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Novel triazene dyes based on *N*-phenylpiperazine: Synthesis, anti-bacterial activity and solvatochromic properties

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ABSTRACT

Eight triazenes and two heterocyclic azo dyes were synthesized by coupling *N*-phenylpiperazine, to various aryl and heteroaryl diazonium salts in moderate to excellent yields. Characterization of the compounds was carried out by using UV–Vis, IR and ^{1}H NMR spectroscopic techniques. The solvatochromic behavior of prepared dyes was discussed using solvent dielectric constant (ε) and Kamlet–Taft polarity parameters. The correlation coefficients were found between the maximum absorption band of dyes (λ_{max}) and solvent polarity parameters. The obtained values indicate that there is a strong positive linear relationship, especially between solvent dipolarity/polarizability scale (π^*) and maximum absorption band of azo dyes. Investigation of antibacterial activity of compounds was done by agar dilution method against 4 pathogenic bacteria.

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1. Introduction

Triazenes are a group of compounds characterized by the presence of a diazoamino moiety (–N=N–N<). These compounds can be obtained by diverse synthetic ways. One of the most widely used methods for the synthesis of triazenes is the coupling reaction of diazonium salts extracted from aromatic amines to amines. However, other methods such as the addition of organometallic reagents to alkyl azides have been used for the preparation of triazene derivatives [1–3].

Triazenes showed a wide range of applications such as medical uses [4], anticancer drugs and DNA alkylating agents in tumor therapy [5,6], polymer and oligomer synthesis [7], photoresponsive systems [8], optical data storage [9], and protecting groups in natural product synthesis [10] and also used to form heterocycles [11,12].

On the other hand, the compounds containing piperazine moiety play a main role in pharmacology and medicinal chemistry and they have been used for drug development [13,14].

Triazene derivatives mainly aromatic triazenes as colorant materials have received large attention due to their reactions with a number of metal ions [15]. Also, they represent cis/trans (Z–E) isomerisation around a nitrogen–nitrogen double bond, induced by exposure to light and offer potential use in light-driven molecular switches. Therefore optical properties and solvatochromic behavior of these dyes depend on several factors, such as the nature of electron donor/acceptor substituents, the cis-trans photo-isomerization in –N–N=N< chromophore, and the interaction of solute–solvent. Thus in the recent years, triazene compounds have been used in many research and applied fields [16,17].

In the present study, we have synthesized eight triazene dyes with donor–acceptor substituents in diazo moiety and two heterocyclic azo dyes based on *N*-phenylpiperazine as coupling component. The solvatochromic behavior of prepared dyes was investigated in different solvents using the empirical solvent polarity parameters. Also, the antibacterial activity of products was evaluated and some dyes showed favorable activities. The synthetic way and chemical structures of triazenes are presented in Scheme 1.

2. Experimental

2.1. Materials and apparatus

The chemicals used in this study were obtained from Merck and Aldrich Chemical Companies and were used without further purification. All melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded on a Shimadzu 8400 FT-IR spectrophotometer. The Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a FT-NMR (500 MHz) Brucker apparatus spectrometer, and the chemical shifts are expressed in δ ppm using TMS as an internal standard. The visible spectra were measured using a Pharmacia Biotech Spectrophotometer.

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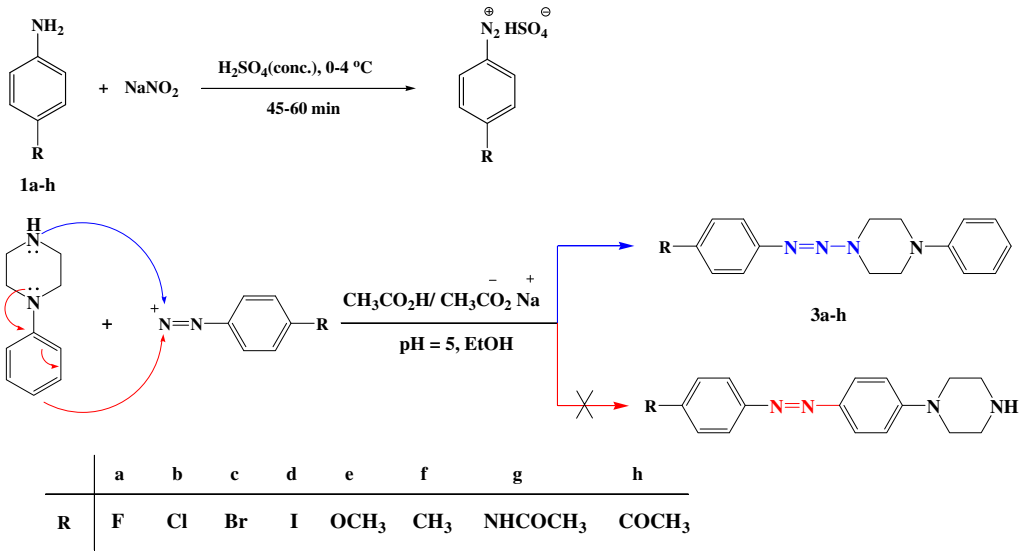


Scheme 1. Synthesis of triazene derivatives (3a–h).

The purity determination of the substrates and reaction monitoring were accompanied by TLC using silica gel SIL G/UV 254 plates.

2.2. Synthesis and characterization

The diazonium salts were prepared in good yield from equimolar mixture of aniline derivatives (1 a–h) (3 mmol) and sodium nitrite (0.207 g, 3 mmol) according to previously described method [18]. After diazotization was complete, the azo liquor was slowly added to a stirred solution of *N*-phenylpiperazine (2) (3 mmol) dissolved in 10 ml of sodium acetate–acetic acid buffer solutions (pH = 5) and 2–5 mL ethanol. The resulting mixture was stirred at 0–4 °C for 2 h in an ice bath. After this stage, the pH of the reaction mixture was maintained at 6.5–7.5 by addition of sodium acetate (CH_3COONa) solution. Then the resulting compounds (3a–h) were filtered and washed with cold water. The products were recrystallized from DMF/H_2O and EtOH/H_2O (Scheme 1).

The same experimental conditions were applied to the synthesis of heterocyclic triazenes using thiazole derivatives. However, in this case, characterization of products with spectrophotometric techniques showed that the azo species are formed (Scheme 2). It has been known that when aromatic amines are the nucleophilic reagents, two pathways are possible: the N–N bond formation (triazenes) and C–N bond formation (azo compounds) according to the reactivity of diazonium salts [19,20]. The structures of the newly synthesized dyes were characterized by IR and ^{1}H NMR spectra studies. The IR spectrum of the dyes 3i and 3j showed significant absorption peaks at range of 3435 cm^{-1} due to N–H stretching vibration. However the triazene dyes 4a–h did not show absorption peak of N–H functional group. The synthesized compounds were found in good agreement with the spectral data. The physical and spectral data of the purified dyes are as follows.

2.2.1. Triazene 3a

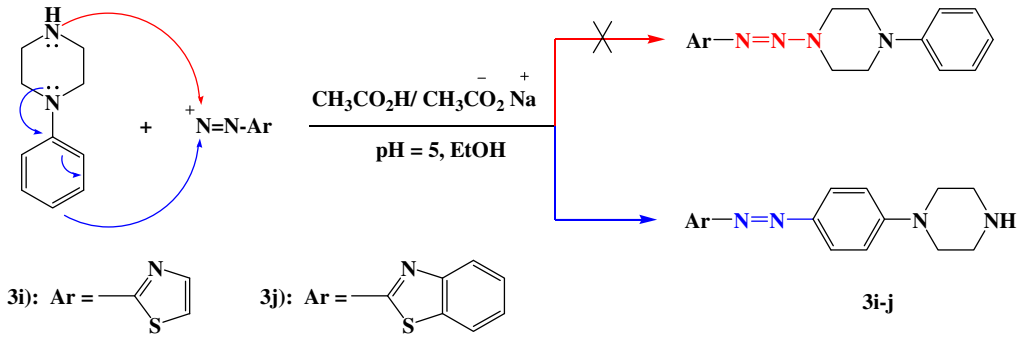
Yellow solid (EtOH/H_2O); yield 80%; m.p. 165–167 °C; IR (KBr) ν cm^{-1}: 3085, 2810, 1596, 1500, 1440, 1360, 1220,1144, 1055, 836, 742, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.41 (t, J = 5.19 Hz, 4H, $-CH_2$), 3.99 (t, J = 4.82 Hz, 4H, $-CH_2$), 6.91 (t, J = 7.35 Hz, 1H, Ar–H), 7.06–7.09 (m, 4H, Ar–H), 7.35 (t, J = 7.96 Hz, 2H, Ar–H), 7.46–7.49 (m, 2H, Ar–H) ppm.

2.2.2. Triazene 3b

Yellow solid (EtOH/H_2O); yield 80%; m.p. 167–169 °C; IR (KBr) ν cm^{-1}: 3097, 2822, 1590, 1500, 1440, 1400, 1360, 1238, 1142, 1080, 830, 742, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.40 (t, J = 5.34 Hz, 4H, $-CH_2$), 4.00 (t, J = 4.76 Hz, 4H, $-CH_2$), 6.97 (t, J = 7.23 Hz, 1H, Ar–H), 7.04 (d, J = 7.50 Hz, 2H, Ar–H), 7.33–7.36 (m, 4H, Ar–H), 7.45 (d, J = 7.57 Hz, 2H, Ar–H) ppm.

2.2.3. Triazene 3c

Yellow solid (EtOH/H_2O); yield 98%; m.p. 187–189 °C; IR (KBr) ν cm^{-1}: 3090, 2820, 1592, 1500, 1440, 1360, 1238, 1141, 1062, 820, 744, 680; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.40 (t, J = 5.18 Hz, 4H, $-CH_2$), 3.90 (t, J = 5.20 Hz, 4H, $-CH_2$), 6.84 (t, J = 7.22 Hz, 1H, Ar–H), 7.02 (d, J = 8.06 Hz, 2H, Ar–H), 7.26 (t, J = 8.83 Hz, 2H,



Scheme 2. Synthesis of heterocyclic azo derivatives (3i–j).

Table 1
Experimental electronic absorption maxima for investigated dyes and solvent parameters.

Solvents	π*	α	β	ε 293 K	λ_{max} (nm)									
					3a	3b	3c	3d	3e	3f	3 g	3 h	3i	3j
Cyclohexane	0.00	0.00	0.00	2.02	307	311	313	314	313	311	330	341	458	470
Diethyl ether	0.27	0.00	0.47	4.27	310	316	320	322	317	315	338	350	471	488
Ethyl acetate	0.54	0.00	0.45	6.08	312	321	325	325	322	321	341	354	478	496
Ethanol	0.54	0.83	0.77	25.33	312	320	322	325	324	322	337	354	489	500
Dioxane	0.55	0.00	0.37	2.22	310	318	325	324	322	321	342	358	482	498
Chloroform	0.58	0.44	0.00	4.81	317	323	330	333	331	340	380	370	475	493
Tetrahydrofuran	0.58	0.00	0.55	7.47	313	322	324	326	325	321	339	355	488	499
Methanol	0.60	0.93	0.62	33.10	311	320	323	324	325	324	338	356	490	501
Glacial acetic acid	0.64	1.12	0.45	6.20	310	318	320	323	330	328	344	352	485	495
Acetone	0.71	0.08	0.48	21.01	314	324	327	328	329	326	346	372	488	508
Acetonitrile	0.75	0.19	0.31	37.5	314	324	326	328	328	326	346	367	491	508
Dichloromethane	0.82	0.30	0.00	9.08	319	327	332	336	335	342	383	377	478	498
DMF	0.87	0.00	0.69	38.25	322	330	341	343	339	340	359	386	494	517
DMSO	1.00	0.00	0.76	47.24	325	334	345	348	342	342	365	391	500	524

Ar – H), 7.35 (d, J = 8.40 Hz, 2H, Ar – H), 7.55 (d, J = 8.05 Hz, 2H, Ar – H) ppm.

2.2.4. Triazene 3d

Yellow solid (EtOH/H_2O); yield 98%; m.p. 193–194 °C; IR (KBr) ν cm^{-1}: 3086, 2810, 1596, 1500, 1440, 1359, 1238, 1142, 1010, 820, 744, 680; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.37 (t, J = 5.35 Hz, 4H, $-CH_2$), 3.89 (t, J = 4.87 Hz, 4H, $-CH_2$), 6.84 (t, J = 7.25 Hz, 1H, Ar – H), 7.02 (d, J = 8.06 Hz, 2H, Ar – H), 7.22 (d, J = 8.81 Hz, 2H, Ar – H), 7.26 (t, J = 7.66 Hz, 2H, Ar – H), 7.72 (d, J = 8.81 Hz, 2H, Ar – H) ppm.

2.2.5. Triazene 3e

Yellow solid (EtOH/H_2O); yield 92%; m.p. 184–186 °C; IR (KBr) ν cm^{-1}: 3080, 2808, 1600, 1500, 1440, 1360, 1240, 1150, 1008, 830, 750, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.39 (t, J = 5.34 Hz, 4H, $-CH_2$), 3.86 (s, 3H, $-OCH_3$), 3.93 (t, J = 4.90 Hz, 4H, $-CH_2$), 6.91–6.97 (m, 3H, Ar – H), 7.03 (d, J = 7.87 Hz, 2H, Ar – H), 7.33 (t, 2H, J = 8.42 Hz, Ar – H), 7.48 (d, J = 7.55 Hz, 2H, Ar – H) ppm.

2.2.6. Triazene 3f

Yellow solid (EtOH/H_2O); yield 94%; m.p. 160–162 °C; IR (KBr) ν cm^{-1}: 3066, 2810, 1600, 1500, 1440, 1360, 1240, 1144, 1008, 820, 740, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.40 (s, 3H, CH_3), 3.41 (t, 4H, J = 5.25 Hz, CH_2), 3.97 (t, J = 5.25 Hz, 4H, CH_2), 6.98 (t, J = 6.91 Hz, 1H, Ar – H), 7.04 (br, 2H, Ar – H), 7.20 (d, J = 8.04 Hz, 2H, Ar – H), 7.35 (t, J = 8.22 Hz, 2H, Ar – H), 7.42 (m, 2H, Ar – H) ppm.

2.2.7. Triazene 3 g

Brown solid (DMF/H_2O); yield 95%; m.p. 196–198 °C. IR (KBr) ν cm^{-1}: 3300 (NH), 3060, 2815, 1680 (C=O), 1600, 1540, 1500, 1444, 1360, 1222, 1142, 1008, 840, 750, 690; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.03 (s, 3H, $-CH_3$), 3.34 (t, J = 5.25 Hz, 4H, $-CH_2$), 3.81 (t, J = 5.04 Hz, 4H, -CH_2), 6.83 (t, J = 7.24 Hz, 1H, Ar – H), 7.00 (d, 2H, J = 8.07 Hz, 2H, Ar – H), 7.24 (t, J = 7.43 Hz, 2H, Ar – H), 7.34 (d, 2H, J = 8.77 Hz, Ar – H), 7.59 (d, J = 8.76 Hz, 2H, Ar – H), 9.95 (s, 1H, – NH) ppm.

Table 2
Correlation between solvatochromic parameters and wavelengths of maximum absorption of dyes in studied solvents.

Correlation coefficients (R)										
Dye no.	3a	3b	3c	3d	3e	3f	3 g	3 h	3i	3j
π	0.67	0.85	0.73	0.74	0.88	0.84	0.75	0.76	0.91	0.95
α	0.07	0.04	0.08	0.05	0.00	0.00	0.04	0.07	0.04	0.00
β	0.55	0.63	0.60	0.62	0.60	0.59	0.52	0.52	0.67	0.66
ε	0.62	0.65	0.55	0.60	0.59	0.61	0.50	0.64	0.60	0.65

2.2.8. Triazene 3 h

Orange solid (EtOH/H_2O); yield 86%; m.p: 175–178 °C. IR (KBr) ν cm^{-1}: 3066, 2820, 1680, 1600, 1500, 1440, 1360, 1240, 1148, 1006, 820, 740, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.64 (s, 3H, $COCH_3$), 3.43 (t, J = 5.31 Hz, 4H, CH_2), 4.08 (t, J = 5.28 Hz, 4H, CH_2), 6.97 (t, J = 7.30 Hz, 1H, Ar – H), 7.02 (d, J = 7.21 Hz, 2H, Ar – H), 7.35 (m, 2H, Ar – H), 7.56 (m, 2H, Ar-- H), 8.00 (m, 2H, Ar – H) ppm.

2.2.9. Azo dye 3i

Red solid (DMF/H_2O); Yield 88%; m.p: 233–235 °C. IR (KBr) ν cm^{-1}: 3415 (NH), 2896, 1680, 1506, 1448, 1360, 1240, 1142, 1073, 908, 818, 744, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.40 (m, 4H, CH_2), 3.92 (m, 4H, CH_2), 6.94 (d, J = 9.3 Hz, 2H, Ar – H), 7.66 (d, J = 3.35 Hz, 1H, Ar – H), 7.78 (d, J = 9.3 Hz, 2H, Ar – H), 7.95 (d, J = 3.3 Hz, 1H, Ar – H) ppm.

2.2.10. Azo dye 3j

Red solid (DMF/H_2O); yield 91%; m.p: 258–260 °C. IR (KBr) ν cm^{-1}: 3435 (NH), 2980, 1680, 1596, 1498, 1450, 1360, 1270, 1240, 1140, 1070, 1030, 910, 812, 754, 680; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.12 (m, 4H, CH_2), 3.70 (m, 4H, CH_2), 7.18 (d, J = 9.2 Hz, 2H, Ar – H), 7.50 (t, J = 6.8 Hz, 1H, Ar – H), 7.56 (t, J = 6.8 Hz, 1H, Ar – H), 7.92 (d, J = 9.2 Hz, 2H, Ar – H), 8.06 (m, 2H, Ar – H) ppm.

2.3. Determination of antimicrobial activity

The biological (antibacterial) activity of the synthesized dyes (3a–j) was evaluated using *Salmonella enterica* (SE), *Micrococcus luteus* (ML), *Bacillus subtilis* (BS) and *Pseudomonas aeruginosa* (PS). The bacteria were grown on nutrient agar plates and aseptically transferred to 3 mL of nutrient broth. After overnight growth at 37 °C, 50 μL of the

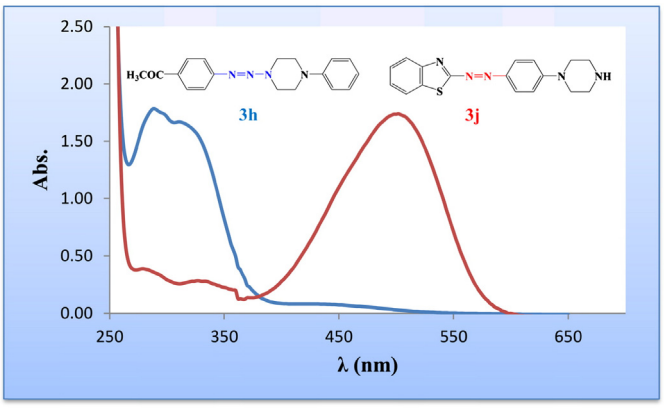


Fig. 1. Electronic absorption spectra of triazene 3 h and azo dye 3j in EtOH.

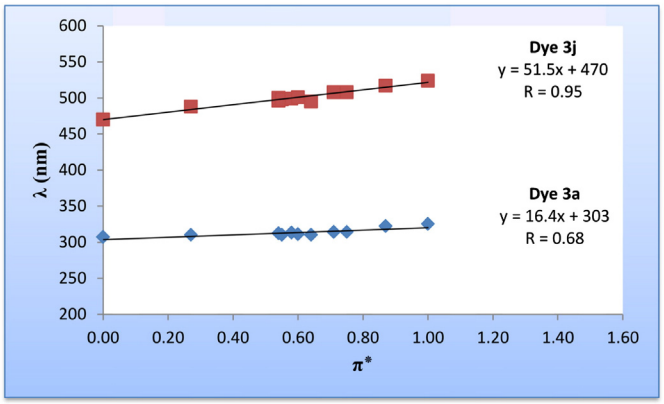


Fig. 2. Variation of λ_{max} (nm) of dyes 3a and 3j as a function of π*.

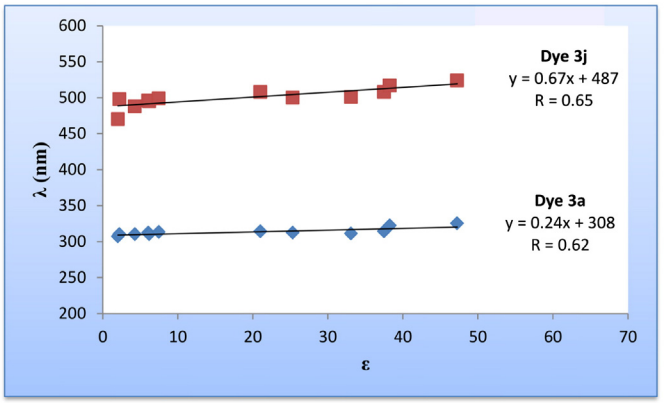


Fig. 4. Variation of λ_{max} (nm) of dyes 3a and 3j as a function of ε.

suspension was transferred on to nutrient agar plates and spread on the surface using a sterile spreader. Wells were bored in to the agar using sterile Pasteur pipette ends. Concentrations of 100 and 150 μg/ml of samples were prepared in DMF. To each well 40 μL of the sample solution (12 μg/well) was added. After incubation overnight at 37 °C, zones of inhibition were measured. Erythromycin and tetracycline disks were used as positive controls.

3. Results and discussion

3.1. The UV–visible spectra and solvatochromic studies

In spite of various studies on the solvatochromic behavior of azo dyes in different solvents, there are only a few investigations on the triazene dyes and more detailed studies are still needed [18,21–24]. In order to study solvent effects on spectral features of the prepared dyes, we recorded their absorption spectra in various solvents with different polarity at a concentration of 10^{-5}–10^{-6} M in the range of 200–700 nm, in which the solvents are arranged in the order of increasing polarity. Also, solvent dielectric constant (ε) and the solvatochromic parameters including dipolarity/polarizability polarity scale (π*), hydrogen bond donation ability (α) and hydrogen bond acceptance ability (β) were taken from literature [25–27]. The results are shown in Tables 1–2 and Figs. 1–4.

As shown in Table 1, the UV–Vis absorption spectra (λ_{max}) of the compounds are influenced by the solvents with different solvatochromic effects. In addition, an increase in the polarity of solvents (except for chlorinated solvents) has caused a bathochromic shift in the absorption maxima of all triazenes (3a–h) and azo dyes 3i–3j. For chlorinated solvents the deviation may be due to unique interactions between the polychlorinated solvents and the solute molecules. Such interactions can be accounted using another specific solvent polarity scale named as delta (δ) that generally is used in multi-parameter correlation.

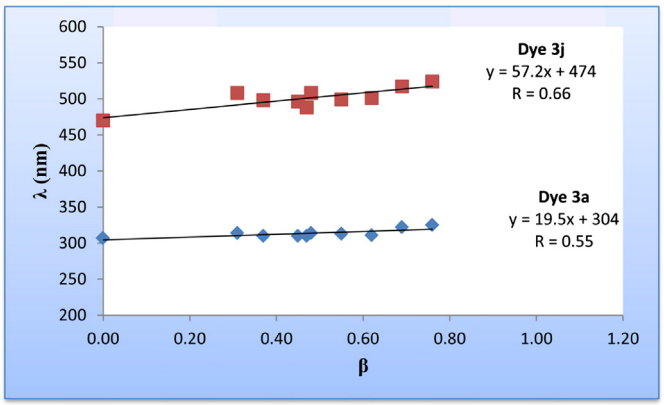


Fig. 3. Variation of λ_{max} (nm) of dyes 3a and 3j as a function of β.

According to Table 1 and Fig. 1, the most solvatochromic shift was observed for azo dye 3j (54 nm). On the other hand, dye 3j, exhibit a red shift as a result of an increase in the solvent polarity scale (π*). These spectral shifts are mainly due to physical intermolecular dye–solvent interactions (such as dipole-dipole, dipole-induced dipole, hydrogen bonding, etc.), that give rise to a better stabilization of the π* orbital as compared to the π orbital in polar solvents. The results have revealed that 3j can be used as an indicator for studied solvents. Also, the changes induced by solvents were evaluated using solvatochromic parameters. Therefore, the correlation coefficient values between maximum wavelength (λ_{max}) of dyes and solvent Kamlet–Taft parameters were obtained (Table 2 and Figs. 2–4). Verification of the results in Table 2 and Figs. 2–4 indicate that the contribution of solvatochromic parameters in absorption shifts of dyes are as π > β > ε > α. Therefore, it can be concluded that the most important contributions to the solvatochromism shift arise from π*, β and ε terms. This effect was attributed to the interaction of polar basic solvents such as DMF and DMSO with nonbonding electron pair on nitrogen atom in coupler component of synthesized dyes, which increases the electron density at the nitrogen atom.

On the other hand the hydrogen bond donating ability (α) has the least contribution to the solvatochromism shift ($R \approx 0$). The observed absorption spectra of all triazenes (3a–h) and azo dyes (3i–j) can be attributed to n → π and/or π → π electronic transitions of –N–N═N< and –N–N– chromophores, respectively [28].

3.2. Biological evaluation

The antibacterial activity of prepared dyes 3a–j was evaluated using *Salmonella enterica* (SE), *Micrococcus luteus* (ML), *Bacillus subtillis* (BS) and *Pseudomonas aeruginosa* (PS). Tetracycline and erythromycin were used as the reference antibacterial agents. The results are given in Table 3. As shown in Table 3, the azo compounds 3i and 3j exhibit strong activities towards *S. enterica* (a Gram-negative bacterium) and *M. luteus* (a Gram-positive bacterium) in compared to triazenes 3a–h. In addition, all of the prepared dyes have exhibited higher levels of antibacterial activity against *B. subtilis* (a Gram-positive bacterium) as revealed by the diameters of their inhibition zones (Table 3). All of the synthesized triazenes exhibit weak antimicrobial activities against *S. enterica* and *P. aeruginosa* (a Gram-negative bacterium).

4. Conclusions

Eight novel triazenes and two heterocyclic azo dyes were synthesized through similar condition in high yields. The results of spectroscopic analysis showed that two synthetic pathways for *N*-phenylpiperazine as nucleophilic reagent are possible: the N–N bond formation (triazenes)

Table 3
Antibacterial activity of the compounds (3a–j).

Dye no.	Antimicrobial activity (zone of inhibition in mm)							
	Salmonella enterica		*Micrococcus luteus*		*Bacillus subtilis*		*Pseudomonas aeruginosa*	
	100 μg/ml	150 μg/ml	100 μg/ml	150 μg/ml	100 μg/ml	150 μg/ml	100 μg/ml	150 μg/ml
3a	–	–	10 (10)[a]	13	12 (11)[a]	15	–	–
3b	–	–	13 (12)[a]	14	14 (14)[a]	17	8	9
3c	–	–	–	–	6	8	–	–
3d	–	–	–	–	8	9	–	–
3e	–	–	6	6	14 (15)[a]	16	6	7
3f	–	–	–	–	14 (13)[a]	17	–	–
3 g	–	–	10 (12)[a]	15	16 (17)[a]	20	11	14
3 h	–	–	–	–	9	11	–	–
3i	14	17	30 (32)[a]	42	12 (12)[a]	15	–	–
3j	6	8	35 (34)[a]	45	18 (20)[a]	21	–	–
Erythromycin	8	–	10	–	12	–	10	–
Tetracycline	7	–	16	–	14	–	18	–

[a] Data of duplicated experiments.

and C – N bond formation (azo compounds) according to the reactivity of diazonium salts. The electronic absorption spectra of dyes were recorded in solvents having different physical–chemical properties. Also, investigations of solvent effects indicate that azo dyes 3i and 3j can be used as solvent polarity indicators. The absorption spectra of triazenes and azo dyes attributed to n → π and/or π → π electronic transitions of – N – N═N< and ═N═N – chromophores, respectively. The tested compounds exhibited a different level of antibacterial activity against four selected bacteria.

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