of heterocyclic azo dyes, coupled with naphthol derivatives, 8-hydroxy quinoline and N,N dimethyl aniline which are biologically active molecules possessing safer

antimicrobial and in vitro antioxidant property. The new dyes of azobenzothiozole derivatives proved to be a safer up to upper most dosage and exhibit a significant antimicrobial and antioxidant activity. The preliminary antimicrobial activity studies revealed that the azo dye having benzothiozole moiety exhibited a potential antimicrobial activity. Hence, it can be concluded that, this class of compounds certainly holds a greater promise in discovering a safer antimicrobial and antioxidant agent.

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