



Uropathogenic *Escherichia coli* chromosomal study of *cnf1* and *fyuA* genes among patients of urinary tract infection in Kirkuk city

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Keywords

Azo- Chalcone, 2, 6-Diaminopyridine, diazonium salt, pyridine.

Abstract

The objective of this work was to prepare azo and azo-chalcones derivatives out of 2, 6- diaminopyridine (2, 6-DAP) which has extensive use in the synthesis of pharmaceutical compounds. The azo-diamine (S₁-S₆) was prepared from the coupling reaction of diazonium salt prepared from various substituted aromatic amines with 2, 6-diaminopyridine. The product (S₆) was reacted in basic condition with one equivalent of different substituted aromatic aldehydes in methanol to give ten new azo-chalcones (S₇-S₁₆) in good yields. The final products were identified by their physical properties besides their UV-visible, FTIR and 1H NMR spectra. Biological activity of the compounds (S₁-S₆) was investigated and compounds (S₁ and S₂) showed high activity against *B. subtilis* and *E. coli* .

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1. Introduction

2, 6-Diaminopyridine (2, 6-DAP) is a heterogeneous aromatic amine which has a reasonable solubility in polar solvents (acetone, methanol, ethanol, isopropanol, ethyl acetate) [1]. It is usually prepared by the amination of pyridine under severe conditions [2]. This compound found its way to application in various fields such as its usage in the synthesis of cosmetic and energetic compounds [3], and as an intermediate agent in the preparation of epoxy dryer, and as mediator in the production of polyamides [4] as well as an intermediate substance in the manufacture of analgesic phenylazo-pyridine hydrochloride[5,6]. Furthermore it was used in flurometric studies of novel photoactive polyamide [7]. Nanosheets, nanofibers and nanotubes of fatty acid derivatives of 2, 6-diaminopyridine were also synthesized [8]. Despite the presence of amino pyridine and diaminopyridine in many biological important molecules (for example folate, antifolate drugs besides cytosine derivatives) [9], the poor solubility of (2, 6-DAP) and its derivatives in water prevent them from crossing the blood brain barrier causing the limitation of their pharmaceutical applications [10]. The Structural,

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thermodynamic and chromatic characteristics of 2, 6-DAP were studied using fast gamma and neutron irradiation [10]. Moreover the photophysical behavior of 2, 6-diaminopyridine has been studied in solvents of different polarity and pH values [11]. Recently results were reported concerning the influence of biofield treatment on the physical, thermal and spectral properties of this compound [12].

Azo dyes are widely used in industry and in our daily lives, and these substances have an impact that cannot be underestimated on both of the bacterial colonies and public health [13]. The azo-Chalcone is thought to be a new class of organic compound, in which the chromatic phenyl azo group or groups, is joined with the colored chalcone part. The combination of the two biological active moieties (azo and chalcone) in a single compound is expected to increase its biological effectiveness or even to enhance its chromatic activity [14-16]. These excellent characterizations give this sort of organic compound the potential to be used, not even in pharmaceutical fields, but rather in other research areas such as electrochemistry, photochemistry and dye industry. The goal of the present study is to incorporate the azo-chalcone moieties into the structure of 2, 6-diaminopyridine as an endeavor to achieve some of the targets mentioned above.

2. Experimental Part

2.1. Methods and Equipments

The chemicals used in this study were bought from Merck, Sigma-Aldrich, and utilized without additional handling. Stuart-SMP11 was used for measuring melting points of the products. PG -92 Spectrophotometer was used to record UV-visible spectra. IR measurements were conducted using KBr discs on a Nicolet 100 FT-IR spectrophotometer. ^1H NMR spectra sheets were recorded as solutions in DMSO on a 500 MHz Bruker, and C^{13} -NMR spectra on 300 MHz, TMS was hold as reference for chemical shifts.

2.2. Synthesis of azo-2, 6-DAP (S_1 - S_6) [17]

(0.01 mol) of substituted aromatic amines was dissolved in (9 mL) of cooled acidic solution prepared by mixing (6 mL) of distilled water with (3 mL) of concentrated hydrochloric acid. To this solution (10 mL) of 1M sodium nitrite solution was added slowly in portions, keeping the temperature range at (0-5) °C. The prepared diazonium salt solution was added in small batches with continuous stirring to another cooled solution at 0 °C prepared by dissolving (0.01 mol) of 2,6-diaminopyridine in (50 mL), (1M) sodium hydroxide maintaining the temperature at range (0- 5) °C. The stirred reaction mixture was left after completing the addition for one hour in ice bath and another hour at room temperature. The precipitate formed, was washed with distilled water, dried and recrystallized from ethanol.

3-(phenyldiazenyl)pyridine-2,6-diamine(S_1): m.p. 73-75 °C, 78%; IR (KBr, Cm^{-1}): 3414 (N-H, asy, sym), 3010 (C-H Ar), 1600 (C=C Ar), 1411 (N=N). λ_{max} EtOH (nm): 241, 316, 398.

3-(p-tolyldiazenyl) pyridine-2,6-diamine(S_2): m.p. 148-150 °C, 82%; IR (KBr, Cm^{-1}): 3413, 3360 (N-H, asy, sym), 3010 (C-H Ar), 1585 (C=C Ar), 1417 (N=N). λ_{max}

EtOH (nm): 240, 313, 398, ¹H-NMR (DMSO): δ = 2.3 (s, 3 H, CH₃), δ = 5.9-7.2 (d, 1 H, pyridine), δ = 6.6 (s, 4 H, NH₂), δ = 5.9-7.6 (m, 4 H, Ar-H).

3-((4-chlorophenyl)diazenyl)pyridine-2,6-diamine(**S₃**): m.p. 178-180°C, 82%; IR (KBr, Cm⁻¹): 3376,3325 (N-H, asy, sym), 3163 (C-H aromatic),1581 (C=C Ar), 1438 (N=N),772(C-Cl). λ_{max} EtOH (nm): 237, 314, 399.

3-((4-bromophenyl) diazenyl)pyridine-2,6-diamine(**S₄**): m.p. 166-168°C, 80%; IR (KBr, Cm⁻¹): 3375 (N-H, asy, sym), 3168 (C-H Ar), 1583 (C=C Ar), 1437 (N=N), 582(C-Br). λ_{max} EtOH (nm): 239, 298, 401.

3-((4-nitrophenyl)diazenyl)pyridine-2,6-diamine(**S₅**): m.p. 250-252 °C, 88%; IR (KBr, Cm⁻¹):3300,3358 (N-H, asy, sym), 3140 (C-H Ar), 1585 (C=C Ar), 1425 (N=N). λ_{max} EtOH (nm): 238, 313, 409.

1-(4-((2,6-diaminopyridine-3-yl) diazenyl) phenyl)ethan-1-one(**S₆**) : m.p. 257-259 °C, (65%) ; IR (KBr, Cm⁻¹): 3400, 3359 (N-H, asy, sym), 3149 (C-H Ar), 1679(C=O), 1589,1494 (C=C Ar), 1415 (N=N). λ_{max} EtOH (nm): 240, 312, 410.

2.3. Synthesis of azo-chalcone (**S₇-S₁₆**) [18]

(0.01 mol, 0.25 g) of the compound (**S₆**) was dissolved in (10 mL) of methanol, the resulting solution was added to (0.01 mol) of the appropriate substituted aromatic aldehyde dissolved in (10 mL) of methanol. (4 mL of 10% NaOH solution) was added to this solution and stirred for 24 hours. The reaction mixture was poured into a small amount of icy water and left in the fridge for one hour. The resulting precipitate was filtrated, washed with a quantity of cold distilled water and left to dry, and then recrystallized from the ethanol.

Note: At salicyaldehyde case, the reaction mixture is heated in a water bath for one hour, before stirring for 24 hours.

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-phenylprop-2-en-1-one (S₇**):** m.p. >252 °C, 71%; IR (KBr, Cm⁻¹):3404 (N-H, asy, sym), 3151 (C-H Ar),1677 (C=O), 1589 (C=C olef.),1456 (C=C Ar), 1415 (N=N). λ_{max} EtOH (nm): 212, 286, 460.

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(2-hydroxyphenyl) prop-2-en-1-one (S₈**):** m. p. 204 dec.°C, 63%; IR (KBr, Cm⁻¹): 3392(N-H, asy, sym), 3153 (C-H Ar),1679 (C=O),1589(C=C olef.), 1415 (N=N). λ_{max} EtOH (nm): 216, 284, 460.

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one (S₉**):** m.p. 200 dec.°C, 74%; IR (KBr, Cm⁻¹): 3494 (N-H, asy, sym), 3153(C-H Ar),1679 (C=O),1589(C=C olef.),1458 (C=C Ar), 1415 (N=N). λ_{max} EtOH (nm): 214, 242 sh, 284, 460.

3-(2-chlorophenyl)-1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)prop-2-en-1-one (S₁₀**):** m.p. >252 °C, 73%; IR (KBr, Cm⁻¹): 3404 (N-H, asy, sym), 3151 (C-H Ar),1676 (C=O),1589(C=C olef.),1456 (C=C Ar), 1420 (N=N). λ_{max} EtOH (nm): 202, 288, 458.

¹H-NMR (DMSO): δ = 8.2 (d, 1 H, α CH=), δ = 7.5 (d, 1 H, β =CH), δ = 7.1-8.0 (m, 8 H, Ar-H), δ = 6.0-7.7 (d, 2 H, pyridin-H), δ = 6.9 (s, 4 H, NH₂).

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (S₁₁): m.p. 210 dec. °C, 70%; IR (KBr, Cm⁻¹) 3402 (N-H, asy, sym), 3148 (C-H Ar), 1677 (C=O), 1591 (C=C olef.), 1458 (C=C Ar), 1425 (N=N) 802 (C-Cl). λ_{max} EtOH (nm): 213, 286, 459.

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one (S₁₂): m.p. 200 dec. °C, 70%; IR (KBr, Cm⁻¹): 339 (N-H, asy, sym), 3151 (C-H Ar), 1697 (C=O), 1591 (C=C olef.), 1458 (C=C Ar), 1415 (N=N). λ_{max} EtOH (nm): 210, 288, 461.

(1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (S₁₃): m.p. 212 dec. °C, 76%; IR (KBr, Cm⁻¹): 3399 (N-H, asy, sym), 3148 (C-H Ar), 1678 (C=O), 1590 (C=C olef.), 1456 (C=C Ar), 1417 (N=N). λ_{max} EtOH (nm): 212, 286, 457. ¹H- NMR (DMSO): δ= 2.5 (s, 3 H, OCH₃), δ= 7.79 (d, 1 H, α CH=), δ= 6.0 (d, 1 H, β =CH), δ= 7.5-7.7 (m, 7 H, Ar-H), δ= 7.6-7.79 (d, 2 H, pyridin-H), δ= 6.9 (s, 4 H, NH₂).

1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (S₁₄): m.p. 200 dec. °C, 78%; IR (KBr, Cm⁻¹): 3398 (N-H, asy, sym), 3151 (C-H Ar), 1679 (C=O), 1589 (C=C olef.), 1458 (C=C Ar), 1416 (N=N). λ_{max} EtOH (nm): 208, 289, 458.

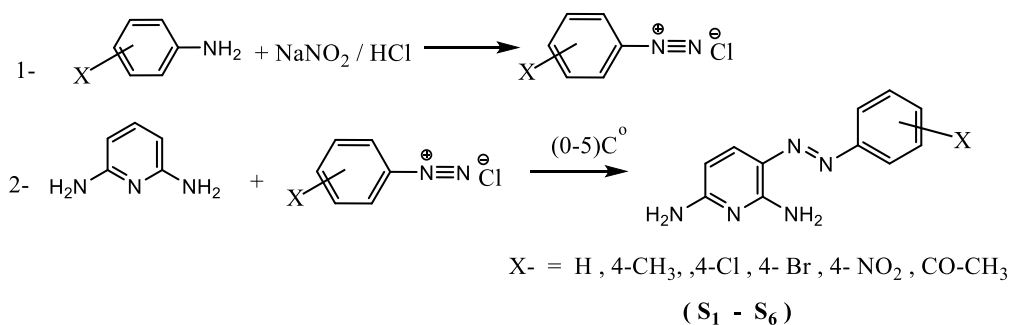
(1-(4-((2,6-diaminopyridin-3-yl)diazenyl)phenyl)-3-(furan-2-yl)prop-2-en-1-one (S₁₅): m.p. 215 dec. °C, 75%; IR (KBr, Cm⁻¹): 3397 (N-H, asy, sym), 3151 (C-H Ar), 1676 (C=O), 1590 (C=C olef.), 1455 (C=C Ar), 1420 (N=N). λ_{max} EtOH (nm): 211, 285, 459.

1-(4-((2,6-diaminopyridin-3-yl)diazenyl) phenyl)-3-(thiophen-2-yl)prop-2-en-1-one (S₁₆): m.p. 200 dec. °C, 71%; IR (KBr, Cm⁻¹): 3394 (N-H, asy, sym), 3137 (C-H Ar), 1679 (C=O), 1589 (C=C olef.), 1456 (C=C Ar), 1420 (N=N). λ_{max} EtOH (nm): 213, 287, 461.

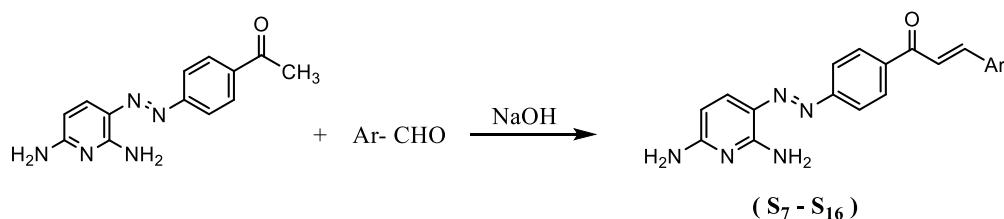
3. Results and Discussions

Coupling of diazonium salts prepared from various substituted aromatic amines (shown in the scheme below) with (2, 6-DAP) were held to synthesize the azo derivatives in the active position 3 of the pyridine ring (S₁-S₆), scheme 1. The compound (S₆), derived from 4-aminoacetophenone, was then reacted in the second step with different substituted aromatic aldehydes in basic condition/MeOH to prepare the azo chalcones (S₇-S₁₆), in good yields, following the traditional way in synthesizing chalcones, scheme 2.

scheme -1- synthesis of azo compound



Scheme -2- synthesis of azo-chalcone



Ar ; C₆H₅ , 2-Cl-C₆H₄ , 2-HO-C₆H₄ , 4-HO-C₆H₄ , 4-CH₃O-C₆H₄ , 4-(CH₃)₂N-C₆H₄ , 4-HO-3-CH₃O-C₆H₃ , 4-NO₂-C₆H₄ , furfuryl , thiophenyl

4. UV-Visible Spectra

The electronic absorption spectra of the azo-(2, 6-DAP),(S₁-S₆) in ethanol solution (10⁻⁴ M) showed intense UV-visible absorption bands in the region in the range of (237-241) nm, assigned to well-known moderate π-π* electronic transition belonging to the aromatic rings[19]. The band at (298-316) nm as a shoulder is due to low energy (π-π*) of N=N group [20, 21], while the third peak at (398-410) nm is assigned to n-π* transition of nitrogen atoms of azo chromophore groups [17]. Assuming that mixing of the forbidden, low (n-π*) frequency band with the stronger; low frequency band responsible for the vivid colors in the azo dyes, may provide an explanation for the intensity of the resultant band [22]. In all the above transitions the position of the mentioned bands was potently influenced by the electronic character and positions of the substituents. In addition, n-π* transition in the azo derivatives is shown to be, in every cases, the low-frequency constituent of the two formerly degenerate n-π* bands which have been split [22, 23]. Almost similar pattern and trend were seen in azo-chalcone compounds (S₇-S₁₆) in which the conjugation pathway within the molecule was further extended by the association of (CO-C=C) to the π-conjugated system resulting in a red-shift of (π-π*) of the traditional band I of the chalcone moiety at (284-288) nm hiding the forbidden (n- π*) transition of the azo chromophore. The less intense Band II of the chalcone part which appears at (202-216) as single peak relating to (π-π*) transition appeared relatively unaffected [24]. The third band which appeared at

(458-460) nm is related to the charge transfer between aromatic rings via the azo group [25].

5. FT-IR Spectra

In Infrared spectra of the compounds (S_1 - S_6) the two bands related to the asymmetric and symmetric stretching vibrations of NH_2 groups appeared at the ranges $(3358-3414) \text{ cm}^{-1}$, and $(3300-3360) \text{ cm}^{-1}$ respectively, whereas single band was registered in the case of (S_1, S_4, S_7) due to the amine group, compatible with the spectrum of the original compound reported at least in one study [26]. The broad band shown at the range of $(3140-3168) \text{ cm}^{-1}$ of all the synthesized azo-diamine compounds (S_1 - S_6) is ascribed mainly to the stretching vibration of $(-CH=)$ belonging to the heterogeneous pyridine ring compared to the band absorbed at (3122 cm^{-1}) for (2, 6-diaminopyridine) [27]. Moreover, medium intensity bands at the wavenumbers $(3202) \text{ cm}^{-1}$, $(3209) \text{ cm}^{-1}$, $(3235) \text{ cm}^{-1}$ of the compounds (S_1, S_7 and S_{10}) respectively which was previously referred to the dimer frequency of the (2, 6-DAP) recorded in the infrared spectra for their solid phases. Various unequal absorption bands were recorded at the range $(1625-1655) \text{ cm}^{-1}$ which are ascribed to the first band of stretching vibration of heterogeneous pyridine ring $\{C=C\}$, $\{C=N\}$ [26,28] compared with a medium intensity band at (1639 cm^{-1}) of (2,6-DAP), indicating that this vibration was affected by the nature of the various substituents on the heterogeneous pyridine ring [19]. The band that was observed at the range $(1581-1600) \text{ cm}^{-1}$, is attributed to the stretching vibration of $(C=C)$ bond of the substituted aromatic ring attached to azo group. Some of the studies concerning (2, 6-diaminopyridine) ascribed the frequencies recorded at $(1603-1605) \text{ cm}^{-1}$ [27, 29] and those absorbed at $(1595) \text{ cm}^{-1}$ to the bending vibration of the $(N-H)$ group [26]. But we think that there are some cases in which the bending vibration of $(N-H)$ may interfere or overlap with the absorption due to $(C=C)$ of the aromatic ring as in the case of compounds (S_3 - S_5). The strength of these bands could be a proof of what we claim, as the strength of the first vibration band of the ring at this site $(1598-1600) \text{ cm}^{-1}$ is usually moderate compared to the strongest band at (1500 cm^{-1}) . The absorption of the contradictory azo group, in its position and intensity, was identified as band with low intensity at the range of $(1400-1438) \text{ cm}^{-1}$ for the compounds (S_1 - S_6) depending on the nature of the substituted groups at terminal aromatic ring. For the azo-chalcones (S_7 - S_{16}) splitting of the band due to the stretching vibration of $(N-H)$ of both of the amine groups of (2, 6-DAP) at the range $(3390-3400) \text{ cm}^{-1}$ was not identified except in the case of the compounds S_{12} and S_{14} . Instead of that a broad band attributed to the asymmetric vibration $(N-H)$ was seen, probably, hiding the asymmetric one beneath it, to be seen finally, as a weak shoulder at a position less than that range by $(80-100) \text{ cm}^{-1}$. Another broad band was shown up in the range of $(3137-3153) \text{ cm}^{-1}$ belonging to the absorption of $(=C-H)$ of the pyridine ring, covering in general, both of the $(=C-H)$ of the two aromatic rings in addition to the olefinic $(C-H)$ of the chalcone part which is expected to appear as a weak band in the range of $(3000-3100) \text{ cm}^{-1}$. The carbonyl group of the chalcone appeared in the expected range at $(1676-1679) \text{ cm}^{-1}$ as medium-to-strong intensity consistent in its position with what is mentioned in literature [16, 18]. The stretching vibration of the olefinic double bond conjugated with the carbonyl group [171], was recorded as medium to strong intensity band

at the range of (1589-1591) cm^{-1} , whereas the vibration of the heterogeneous pyridine ring pattern at (1645-1647) cm^{-1} was observed as low-intensity bands.

6. NMR spectra

The aromatic protons of benzene rings appeared as multiple signal at δ (5.9-7.2) ppm for S_2 interfering with two left pyridine protons recorded as doublet at the range δ (5.9-7.2) ppm. While the aromatic protons of S_{10} was identified as multiple signal at δ (7.1-8) ppm and pyridine ring protons was shown as doublet at δ (6-7.7) ppm. The aromatic ring of S_{13} appeared at δ (7.5-7.7) ppm along with the protons of pyridine ring which appeared at δ (7.6-7.8) ppm. The four protons of the two amino groups of pyridine ring appeared as single signal at 6.6ppm for S_2 , δ 6.9 ppm for S_{10} and δ 7 ppm for S_{13} . The three protons of methyl of acetyl group in S_2 appeared as single signal at δ 2.3ppm and single one at δ 2.5 ppm ascribed to methoxy group of S_{13} . The α , β protons of chalcones appeared as two doublet signal, the first one at δ 8.2 ppm due to the α proton and the other at δ 7.5 ppm due to the β proton in the case of S_{10} , whereas the analogues two α , β protons in the case of S_{13} appeared at δ 7.8 ppm and δ 6.9 ppm respectively.

7. Microbiological investigations

The synthesized compounds were screened for their *in vitro* antimicrobial activity against Gram (+) strain, *Bacillus subtilis* and Gram (-ve) strain, *Escherichia coli* using the Agar dilution method, DMSO as solvent and control [31]. The zone of inhibition around the disc was measured (in mm) as explained beneath the Table 1. The examination of antimicrobial activity of the synthesized compounds reveal that compound S_1 has high activity against the two types of bacteria; with moderate activity towards the Gram positive bacteria and high activity towards the Gram negative one for compound S_2 . But none of the other compounds (S_3 - S_6) had any antibacterial activities when compared with the control. The anti-bacterial activity of the azo-(2, 6-DAP) S_1 and S_2 might be due to the effect of the biodegradation product of the azo group in both of the compounds (the biological active aromatic amine in the case of S_1 and chloro-substituted aromatic amine in the case of S_2), which might be easily liberated due to the action of the bacterial enzymes [13].

Table 1: Antibacterial activity data of the synthesized compounds (S_1 - S_6)

Compd. No.	B. subtilis G + ve	E. coli G - ve
S_1	+++	+++
S_2	++	+++
S_3	-	-
S_4	-	-
S_5	-	-
S_6	-	-

Key to interpretation: Less than 10 mm=inactive (-), 10-15 mm=slightly active (+), 15-20 mm= moderately active (++) , more than 20 mm=highly active (+++).

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