Turkish Journal of Chemistry
http://journals.tubitak.gov.tr/chem/
Research Article

Turk J Chem
(2015) 39: 998 – 1011

doi:10.3906/kim-1412-46

New tetrazolic azo dyes linked to (thio)barbiturate and electron-rich aromatics as potential antimicrobial agents

Nader NOROOZI PESYAN[1,*], Davoud SOLEIMANI[1], Nima HOSSEINI JAZANI[2]
[1]Faculty of Chemistry, Urmia University, Urmia, Iran
[2]Department of Microbiology, Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Received: 17.12.2014 • **Accepted/Published Online:** 22.03.2015 • **Printed:** 30.10.2015

Abstract: A series of new tetrazolic azo dyes based on (thio)barbiturate and electron-rich aromatics were synthesized in excellent yield. The electron-donor and tetrazole ring moieties were linked by a *p*-phenylazo bridge and the structural characterizations were achieved by FT IR, ^{1}H and ^{13}C NMR, and UV-visible spectrometry. The antibacterial activity of the synthesized compounds was tested against gram-positive and gram-negative bacterial strains, namely *Acinetobacter calcoaceticus* (ATCC23055), *Escherichia coli* (ATCC2592), *Pseudomonas aeruginosa* (ATCC27853), and *Staphylococcus aureus* (ATCC25923). As a result, potential antimicrobial effects were seen for some of the synthesized compounds.

Key words: Tetrazole, azo dye, (thio)barbituric acid, antimicrobial effect

1. Introduction

Azo dye derivatives are considered important compounds from biological and medicinal viewpoints; some examples are catechol diazo dyes as substrates for the enzyme catechol-*O*-methyltransferase (**9**);[1] as potent tyrosinase inhibitors (**10**);[2] as potent and selective inhibitors of the tumor-associated isozymes IX and XII over the cytosolic isoforms I and II (**11**);[3] antimicrobial, anti-HCV, anti-SSPE, antioxidant, and antitumor activities of arylazobenzosuberones (**12**);[4] some novel arylazopyrazolodiazine and triazine analogs having an antitumor effect (**13** and **14**);[5] antimicrobial and cytotoxic arylazoenamines (**15** and **16**);[6] antiviral and cytotoxic activities (**17**);[7] phenylimino-10*H*-anthracen-9-ones as novel antimicrotubule agents (**18**);[8] novel azo-resveratrol as a potent tyrosinase inhibitor (**19**);[9] antifungal agents (**20** and **21**);[10] (Figure 1), carbonic anhydrase inhibitors;[11] β-aggregation inhibitors[12] etc.

In addition, many other compounds containing the tetrazole functional group are also known for their medicinal and biological effects. This functional group can take different roles including as a ligand in coordination chemistry, as a metabolically stable surrogate for a carboxylic acid group in medicinal chemistry,[13,14] and as a carboxylic acid isostere.[15] 5-Substituted-tetrazoles are reported to possess antibacterial,[16] antifungal,[17] antiviral,[18] analgesic,[19–23] anti-inflammatory,[24,25] antiulcer,[26] and antihypertensive activities;[27] for instance, irbesartan (**22**), valsartan (**23**), tasosartan (**24**), and losartan (**25**) (Figure 1). Moreover, it needs to be added that the whole tetrazole function is metabolically stable.[28]

*Correspondence: n.noroozi@urmia.ac.ir

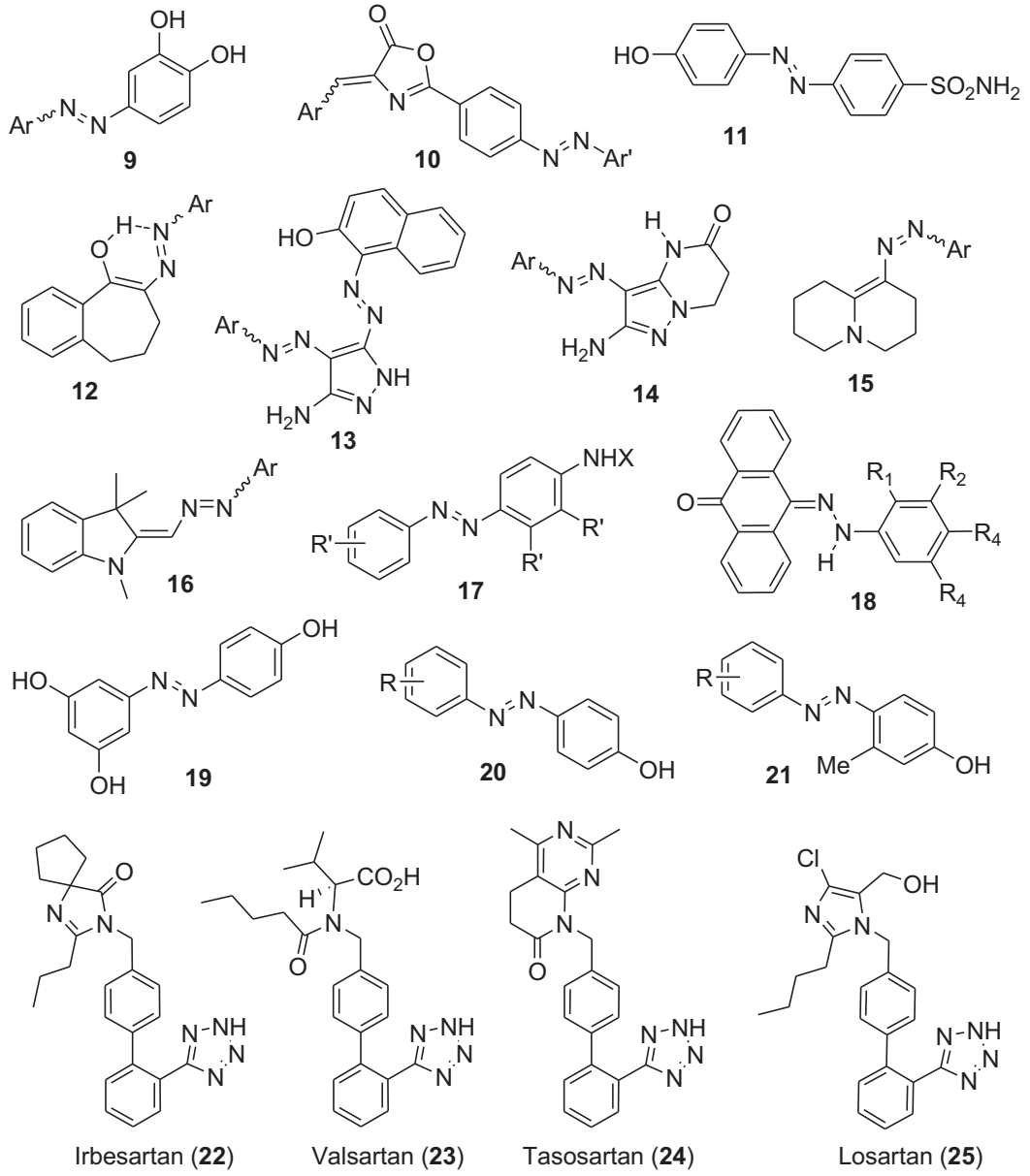


Figure 1. Structure of some drugs based on azo dyes and tetrazole.

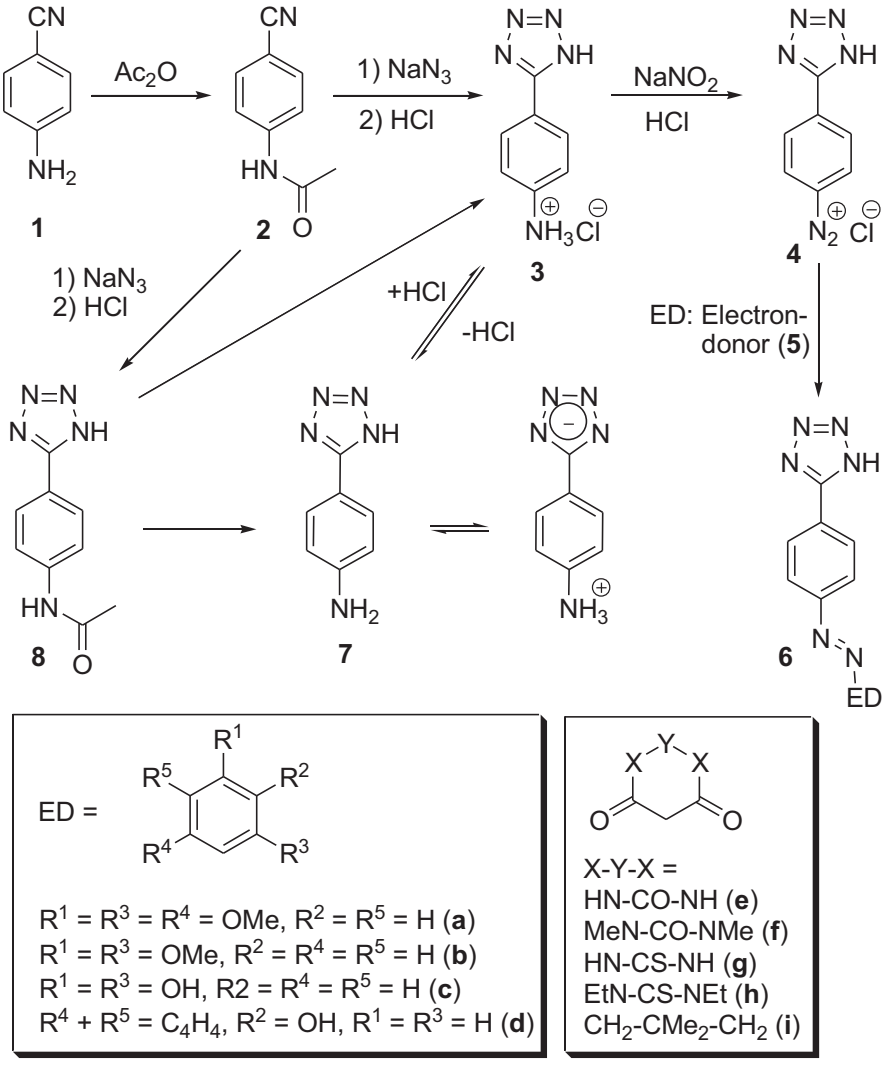
In addition to medicinal applications, azo dyes are also used as colorimetric sugar sensors,[29] MRI contrast agents,[30] and even in high technology fields such as electronic devices, linear and nonlinear optics, reprography, and sensors.[31–34] Based on these concepts, in this study we designed and colligated the tetrazole and azo functional groups linked to an electron donor in the molecule in order to evaluate their antimicrobial effects.

2. Results and discussion

2.1. Chemistry

This article describes the synthesis of (*E*)-1-(4-(1*H*-tetrazol-5-yl)phenyl)-2-aryldiazenes (**6a**–**6d**), (*E*)-5-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione and their sulfur analogues (**6e**–**6h**),

and (*E*)-2-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)-5,5-dimethylcyclohexane-1,3-dione (**6i**) in the reaction of 4-amino benzonitrile (**1**) as a starting material in three steps in good to excellent yield (Scheme 1; Table 1). In these reactions, there is no need for separation or purification of 4-(1*H*-tetrazol-5-yl) benzenaminium chloride (**3**), which is considered an advantage for the synthesis of tetrazole-based azo dyes in current studies. Due to this advantage, the experimental work-up to determine azo dyes **6** will be very easy.



Scheme 1. Synthesis of azo dyes based on tetrazole and electron donors.

To determine **3** as a key material, the amino group in **1** should be protected initially. For this aim, the reaction of **1** with acetic anhydride obtained *N*-(4-cyanophenyl) acetamide (**2**). The IR spectrum of **2** shows a peak in the frequency of 1671 cm^{-1} for carbonyl and the stretching vibration of NH of the amide group appeared at the frequency of 3326 and 3256 cm^{-1}, while the stretching of the nitrile group appeared at the frequency of 2221 cm^{-1}. The appearance of the carbonyl stretching of the acetamido group supports the formation of **2**. The ^{1}H NMR spectrum of **2** showed a singlet at δ 2.01 ppm (CH_3–CO–), a multiplet at δ 7.75 ppm, and a singlet at δ 10.38 ppm (–NH–CO–). The ^{13}C NMR spectrum of this compound showed seven distinct peaks, of which the ones at δ 24.2 and 169.2 ppm corresponded to methyl and carbonyl groups,

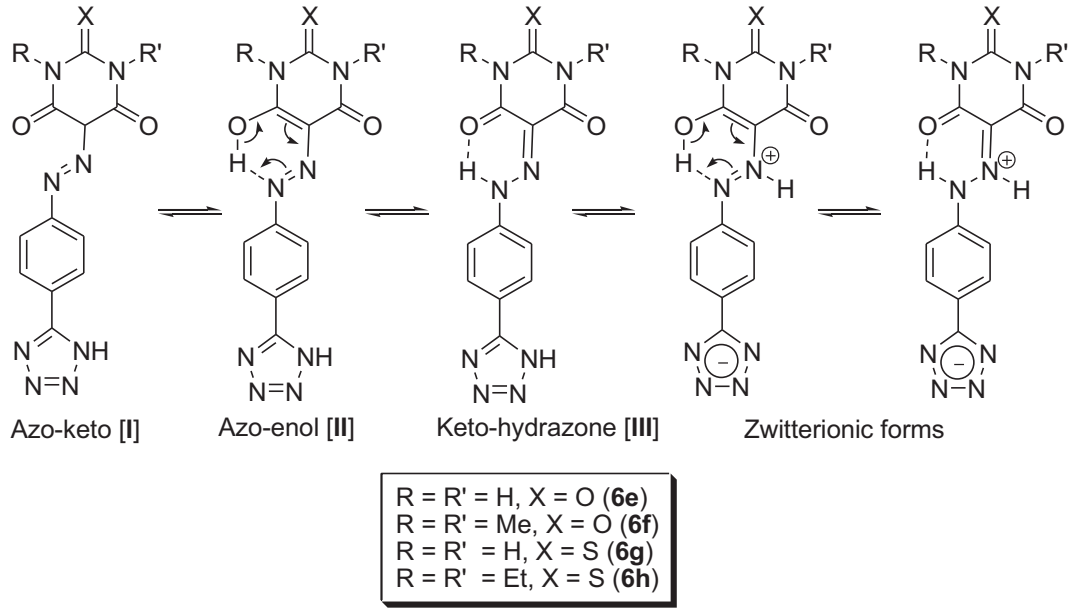
respectively. The peaks at δ 143.5, 133.3, 119.1, and 118.9 ppm and at δ 104.7 ppm corresponded to phenyl and CN groups, respectively (see Experimental part and Supplementary material; on the journal's website). The cycloaddition reaction of **2**, with sodium azide and followed by concentrated hydrochloric acid, afforded **3** through compound *N*-(4-(1*H*-tetrazol-5-yl)phenyl)acetamide (**8**). Compound **8** was isolated for its structural characterization. The IR spectrum of **8** showed a peak at the frequency of 1678 cm^{-1} for the carbonyl group and the stretching vibration of the NH group appeared at the frequency of 3311 and 3267 cm^{-1}. The tetrazolic NH stretching appeared at the frequencies of 2471–3195 cm^{-1}.[35]

Compound **2** can also convert to **3** simultaneously in one step and without the need for separation of **8** (formation of tetrazole followed by deprotection reaction). The IR spectrum of **3** showed a peak at the frequency of 2470 to 3134 cm^{-1} for the tetrazolic NH group and a peak at the frequency of 3383 cm^{-1} corresponded to ammonium salt moiety, while some peaks of this group overlapped with the peaks of the tetrazolic NH group. The ^{1}H NMR spectrum of **3** showed a broad singlet at δ 6.73 ppm that corresponded to the sum of tetrazolic NH, while ammonium salt protons indicated two doublets at δ 7.29 and 8.04 ppm that corresponded to phenyl protons. The ^{13}C NMR spectrum of **3**, on the other hand, showed five distinct peaks (see Experimental part and Supplementary material).

Compound 4-(1*H*-tetrazol-5-yl)aniline (**7**) can also be obtained from **3** under natural conditions (naturalized by Na_2CO_3). The IR spectrum of **7** showed two peaks at the frequencies of 3485 and 3385 cm^{-1} for the primary amino group and the stretching vibration of the tetrazolic NH group appeared at the frequencies of 3357 to 3213 cm^{-1} (see Experimental part and Supplementary material). Compound **7** can also be found in zwitterionic form.

There is no need to separate and purify **3** from the crude reaction mixture in the azo dye synthesis. This feature is the most favorable advantage of the reaction process (except for the spectroscopic analysis of **3**). The salt of **3** converted to diazonium salt of **4** by the use of sodium nitrite added to the reaction mixture at 0 °C. Finally, azo dyes (**6**) were precipitated by dropwise addition of diazonium salt into the solution of corresponding electron donors (ED, **5**) at 0 °C (Scheme 1 and see Experimental part). Representatively, the IR spectrum of **6e** showed peaks at the frequencies of 3478 cm^{-1} for NH/OH, 3200 and 3075 cm^{-1} for BA-NH groups, 2479–2846 for tetrazolic NH, and 1743 and 1692 cm^{-1} for carbonyl groups of the barbituric acid ring moiety. The ^{1}H NMR spectrum of this compound showed a singlet at δ 14.10 ppm for NH/OH and two singlets at δ 11.55 and 11.32 ppm that corresponded to different chemical shifts of BA-NH groups. In addition, two doublets at δ 7.77 and 8.09 ppm occurred in the phenyl ring. It seems that the peak of the tetrazole-NH group overlaps with the DMSO-water peak at δ 3.58 ppm as a broad singlet. In many tetrazolic compounds, the tetrazole-NH proton can be detected by adding a drop of D_2O (judging the appearance of the DOH peak at 3.99 ppm (variable)).[36] The ^{13}C NMR spectrum of **6e** showed nine distinct peaks: two peaks at δ 161.9, 159.7, and 154.9 ppm corresponded to different chemical shifts of carbonyl groups and that at δ 149.7 ppm corresponded to tetrazolic carbon atoms. The peaks at δ 143.5 and 121.1 are of quaternary carbon atoms, and those at δ 128.4 and 117.2 are of phenyl CH carbons. Finally, the peak at δ 119.0 is of C=N and/or =C–N carbon atom on the BA ring moiety (see Experimental part and Supplementary material).

Due to the formation of the intramolecular H-bond in azo-enol and/or keto-hydrazone forms and also the restricted rotation about the C=N bond in keto-hydrazone form, the two carbonyl groups along with the two substituents on the *N*,*N*-disubstituted (thio)barbituric acid ring moiety have been found in the results of different chemical shifts (Scheme 2).[37–39]



Scheme 2. Possible tautomeric forms of tetrazolic azo dyes based on symmetrical (thio)barbituric acids.

Representatively, the UV-visible spectra of azo dye **6c** are shown in Figure 2. These spectra are recorded in acetone (A) and ethanol (B) as aprotic and protic solvents, respectively, over the range of λ between 250 and 600 nm using two solvents in concentrations $\approx 10^{-4}$–10^{-5} mol L^{-1} (M); for more information, see Experimental part and Supplementary material. It was observed that despite there being some absorption spectra in acetone and ethanol (**6a**, **6d**, **6e**, and **6i**), they did not change significantly except for dyes **6b**, **6c**, **6f**, **6g**, and **6h**. Representatively, in 1.7×10^{-4} M, the λ_{max} values of dye **6c** in acetone and ethanol appeared at 331 (log ε = 1.027) and 308 nm (log ε = 2.529), respectively. Obviously, the λ_{max} values of dye **6c** in ethanol as a protic solvent hypsochromically shifted with respect to the λ_{max} of acetone in higher concentrations (Figure 2A and 2B). In low concentrations of ethanol (8.7×10^{-6} to $3.5 \times 10^{-5} M$), dye **6c** showed two λ_{max} at 290 and 332 nm, while from 1.0×10^{-5} to $3.5 \times 10^{-4} M$ it showed one distinct λ_{max} that bathochromically shifted

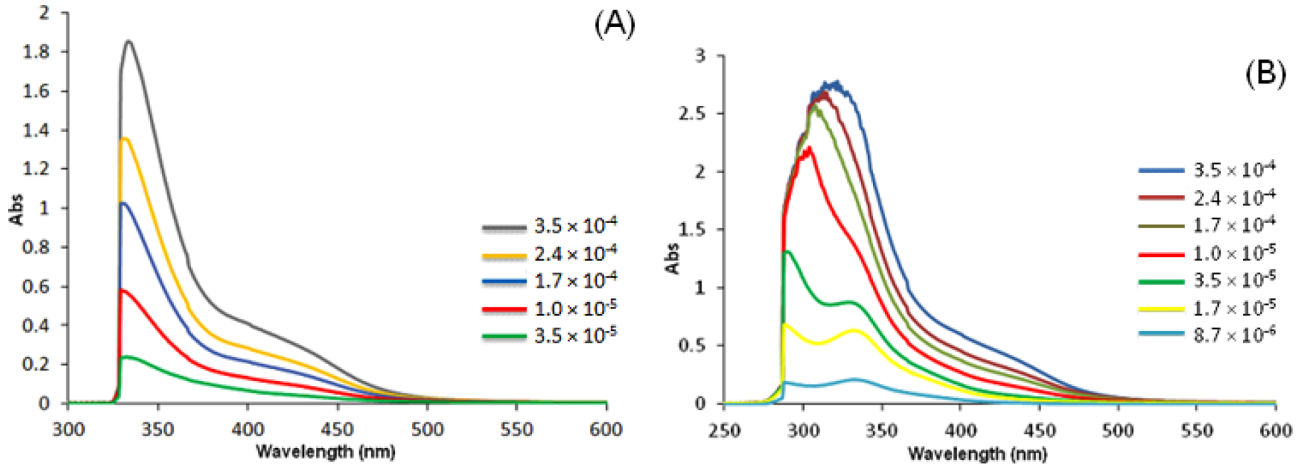
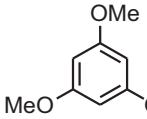
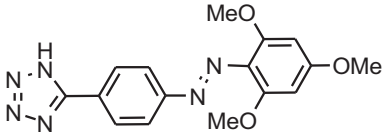
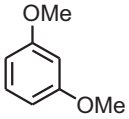
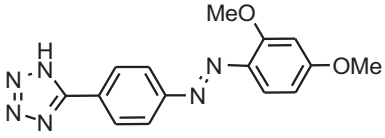
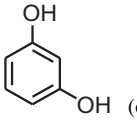
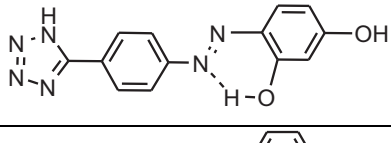
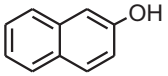
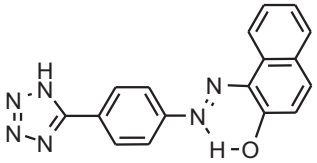
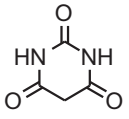
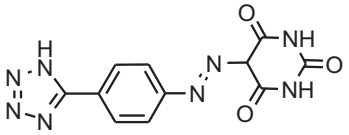
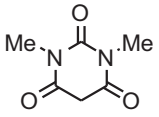
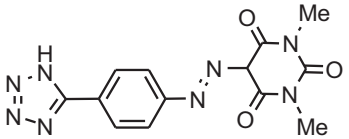
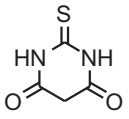
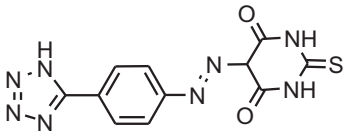
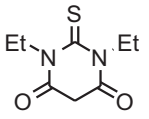
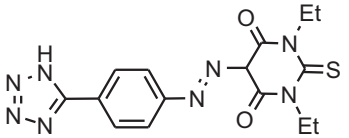
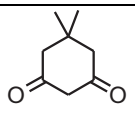
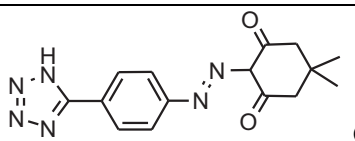


Figure 2. UV-visible spectrum of **6c** in acetone (A) and in ethanol (B) in various concentrations (M).

with increasing concentration (Figure 2B). In contrast, in acetone as an aprotic solvent, **6c** showed a distinct λ_{max} that bathochromically shifted slightly in higher concentrations (Figure 2A) (for more information, see Experimental part and Supplementary material). The λ_{max} values of dyes **6a**–**6i** are summarized in Table 1.

Table 1. Structure, yields, and λ_{max} of new tetrazolic azo dyes linked to (thio)barbiturate and electron-rich aromatics (**6a**–**6i**).

Entry	Electron-donor (ED, **5**)	Tetrazolic azo dye (**6**)	λ_{max} (nm), (EtOH*, acetone**)	Yield[a] (%)
1	 (**a**)	 (**a**)	307, 360 (*) 360, 458 (**)	50
2	 (**b**)	 (**b**)	313, 363 (*) 360 (**)	70
3	 (**c**)	 (**c**)	288, 331 (*) 332 (**)	60
4	 (**d**)	 (**d**)	363, 496 (*) 382, 479 (**)	57
5	 (**e**)	 (**e**)	292, 330 (*) 333 (**)	55
6	 (**f**)	 (**f**)	317, 408 (*) 391 (**)	60
7	 (**g**)	 (**g**)	329, 366, 402, 465 (*) 337, 366, 402, 455 (**)	64
8	 (**h**)	 (**h**)	329, 360, 401, 456 (*) 342, 366, 402, 462 (**)	60
9	 (**i**)	 (**i**)	288, 365, 397, 431 (*) 366, 402, 421 (**)	15

[a] Isolated yields.

2.2. Antimicrobial activities of 6a–6i

As outlined in Table 2, among all of the derivatives, compounds **6a**, **6b**, **6c**, **6d**, **6g**, and **6h** exhibited a good and broad spectrum of antimicrobial activities against the four bacterial species *Acinetobacter calcoaceticus* (ATCC23055), *Escherichia coli* (ATCC2592), *Pseudomonas aeruginosa* (ATCC27853), and *Staphylococcus aureus* (ATCC25923), tested at the concentration of 100 μg/μL. For example, compounds **6c** and **6e** showed potential inhibitory effects against the four above-mentioned bacterial strains. Compound **6b** only inhibited the growth of *P. aeruginosa*. Compounds **6a** and **6h** affected *A. calcoaceticus* and *S. aureus* while compound **6g** only affected *A. calcoaceticus* (Table 2). Table 3 shows the antimicrobial activities against the four above-mentioned bacterial species by six known standard antibiotics as a model test. The results derived from Table 2 are comparable with those from Table 3. Representatively, the image of antimicrobial test results for the above-mentioned bacterial species is shown in Figure 3.

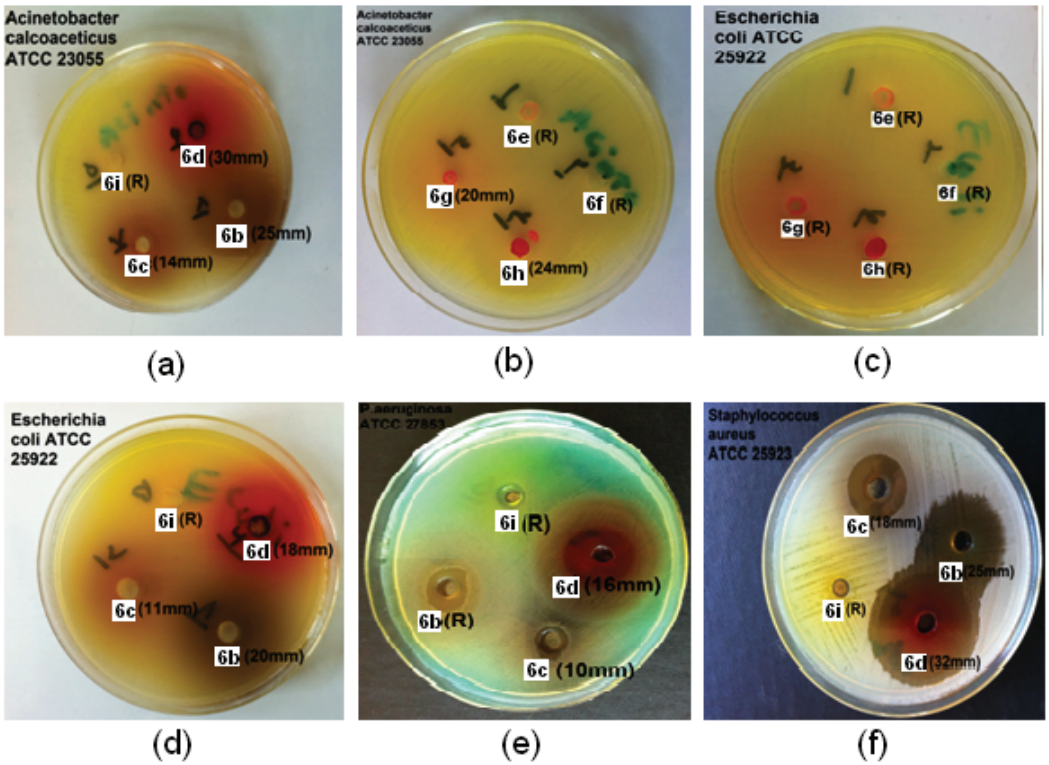


Figure 3. Representatively, antimicrobial test results for *Acinetobacter calcoaceticus* ATCC23055 (a and b), *Escherichia coli* ATCC25922 (c and d), *Pseudomonas aeruginosa* ATCC27853 (e), and *Staphylococcus aureus* ATCC25923 (f). R in parentheses (R) indicated resistant.

3. Experimental

3.1. General procedures

Melting points were measured by a digital melting point apparatus (Electrothermal) and were corrected. IR spectra were determined in the region 4000–400 cm^{-1} on a NEXUS 670 FT IR spectrometer by preparing KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 FT NMR at 400 and 100 MHz,

respectively (University of Tabriz, Tabriz, Iran). ^{1}H and ^{13}C NMR spectra were obtained in solution in DMSO-d_6 and/or in $CDCl_3$ as solvent using TMS as internal standard. The data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or unresolved, bs = broad singlet, coupling constant(s) in Hz integration. All reactions were monitored by TLC with silica gel-coated plates (EtOAc:n-hexane/8:10/v:v). UV-visible spectra were recorded on a T80 UV-vis (PG instruments Ltd) spectrometer (Urmia University, Urmia, Iran). Compounds **1**, **5a**–**5i**, sodium azide, sodium nitrite, hydrochloric acid, and the solvents used were purchased from Merck and Aldrich without further purification.

Table 2. Antimicrobial activity of some potential tetrazolic azo dyes.

Compd.	*Acinetobacter calcoaceticus*[a] ATCC23055	*Escherichia coli*[a] ATCC25922	*Pseudomonas aeruginosa*[a] ATCC27853	*Staphylococcus aureus*[b] ATCC25923
6a	14 mm[c]	R[d]	R	14
6b	25	20	R	20
6c	14	11	10	18
6d	30	18	16	32
6e	R	R	R	R
6f	R	R	R	R
6g	20	R	R	R
6h	24	R	R	15
6i	R	R	R	R

[a] Gram-negative. [b] Gram-positive. [c] Scale is based in millimeter radius. [d] Resistant.

Table 3. Antimicrobial activities against the four bacterial species by six standard drugs as a model test.

Bacterial strains	Erythromycin (5 μg)	Cephalothin (30 μg)	Ampicillin (10 μg)	Trimethoprim/ sulfamethoxazole	Ciprofloxacin	Imipenem (10 μg)
Pseudomonas aeruginosa ATCC27853	NT[a]	R[b]	R	R	R	31 mm
Acinetobacter calcoaceticus ATCC23055	NT	NT	18 mm	25 mm	R	NT
Escherichia coli ATCC25922	NT	20 mm	14 mm	24 mm	NT	NT
Staphylococcus aureus ATCC25923	20 mm	27 mm	11 mm	22 mm	NT	NT

[a]Not tested. [b] Resistant.

3.2. General procedure for the preparation of 2

3.2.1. *N*-(4-Cyanophenyl)acetamide (2)

To a 50-mL round bottom flask equipped with a magnetic stirrer were added consecutively *p*-aminobenzonitrile (2.36 g, 20 mmol) and acetic anhydride (dropwise 50 mmol) and then the reaction mixture was refluxed for 30 min. After cooling, the reaction mixture was poured into a beaker containing 30 mL of cool distilled water and white solid precipitated. The mixture was boiled until decomposition of the acetic anhydride residue. The precipitate was filtered out and washed with a mixture of cool ethanol and water (2.93 g, 92% yield).

Colorless solid, mp 206–208 °C; FT IR (KBr) 3303 (NH), 3256, 3183, 3109, 3053 (CH-ar.), 2932 (CH-aliph.), 2221 (CN), 1671 (CO) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.09 (s, 3H, CH_3), 7.75 (s, 4H, C-ar.), 10.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 24.2 (CH_3), 104.7 (CN), 118.9 (CH-ar.), 119.1 (C-ar.) 133.3 (CH-ar.), 143.5 (C-ar.), 169.2 (CO).

3.3. General procedure for the preparation of 8

3.3.1. *N*-(4-(1*H*-tetrazol-5-yl)phenyl)acetamide (8)

In a 50-mL round bottom flask equipped with a magnetic stirrer and an oil-bath, a mixture of *p*-cyanoacetanilide (2.93 g, 18.3 mmol), sodium azide (2.37 g, 36.5 mmol), and ammonium chloride (0.53 g) as a catalyst was dissolved in 20 mL of DMF and refluxed for 12 h. The reaction progression was controlled by thin layer chromatography (TLC) with the solvent mixture of EtOAc:cyclohexane:methanol/8:10:2 (V/V). The reaction color turned pale yellow and the solvent was removed under reduced pressure by a rotary evaporator. The viscous residue was put on an ice-bath, hydrochloric acid (2 M) was added dropwise until the pH was 1, and the white solid was precipitated, filtered out, and recrystallized with the mixture of methanol and water (3.1 g, 84% yield).

Colorless solid, mp 289–300 °C; FT IR (KBr) 3311, 3267 (NHCO), 3195, 3129, 3069, 2985, 2920, 2854, 2706, 2615, 2471 (N_4H, CH-ar.), 1678 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.09 (s, 3H, CH_3), 3.44 (bs, 1H), 7.78 (d, 2H, J = 8.3 Hz, CH-ar.), 7.96 (d, 2H, J = 8.3 Hz, CH-ar.), 10.26 (s, 1H, NHCO); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 24.2 (CH_3), 118.4 (C-ar.), 119.2 (CH-ar.), 127.7 (CH-ar.), 141.9 (C-ar.), 155.0 (C-tetrazole), 168.9 (C=O).

3.4. General procedure for the preparation of 7

3.4.1. 4-(1*H*-tetrazol-5-yl)aniline (7)

Colorless solid, mp 265–267 °C; FT IR (KBr) 3485, 3385 (NH_2), 3213, 3142, 3097, 3062, 3024, 3003, 2938, 2859, 2792, 2753, 2632, 2496, 2357 (N_4H, CH-ar.), 1622 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.36 (bs, 2H, overlapped with the DMSO's water peak), 5.77 (bs, 1H), 6.68 (d, 2H, J = 8.5 Hz, CH-ar.), 7.68 (d, 2H, J = 8.5 Hz, CH-ar.); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 113.6 (CH-ar.), 128.2 (CH-ar), 142.0 (C-ar.), 151.6 (C-ar.), 160.0 (C-tetrazole).

3.5. General procedure for the preparation of 3

3.5.1. 4-(1*H*-tetrazol-5-yl)benzenaminium chloride (3)

In a 50-mL round bottom flask equipped with a magnetic stirrer and an oil bath, *N*-(4-(1*H*-tetrazol-5-yl)phenyl)acetamide (2.6 g, 12.8 mmol) in 20 mL of hydrochloric acid (4 M) was refluxed for 5–6 h. After cooling, the solvent was evaporated and white solid precipitated (2.04 g, 80% yield).

Colorless solid, mp 251–253 °C; FT IR (KBr) 3383, 3263, 3044, 3007, 2979, 2922, 2854, 2769, 2758, 2470 (N_4H, NH_3^+, CH-ar.), 1620, (C=C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.29 (d, 2H, J = 8.4 Hz, CH-ar.), 8.04 (d, 2H, J = 8.4 Hz, CH-ar.), 6.73 (bs, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 119.2 (C-ar.), 120.6 (CH-ar.), 128.4 (CH-ar.), 140.5 (C-ar.), 154.9 (C-tetrazole).

3.6. General procedure for the synthesis of tetrazolic azo dyes 6a–6d

In a 50-mL beaker equipped with an ice-salt bath, 4-(1*H*-tetrazol-5-yl)benzenaminium chloride (1.0 mmol) was dissolved in 5 mL of distilled water at 0 °C. Then the solution of sodium nitrite (2.0 mmol) in 10 mL of water was added dropwise into the beaker over 30 min at 0 °C and afterwards 5 mL of diluted HCl was added to the reaction mixture and this made it a clear solution at 0 °C. In the other vessel, 1 mmol of an electron donor

was dissolved in 10 mL of 10% sodium hydroxide. Finally, the solution of diazonium salt was added dropwise into the basic solution of electron donor. As a result, red solid dye was precipitated, filtered out, washed with distilled water, recrystallized with methanol, and dried.

3.6.1. (*E*)-1-(4-(1*H*-tetrazol-5-yl)phenyl)-2-(2,4-dimethoxyphenyl)diazene (6b)

Red solid, mp 222–224 °C (decomp.); FT IR (KBr) 3536, 3423, 3189, 3084, 3022, 2979, 2918, 2838, 2765, 2639 (N_4H, CH-ar.), 1605 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 6.66 (dd, 1H, ^3J = 9.9 Hz, ^4J = 1.2 Hz, CH-ar.), 6.81 (d, 1H, J = 1.2 Hz, CH-ar.), 7.69 (d, 1H, J = 9.0 Hz, CH-ar.), 7.98 (d, 2H, J = 7.5 Hz, CH-ar.), 8.22 (d, 2H, J = 7.5 Hz, CH-ar.); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.8 (OCH_3), 56.2 (OCH_3), 99.1 (CH-ar.), 106.6 (CH-ar.), 116.0 (C-ar.), 117.5 (CH-ar.), 123.1 (CH-ar.), 128.1 (CH-ar.), 130.0 (C-ar.), 135.9 (C-ar.), 153.8 (C-tetrazole), 159.3 (**C**-OCH_3), 164.4 (**C**-OCH_3); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 313, 363 nm, (2.122, 1.793); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 360 nm, (1.531).

3.6.2. (*E*)-4-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)benzene-1,3-diol (6c)

Red solid, mp 160–162 °C (decomp.); FT IR (KBr) 3522 (OH), 3405 (OH), 3173, 2977, 2813, 2756, 2689 (N_4H, CH-ar.), 1696, 1660 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.37 (d, 1H, J = 10.8 Hz, CH-ar.), 6.48 (d, 1H, J = 10.8 Hz, CH-ar.), 7.79 (d, 2H, J = 11.0 Hz, CH-ar.), 7.83 (d, 2H, J = 10.8 Hz, CH-ar.), 13.84 (bs, 2H, OH), 14.0 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 128.6 (CH-ar.), 129.3 (CH-ar.), 130.7 (CH-ar.), 131.0 (CH-ar.), 145.0 (C-ar.), 146.8 (CH-ar.), 147.1 (C-ar.), 173.2 (C-ar.), 177.6 (C-ar.), 178.8 (C-ar.), 182.5 (C-ar.); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 288, 331 nm, (1.291, 0.631, up to 3.5 $\times$ $10^{-5}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 332 nm, (1.838).

3.6.3. (*E*)-1-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)naphthalen-2-ol (6d)

Red solid, mp 69–71 °C; FT IR (KBr) 3433 (OH), 3062, 3029, 2926, 2860, 2759, 2623 (N_4H, CH-ar.), 1615 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (d, 1H, J = 9.5 Hz, CH-napht.), 7.46 (t, 1H, J = 9.8 Hz, CH-napht.), 7.61 (t, 1H, J = 7.9 Hz, CH-napht.), 7.73 (d, 1H, J = 7.6 Hz, CH-napht.), 7.90 (d, 1H, J = 9.5 Hz, CH-napht.), 8.00 (d, 2H, J = 8.4 Hz, CH-ph), 8.15 (d, 2H, J = 8.4 Hz, CH-ph), 8.50 (d, 1H, J = 8.1 Hz, CH-napht.), 15.85 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 118.7 (CH-ar.), 121.7 (CH-ar.), 122.1 (C-ar.), 125.0 (CH-ar.), 126.6 (CH-ar.), 128.0 (C-ar.), 128.5 (CH-ar.), 129.1 (CH-ar.), 129.4 (C-ar.), 130.0 (C-ar.), 130.4 (C-ar.), 132.7 (CH-ar.), 141.7 (CH-ar.), 145.7 (C-tetrazole), 174 (C-OH); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 363, 490 nm, (1.772, 1.273, up to 1.5 $\times$ $10^{-4}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 382, 479 nm, (1.212, 0.933).

3.7. General procedure for the synthesis of tetrazolic azo dyes 6e–6h and 6i

In a 50-mL beaker equipped with an ice-salt bath, 4-(1*H*-tetrazol-5-yl)benzenaminium chloride (1.0 mmol) was dissolved in 5 mL of distilled water at 0 °C. Then the solution of sodium nitrite (2.0 mmol) in 10 mL of water was added dropwise to the beaker over 30 min at 0 °C and afterwards 5 mL of diluted HCl was added to the reaction mixture and this made it a clear solution at 0 °C. In the other vessel, 1 mmol of an electron-donor (barbituric acid and its derivatives) was dissolved in 10 mL of water. Finally, the solution of diazonium salt

was added dropwise to the solution of electron donor. As a result, yellow solid dye was precipitated, filtered out, washed with distilled water, recrystallized with methanol, and dried.

3.7.1. (*E*)-5-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (6e)

Yellow solid, mp 245 °C; FT IR (KBr) 3478 (OH/NH), 3323, 3200 (OH), 3075, 2846, 2769, 2687, 2629, 2574, 2507, 2479 (N_4H, CH-ar.), 1743, 1692, 1664 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, 2H, J = 8.3 Hz, CH-ar.), 8.09 (d, 2H, J = 8.3 Hz, CH-ar.), 11.40, 11.32 (2s, 1H, NH-BA), 11.55 (s, 1H, NH-BA), 14.10 (s, 1H, NH/OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 117.2 (CH-ar.), 119.0 (C-BA), 121.1 (C-ar.), 128.4 (CH-ar.), 143.5 (C-ar.), 149.7 (C-tetrazole), 154.9 (CO), 159.7 (CO), 161.9 (CO); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 292, 330 nm, (1.293, 0.870, up to 3.5 $\times$ $10^{-5}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 333 nm, (1.852).

3.7.2. (*E*)-5-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (6f)

Yellow solid, mp 108–110 °C; FT IR (KBr) 3430 (OH/NH), 3093, 3065, 2961, 2929, 2762, 2500 (N_4H, CH-ar.), 1724, 1676, 1645 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.24 (s, 6H, $2NCH_3$), 7.82 (d, 2H, J = 8.5 Hz, CH-ar.), 8.12 (d, 2H, J = 8.5 Hz, CH-ar.), 14.16 (s, 1H, NH/OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.3 (NCH_3), 28.2 (NCH_3), 117.4 (CH-ar.), 118.5 (C-BA), 121.9 (C-ar.), 128.4 (CH-ar.), 134.0 (C-ar.), 143.4 (C-tetrazole), 150.6 (CO), 158.6 (CO), 160.3 (CO); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 317, 408 nm, (0.517, 0.200, up to 3.0 $\times$ $10^{-5}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 391 nm, (1.662).

3.7.3. (*E*)-5-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)-2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)-dione (6g)

Yellow solid, mp 178–180 °C; FT IR (KBr) 3463 (OH/NH), 3328, 3212, 3152, 2923, 2875, 2769, 2725, 2685, 2626, 2569, 2530 (N_4H, CH-ar.), 1682, 1664 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, 2H, J = 8.6 Hz, CH-ar.), 8.12 (d, 2H, J = 8.6 Hz, CH-ar.), 12.50 (s, 1H, NH), 12.66 (s, 1H, NH), 14.18 (s, 1H, NH/OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 117.7 (CH-ar.), 119.9 (C-TBA), 121.5 (C-ar.), 128.5 (CH-ar.), 143.4 (C-ar.), 155.0 (C-tetrazole), 158.3 (CO), 159.9 (CO), 177.7 (CS); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 329, 366, 402, 465 nm, (1.911, 2.077, 1.720, 1.804, up to 2.5 $\times$ $10^{-4}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 337, 366, 402, 455 nm, (1.660, 2.157, 1.728, 1.803).

3.7.4. (*E*)-5-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)-1,3-diethyl-2-thioxo-dihydropyrimidine-4,6(1*H*,5*H*)-dione (6h)

Yellow solid, mp 241–243 °C; FT IR (KBr) 3441 (OH/NH), 3167, 3101, 2979, 2934, 2872, 2759, 2618, 2463 (N_4H, CH-ar.), 1702, 1646 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.17 (t, 6H, J = 7.3 Hz, $2NCH_2C\mathbf{H}_3$), 4.42 (q, 4H, J = 7.3 Hz, $2NC\mathbf{H}_2CH_3$), 7.90 (d, 2H, J = 7.5 Hz, CH-ar.), 8.13 (d, 2H, J = 7.5 Hz, CH-ar.), 14.31 (bs, 1H, NH/OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.1 ($2NCH_2\mathbf{C}H_3$), 35.3 ($2N\mathbf{C}H_2CH_3$), 112.0 (C-DETBA), 118.0 (CH-ar.), 119.4 (C-ar.), 120.1 (C-ar.), 128.5 (CH-ar.), 150.5 (C-tetrazole), 160 (CO), 164.0 (CO), 177.9 (CS); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 329, 360, 401, 456 nm, (1.975, 1.847, 1.811,

1.795, up to 3.2 $\times$ $10^{-4}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 342, 366, 402, 462 nm, (1.790, 1.948, 1.728, 1.803, up to 1.1 $\times$ $10^{-4}M$).

3.7.5. (*E*)-2-((4-(1*H*-tetrazol-5-yl)phenyl)diazenyl)-5,5-dimethylcyclohexane-1,3-dione (6i)

Yellow solid, mp 240–242 °C (decomp.); FT IR (KBr) 3435 (OH/NH), 3094, 2956, 2929, 2871, 2714, 2602, 2553, 2490, 2322 (N_4H, CH-ar.), 1673 (C=O), 1616 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (s, 6H, –C(C**H**$_3$)$_2$–), 2.59 (s, 2H, –C**H**$_2$–), 2.67 (s, 2H, –C**H**$_2$–), 3.40 (bs, 1H, NH-tetrazole, overlapped with the DMSO's water peak), 7.81 (d, 2H, J = 8.4 Hz, CH-ar.), 8.10 (d, 2H, J = 8.4 Hz, CH-ar.), 14.75 (s, 1H, NH/OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.0 (–C(**C**H$_3$)$_2$–), 30.2 (–**C**(CH$_3$)$_2$–), 51.8 (–**C**H$_2$–), 52.0 (–**C**H$_2$–), 117.7 (CH-ar.), 121.5 (C-ar.), 128.4 (CH-ar.), 130.8 (C-ar.), 143.7 (C-ar.), 154.9 (C-tetrazole), 192.8 (C=O), 197.2 (C=O); UV-visible data (EtOH): λ_{max}, (log ε_{max}) = 288, 365, 397, 431 nm, (0.820, 2.979, 1.949, 1.816, up to 2.5 $\times$ $10^{-4}M$); UV-visible data (acetone): λ_{max}, (log ε_{max}) = 366, 402, 421 nm, (2.971, 1.751, 1.793).

3.8. Biology

3.8.1. Materials and methods

3.8.2. Bacterial strains

The antibacterial activity of the synthesized compounds was tested against the gram-positive and gram-negative bacterial strains *Acinetobacter calcoaceticus* (ATCC23055), *Escherichia coli* (ATCC2592), *Pseudomonas aeruginosa* (ATCC27853), and *Staphylococcus aureus* (ATCC25923).

3.8.3. Preparation of the test compound and antibacterial activity assays

The antibacterial activity of the compounds was assayed by Parekh et al.'s[40] method with some modifications. In brief, solutions with 100 μg/μL concentrations of each compound in DMSO (Merck) were prepared. A full loop of the defined strain was inoculated in 25 mL of nutrient broth medium and incubated for 24 h in 37 °C. Mueller Hinton agar (MHA) (Merck) plates were prepared according to the manufacturer's recommendations by dissolving 34 g of the medium in 1000 mL of distilled water. Then 30 mL of autoclaved media were added to a 10-cm plate. Inoculation of each strain was done by the pour-plate method. Next 200 μL of the activated strain was added to the MHA medium at 45 °C and after proper homogenization was distributed into a petri dish. The complete microbiological procedures were performed in a laminar airflow in order to maintain aseptic conditions. After solidification of the media, a well was made in the MHA with a sterile glass tube (6 mm) and 50 μL of the drug compound was added to the well. Then 50 μL of DMSO was inoculated into another well as a negative control. The antibacterial activities of the drug compounds were determined by measuring the inhibition zone formed around each well against the defined bacterial strain. Erythromycin and cephalothin were used as standard drugs for antibacterial effects against gram-positive bacteria while ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin were used against gram-negative bacteria and imipeneme was used for *P. aeruginosa*.

4. Conclusion

In summary, new series of tetrazolic azo dyes based on (thio)barbiturates and electron-rich aromatics were synthesized and their structures were characterized employing spectroscopic techniques. Their antimicrobial properties were evaluated on gram-positive and gram-negative bacterial strains in detail.

Acknowledgments

We gratefully acknowledge financial support from the Research Council of Urmia University and Urmia University of Medical Sciences.

Supplementary material

Full characterization data of compounds **6a**–**6i** and antibacterial activity assays are available.

References

1. Bailey, K.; Cowling, R.; Tan, E. W.; Webb, D. *Bioorg. Med. Chem.* **2004**, *12*, 595–601.
2. Hamidian, H.; Tagizadeh, R.; Fozooni, S.; Abbasalipour, V.; Taheri, A.; Namjou, M. *Bioorg. Med. Chem.* **2013**, *21*, 2088–2092.
3. Carta, F.; Maresca, A.; Scozzafava, A.; Vullo, D.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 7093–7099.
4. Farghaly, T. A.; Abdalla, M. M. *Bioorg. Med. Chem.* **2009**, *17*, 8012–8019.
5. El-Shafei, A.; Fadda, A. A.; Khalil, A. M.; Ameen, T. A. E.; Badria, F. A. *Bioorg. Med. Chem.* **2009**, *17*, 5096–5105.
6. Tonelli, M.; Boido, V.; Canu, C.; Sparatore, A.; Sparatore, F.; Paneni, M. S.; Fermeglia, M.; Pricl, S.; Colla, P. L.; Casula, L.; et al. *Bioorg. Med. Chem.* **2008**, *16*, 8447–8465.
7. Tonelli, M.; Vazzana, I.; Tasso, B.; Boido, V.; Sparatore, F.; Fermeglia, M.; Paneni, M. S.; Posocco, P.; Pricl, S.; Colla, P. L.; et al. *Bioorg. Med. Chem.* **2009**, *17*, 4425–4440.
8. Prinz, H.; Schmidt, P.; Böhm, K. J.; Baasner, S.; Müller, K.; Gerlach, M.; Günther, E. G.; Unger, E. *Bioorg. Med. Chem.* **2011**, *19*, 4183–4191.
9. Song, Y. M.; Ha, Y. M.; Kim, J. A.; Chung, K. W.; Uehara, Y.; Lee, K. J.; Chun, P.; Byun, Y.; Chung, H. Y.; Moon, H. R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7451–7455.
10. Xu, H.; Zeng, X. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4193–4195.
11. Maresca, A.; Carta, F.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4929–4932.
12. Lin, S. J.; Shiao, Y. J.; Chi, C. W.; Yang, L. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1173–1176.
13. Singh, H.; Chawla, A. S.; Kapoor, V. K.; Paul, D.; Malhotra, R. K. *Prog. Med. Chem.* **1980**, *17*, 151–183.
14. Potewar, T. M.; Siddiqui, S. A.; Lahoti, R. J.; Srinivasan, K. V. *Tetrahedron Lett.* **2007**, *48*, 1721–1724.
15. Herr, R. J. *Bioorg. Med. Chem.* **2002**, *10*, 3379–3393.
16. Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; et al. *J. Med. Chem.* **2000**, *43*, 953–970.
17. Rostom, S. A. F.; Ashour, H. M. A.; Abd El Razik, H. A.; Abd El Fattah, A. E. H.; El-Din N. N. *Bioorg. Med. Chem.* **2009**, *17*, 2410–2422.
18. Poonian, M. S.; Nowoswiat, E. F.; Blount, J. F.; Kramer, M. J. *J. Med. Chem*. **1976**, *19*, 1017–1020.
19. Rajasekaran, A.; Thampi, P. P. *Eur. J. Med. Chem.* **2004**, *39*, 273–279.
20. Maxwell, J. R.; Wasdahl, D. A.; Wolfson, A. C.; Stenberg, V. I. *J. Med. Chem.* **1984**, *27*, 1565–1570.
21. Lee, K.-H.; Park, .-E.; Min, K.-H.; Shin, Y.-J.; Chung, C.-M.; Kim, H.-H.; Yoon, H.-J.; Kim, W.; Ryu, E.-J.; Shin, Y.-J.; et al. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5567–5571.
22. Vicini, P.; Amoretti, L.; Barocelli, E.; Chiavarini, M.; Impicciatore, M. *Farmaco* **1986**, *41*, 111–118.
23. Stewart, K. D.; Loren, S.; Frey, L.; Otis, E.; Klinghofer, V.; Hulkower, K. I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 529–534.
24. Dekhane, D. V.; Pawar, S. S.; Gupta, S.; Shingare, M. S.; Patil, C. R.; Thore, S. N. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6527–6532.

25. Pande, K.; Tandon, M.; Bhalla, T. N.; Parmar, S. S.; Barthwal, J. P. *Pharmacol.* **1987**, *35*, 333–338.
26. Terashima, K.; Tanimura, T.; Shimamura, H.; Kawase, A.; Uenishi, K.; Tanaka, Y.; Kamisaki, I.; Ishizuka, Y.; Sato, M. *Chem. Pharm. Bull.* **1995**, *43*, 1042–1044.
27. Hayao, S.; Havera, H. J.; Strycker, W. G.; Leipzig, T. J.; Rodriguez, R. *J. Med. Chem.* **1965**, *10*, 400–404.
28. Palazzi, A.; Stagni, S.; Selva, S.; Monari, M. *J. Organometall. Chem.* **2003**, *669*, 135–140.
29. Egawa, Y.; Gotoh, R.; Niina, S.; Anzai, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3789–3792.
30. Yamamoto, T.; Ikuta, K.; Oi, K.; Abe, K.; Uwatoku, T.; Hyodo, F.; Murata, M.; Shigetani, N.; Yoshimitsu, K.; Shimokawa, H.; et al. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2787–2790.
31. Zollinger, H. *Color Chemistry Synthesis, Properties and Application of Organic Dyes and Pigments*; 2nd ed., VCH: Weinheim, Germany, 1991.
32. Zollinger, H. *Color Chemistry*; 3rd ed., VCH, Weinheim, Germany, 2003.
33. Viscardi, G.; Quagliotto, P.; Barolo, C.; Caputo, G.; Digilio, G.; Degani, I.; Barni, E. *Dyes Pigm.* **2003**, *57*, 87–95.
34. Fraleoni-Morgera, A.; Della-Casa, C.; Costa-Bizzarri, P.; Lanzi, M.; Missiroli, A. *Macromolecules* **2005**, *38*, 3170–3175.
35. Pagacz-Kostrzewa, M.; Mucha, M.; Weselski, M.; Wierzejewska, M. *J. Photochem. Photobiol. A Chem.* **2013**, *251*, 118–127.
36. Noroozi Pesyan, N. *Magn. Reson. Chem.* **2011**, *49*, 592–599.
37. Noroozi Pesyan, N. *Magn. Reson. Chem.* **2009**, *47*, 953–958.
38. Noroozi-Pesyan, N.; Khalafy, J.; Malekpoor, Z. *Prog. Color Colorants Coat.* **2009**, *2*, 61–70.
39. Noroozi-Pesyan, N.; Khalafy, J.; Malekpoor, Z. *J. Chin. Chem. Soc.* **2009**, *56*, 1018–1027.
40. Parekh, J.; Inamdhar, P.; Nair, R.; Baluja, S.; Chanda, S. *J. Serb. Chem. Soc.* **2005**, *70*, 1155–1162.