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Synthesis, Characterization, Solvatochromic and Biological studies of novel Benzothiazole based azo dyes

Maliyappa MR, J Keshavayya*, Shoukat Ali R.A, Harisha S
Department of PG Studies and Research in Chemistry, Kuvempu University,
Janna Sahyadri, Shankaragatta-577451, Shimoga, Karnataka, India
***Corresponding author: E-Mail: jkeshavayya@gmail.com**

ABSTRACT

In this present research investigation, we have synthesized novel benzothiazole based azo dyes and its derivatives. Which were prepared by diazotization of 2-amino-6-substituted benzothiazoles and coupling with 4-imino-3, 4-dihydro-2H-pyrimido [2,1-b][1,3]benzothiazole-2-one in neutral media under suitable experimental conditions. Synthesized compounds were characterized by various spectroscopic techniques like Uv-Visible (electronic spectral), IR, NMR and Mass Spectrometry. The newly synthesized colored compounds are screened for their biological activities like *in vitro* antimicrobial such as antibacterial and antifungal activities. Out of four synthetic derivatives [5a-d], 5b and 5d shows fine anti-bacterial activity and compounds 5b and 5c shows anti-fungal activity.

KEY WORDS: Benzothiazole, Antimicrobial, Azo dyes, Solvatochromic studies.

1. INTRODUCTION

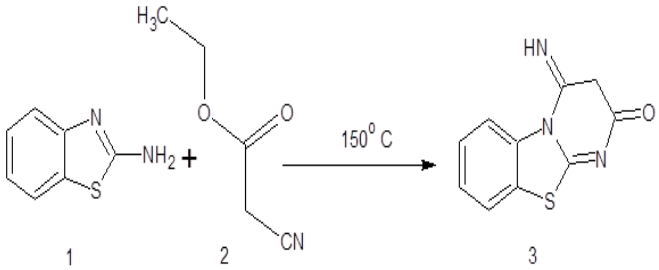
In the past decade, an increase in the uses of antibacterial drugs considerably decreases the happening of infection (Ziga Jakopin, 2017). This issue is successfully accomplished by developing the new target drug (Eakin, 2012). The fused benzothiazole ring present in different marine or terrestrial natural compounds which are biologically useful. Fused benzothiazole rings are structurally fashionable agents and have more number of applications such as fluorescence (YangYang, 2014), second-order non-linear optical (NLO) materials (Nabeshima, 1997), Liquid crystals (Rabiul Karim, 2013), Dye fastness, leather (Vijayaraghavan, 2006) etc. Now a days, benzothiazole based molecules have been extensively used for pharmaceutical and biological activities like anti-inflammatory (Tewari, 2014), antimicrobial (Ashok, 2016), antimycobacterial (Kogi, 2002), antifungal (Raghavendra, 2013), antitumor (Wei, 2012), local anesthetic (Hadjipaulou, 1993) and antimalarial (Nelson, 1982) medicinal agents. In our research laboratory intensive research has been carried out based on benzothiazole moiety (Keerthi kumar, 2013) because of its potential biological activities. In this paper, we describe the biological evaluation of newly synthesized benzothiazole based dispersed azo dyes. Obtained by diazotization of 2-amino-6-substituted benzothiazoles and followed by coupling with 4-imino-3, 4-dihydro-2H-pyrimido[2,1-b][1,3]benzothiazole-2-one.

2. MATERIALS AND METHODS

The chemicals used for the synthesis obtained from Sigma Aldrich Chemical Company are of analytical grade. Melting point of compounds was recorded in electronic melting point apparatus. The elemental analysis was carried out by using ThermoFinnigan with K factor calibration method. Absorption spectra were recorded using a UV-1800 Shimadzu, UV spectrophotometer in different solvents at known concentration. FTIR spectra were measured using KBr pellet on a Bruker FT-IR spectrophotometer. ^{1}H-NMR spectra were measured at 400 MHz in DMSO-d_6 solution on a Bruker spectrometer.

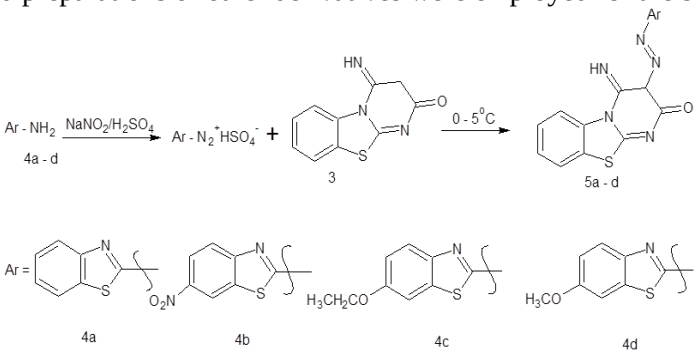
Synthesis:

Preparation of 4-imino-3, 4-dihydro-2H-pyrimido[2,1-b][1,3]benzothiazole-2-one (3): The compound was synthesized by 2-aminobenzothiazole (0.002mole) (Ashok and Awale, 2016) with slight excess of ethyl cyanoacetate in solvent free conditions (Eakin, 2012). The reaction mixture was heated along with stirring at 150^{o}C for 2h, and cooled at room temperature with stirring and diluted with 100 mL ethanol. The separated precipitate was filtered, washed with alcohol for a number of times, and dried. The Obtained % yield was 80 and used as coupling component for the azo dye preparation. The synthetic route was shown in scheme-1. Molecular Formula: $C_{10}H_7N_3OS$; FTIR (in KBr cm^{-1}): 3179(=NH), 3064(Ar-H) ,1661(C=N), 2918(Aliphatic C-H), 1717(C=O) ^{1}HNMR (DMSO-d6 400 MHz):4.1 (s 2H, CH_2), 7.3-8.0 (Ar-m 4H), 12.7 (s, 1H =NH); Elemental analysis: Found(%):C-54.95, H-3.16, N-20.21, S-13.95 Calc(%): C-55.29, H-3.25, N-19.34, S-14.76.



Scheme.1. Synthetic route of 4-imino-3, 4-dihydro-2H-pyrimido[2,1-b][1,3]benzothiazole-2-one

General procedure for the synthesis of azo dyes 5(a–d): 2-aminobenzothiazole 4a (0.002mole) was dissolved in a mixture of glacial acetic acid (6ml) and propionic acid (2ml) in the ratio 3:1 and was rapidly cooled in an ice bath to 0-5ºC. Above mixture was added to a cold solution {prepared from sodium nitrite (0.002mole) and concentrated sulphuric acid (7 ml) at 70ºC for 5 min} in portions. Then it was stirred for an additional 1.5 h at 0-5ºC. After diazotisation was completed, the diazonium salt was slowly added to a vigorously stirred solution of 4-imino-3, 4-dihydro-2H-pyrimido [2,1-b][1,3]benzothiazole-2-one 3 (0.002mole) which was dissolved in a glacial acetic acid (6ml) and propionic acid (3ml) mixture. By addition of sodium carbonate the reaction mixture was brought to neutral. Then the resulting mixture was stirred for 1 h at 0-5ºC and 30 min for room temperature. Obtained solid was separated by filtration, washed with hot water and dried. Recrystallization from ethanol gave orange solid. The synthetic route was shown in scheme.2. And preparations of other derivatives were employed for the similar procedure.



Scheme.2. Synthetic route of azo dyes 5a-d

3-[(E)-1,3-benzothiazol-2-yldiazenyl]-4-imino-3,4-dihydro-2H-pyrimido[2,1-b][1,3]benzothiazol-2-one (5a): yield: 81%; Molecular formula: $C_{17}H_{10}N_6OS_2$; FTIR (in KBr cm^{-1}): 1538(N=N), 3063 (Ar-H), 3222(=NH), 1601(C=N); ^{1}HNMR (DMSO-d6 400 MHz):4.0 (s 1H, CH), 7.1-8.5 (Ar-m 8H), 12.6 (s 1H =NH); Elemental analysis: Found(%):C-53.15, H-2.12, N-50.93, S-17.12; Calc(%): C-53.95, H-2.66, N-22.21, S-16.95.

4-imino-3-[(*E*)-(6-nitro-1,3-benzothiazol-2-yl)diazenyl]-3,4-dihydro-2*H*-pyrimido[2,1 *b*][1,3]benzothiazol-2-one (5b): yield: 76%; Molecular formula: $C_{17}H_9N_7O_3S_2$; FTIR (in KBr cm^{-1}): 1552(N=N), 3068(Ar -H), 3360(=NH), 1672(C=O), 1599(C=N); ^{1}HNMR (DMSO-d6 400 MHz): 4.3 (s 1H), 7.2-8.5(Ar-m 7H) 12.6(s NH); Elemental analysis: Found(%): C-48.12, H-2.95, N-23.12, S-15.02; Calc(%): C-48.22, H-2.14, N-23.16, S-15.15.

3-[(*E*)-(6-ethoxy-1,3-benzothiazol-2-yl)diazenyl]-4-imino-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-2-ol (5c): yield: 78%, Molecular formula: $C_{19}H_{14}N_6O_2S_2$; FTIR (in KBr cm^{-1}): 1243(-C-N-), 1516(-N=N-),1618(-C=N), 3084(Ar-CH), 3375(-OH); ^{1}HNMR (DMSO-d6 400 MHz): 1.3-1.4 (t, 3H), 4.0-4.1(q, 2H), 6.9-7.9 (Ar-m, 7H), 10.5-11.3 (s, 2H); Elemental analysis: Found(%):C-54.25, H-3.14, N-20.25, S-14.96; Calc(%):C-54.01, H-3.34, N-19.89, S-15.18.

4-amino-3-[(*E*)-(6-methoxy-1,3-benzothiazol-2-yl)diazenyl]-2*H*-pyrimido[2,1-*b*][1,3]benzothiazol-2-one (5d): yield: 80%; Molecular formula: $C_{18}H_{12}H_6O_2S_2$; FTIR (in KBr cm^{-1}): 1540(N=N), 3201(Ar –H), 3391(NH_2),1669(C=O), 1599(C=N); ^{1}HNMR (DMSO-d6 400 MHz): 4.1 (s, 3H), 4.3(s, 2H), 7.0-8.0(Ar-m, 7H); Elemental analysis: Found(%): C-52.82, H-2.15, N-19.96, S-14.98; Calc(%): C-52.93, H-2.96, N-20.58, S-15.70.

Bacterial strains: The antimicrobial activity of dyes 5a-d was tested according to the previous reported procedure (Keerthi Kumar, 2013) by agar disc diffusion method which was carried out in our research laboratory.

3. RESULTS AND DISCUSSION

Heterocyclic azo dyes 5(a-d) were synthesized by diazotization of 2-amino-6-substitutedbenzothiazole (4a-d) derivatives with 4-imino-3, 4-dihydro-2H-pyrimido [2,1- b][1,3]benzothiazole-2-one 3, prepared by condensation of 2-aminobenzothiazole (Ashok and Awale, 2016), with excess of ethylcyanoacetoacetate under solvent free condition (Eakin, 2012). The synthetic dyes are characterized by spectroscopic and analytical techniques spectral data shown in table.1.

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Table.1. Physical properties and synthetic data for compounds

Comp. code	Mol. formula	Molecular Wt.	Yield %	Melting point in ^{o}C	Color
3	$C_{10}H_7N_3OS$	217.2	80	220-222	Pale yellow
5a	$C_{17}H_{10}N_6OS_2$	355.8	81	243-245	Orange
5b	$C_{17}H_{14}N_6OS_2$	423.4	76	251- 253	Red
5c	$C_{19}H_{14}N_6O_2S_2$	382.4	78	225-227	Orange
5d	$C_{18}H_{12}H_6O_2S_2$	366.3	80	233-235	Yellow-Orange

Effects of solvent polarity properties of dyes: The UV-Vis spectra (Fig.1-4) of the synthesized azo dyes (5a-d) were recorded in different solvents in the wavelength range 200-800 nm and the obtained spectral data are displayed in table.2.

It was observed that change in solvent polarity some dyes either have significant or influence on absorption maxima. The electronic spectra of some azo dyes are depending on the structure and substituted functional group. Compounds 5a and 5b represents T_1 state in all the solvents but the compounds 5c and 5d represents, T_2 and T_3 in the solvents DMSO and DMF, respectively. The azo dye 5d showed three absorbance peaks in DMSO and DMF solvents, others have single absorbance in all used solvents. This characteristic can be certified due to the formation of azo/hydrazone tautomerism (Scheme.3).

We know that another important factor apart from the solvent effect, the nature of the electron substitution is strongly influenced the azo/hydrazone tautomerism. When the electron donor group present in the compound which enhance the polarization. For example an electron-donor substituent (-OCH_3) in the dye 5c benzothiazole ring enhances rich aromaticity, it extended to the whole molecule through hydrazone form. It was depends on the direction of change the position of the substituent and virtual strength, because the conjugated system created whole the molecule. These explanations are in concurrence with literature conclusions. Therefore, further we can imagine a shift of the tautomeric equilibrium toward the hydrazone form but not in the azo/keto form (Selinay Eriskin 2014).

Table.2. Influence of solvent on λ_{max} (nm) of dyes

Dyes	5a	5b	5c	5d
DMSO	368	400.5	444.5	378, 531, 662.7
Acetone	362.5	382.5	437.5	368.5
Chloroform	388.5	386.5	460	364.5
Ethanol	369	389	441.5	366
Methanol	365.5	389	439.5	364.5, 465.5
n-hexane	364.5	366.5	431.5	360.5
Ethyl acetate	364.5	382	435.5	364.5
1,4 dioxane	364.5	383.5	432.5	364
DMF	405.5	492.5	431.5	375, 533, 663

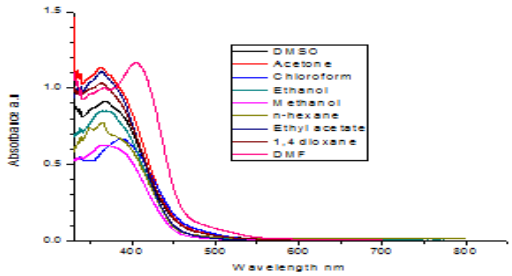


Figure.1. UV-Vis spectra of the dye 5a in various solvents

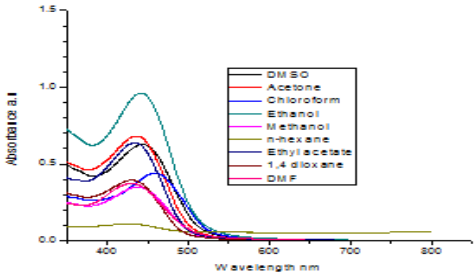


Figure.2. UV-Vis spectra of the dye 5b in various solvents

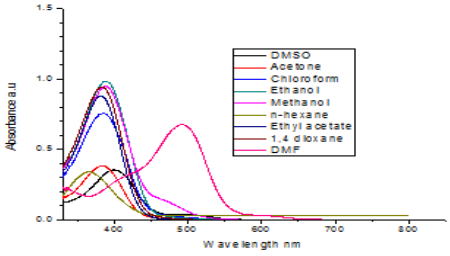


Figure.3. UV-Vis spectra of the dye 5c in various solvents

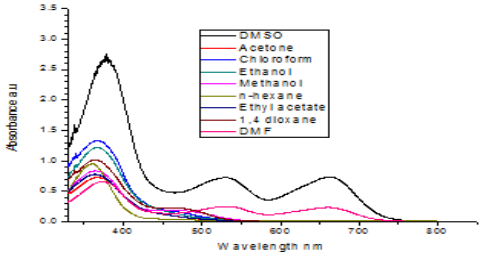
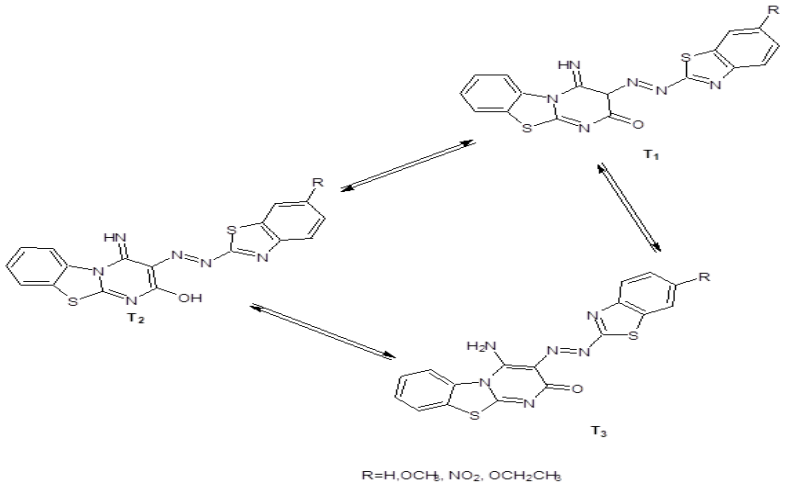


Figure.4. UV-Vis spectra of the dye 5d in various solvents



Scheme.3. Tautomeric form of azo dye

Mass spectra: The mass spectra of compound 3 and all azo dyes are recorded by passing high energy radiation at 70 eV. All the dyes shows a parent ion peak at m/z =218, 381, 409, 423, 379 (R.I. = 100%) which may be due to molecular formulas. The other molecular ion abundance range from 1.0% to 100% may be due to the fragmentation of 3 and azo dyes molecules.

IR Spectra: The FT-IR spectra of synthesized compound showed an intense (–OH) band at 3222 cm^{-1}, and a band (=NH) located at 3063cm^{-1} showed intense carbonyl bands at 1626-1683cm^{-1}. It can be suggested that in the stable solid state these compounds do not be present as the azo-enol form. The IR spectra of dye 5c showed characteristic hydroxyl (–OH) bands at 3424–3385 cm^{-1}, but did not showed any bands for carbonyl (C=O) group. It can suggest that this compound was only in imino/azo/enol form (T_2) in solid state.

^{1}H NMR spectra: The ^{1}H NMR spectra have been recorded for the compound 3 and azo dyes (5a-d).The spectrum of compound 3 displayed CH_2 singlet at 4.1ppm. The aromatic ring protons appeared as a set of multiplet in the region 7.3-8.0. The pyramidine ring protons appeared a sharp peak at 12.7. Similarly, in the spectrum of the azo dye compound 5a, the diazo aromatic protons appeared as a multiplet at 7.2-8.0 ppm, =NH at 12.5ppm. 1H NMR spectra of 5d showed only $–NH_2$ protons Apart from these dyes, 5b was shows amino azo keto form; all the protons of the other dyes were found as to be in their expected region listed**.**

Antimicrobial activity: In the present study, a total of four compounds were teste for *in vitro* antibacterial and antifungal activities at 100 µg/mL concentration against microbial strains. The determination of antimicrobial activity was used by Agar diffusion method. In study Streptomycin was used as a standard reference for antibacterial and Metalaxyl for antifungal, respectively. The result of antimicrobial activity was calculated as the average diameter inhibition zones (IZ) in mm. With respect to the control, most of the newly synthesized compounds showed excellent antimicrobial activities. The results are tabulated in tables 3, 4 and 5.

In general, the results revealed that 5b and 5d compounds exhibited better antibacterial activities than other substituted compounds. The MIC of the synthesized compounds 5c against *A. flavus*, extremely inhibited organisms is reported in Table.6. Compounds 5b showed good MIC 100µg/mL against *C. albicans.*

Table.3. Antibacterial results:

Sample code	***E. coli***		***B. subtilis***	
	25mg/ml	50mg/ml	25mg/ml	50mg/ml
5a	1.3±0.23	2.0±0.24	2.0±0.12	2.2±0.21
5b	1.4±0.22	1.7±0.23	1.8±0.1	2.2±0.4
5c	1.8±0.2	1.3±0.5	1.2±0.21	2.5±0.24
5d	1.5±0.1	2.0±0.30	1.6±0.24	1.8±0.29
Streptomycin	2.0±0.25	2.6±0.28	2.2±0.32	2.6±0.35

Table.4. Minimum inhibitory concentrations of the compounds

Sample code	**Concentration in mg/ml**	
	E.coli	**B.subtilis**
5a	2.0±0.25	2.2±0.25
5b	2.0±0.5	2.1±0.12
5c	2.2±0.23	2.2±0.4
5d	2.0±0.30	2.2±0.4
Streptomycin	2.2±0.26	2.4±0.30

Table.5. Antifungal activity by food-poisoning method

Sample code	*A. flavus* % inhibition		*C. albicans* % inhibition	
	50µg/ml	100µg/ml	50µg/ml	100µg/ml
5a	35	72	21	57
5b	44	80	25	66
5c	38	68	55	70
5d	33	65	25	70
Metalaxyl	46	82	35	70

4. CONCLUSION

We reported here four new heterocyclic dispersed azo dyes having a common benzothiazole skeleton. Synthesized dyes were characterized by IR, UV-Visible, ^{1}HNMR and mass spectrometric techniques. Because of the interaction of polar solvents and hetero or hydrogen atom present in the molecules, solvatochromic performance of these dyes observed was very strong, which was examined in different polar solvents. Hydrogen bond formation mainly affects the chromo tropic phenomenon's which are mainly observed in the azo dyes. Further synthesized dyes 5a-d was screened for *in vitro* antimicrobial properties against Gram-positive and Gram-negative bacteria and the results are interpreted under results and discussion heading.

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