



## Evaluation of the Dyeing Performance and Antimicrobial Activity of Dyed Wool Fabric with New Azo Dyes

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### Abstract

A series of novel mono and diazothienopyrazolone dyes were successfully synthesized and characterized, the coloration performance of the prepared dyes was assessed on wool fabrics. The structures of the synthesized dyes were characterized and confirmed by making use of IR, UV, <sup>1</sup>HNMR and <sup>13</sup>CNMR spectrophotometers with a view to determine and confirm their chemical structure. Antimicrobial activity of the dyes fabrics has been investigated, the dyeing performance of the as prepared dyes in terms of color strength (K/S), CIE lab as well as fastness properties (washing, rubbing, perspiration and light) of the dyed wool fabrics were also investigated. The synthesized dyes exhibited high and homogeneous dyeing performance, antimicrobial activity and the fastness properties of the dyed fabrics were ranging from good to excellent.

Keywords: Dyeing, Antimicrobial activity, Azo Dyes, UV-Visible Spectra, Fastness Properties, Textile Dyeing, and Wool Fiber.

### 1. Introduction

Azo dyes and its derivative compounds considered the most widely used class of organic dyes due to their various industrial applications such as paper, leather and textile coloration <sup>[1-4]</sup>.

Dye chemistry extensively using variety of heterocyclic compounds which being mainly used in textile applications. Recently dyes are now produced in a full range of colorants with no hetero aromatic diazo based colorants. Heterocyclic-based dyes are mainly derived from the diazo compounds containing five-member rings with one or more nitrogen heteroatoms, and then the rings are being fused to obtain another aromatic ring <sup>[5,6]</sup>.

Azo dyes have shown high stability and good staining properties. Azo dyes exhibit a full range of high-intensity and bright colors with moderate to good fastness properties, making it economically covering for the usage of more than half of all commercial dyes.

Azo dyes cover a wide application in dyeing of proteinic fibers such as wool, silk cashmere, and angora <sup>[7]</sup>.

Wool fibers are attached to the dyes through electrostatic bonds between the protonated cationic amino groups of the wool (-NH<sub>2</sub>) and the sulphonate anionic groups of the dye (Dye-O-)<sup>[8]</sup>. The structure effect on the alkali stability of the prepared dyes is extensively investigated at various alkali concentrations by comparing K/S, L\*, a\*, b\* and C\* for the dyed fabrics <sup>[9]</sup>.

Nowadays antimicrobial textiles demands are increasing dramatically and searching for a multiple antimicrobial agent attracts scientist worldwide <sup>[10,11]</sup>. Thiophene nucleus is regarded as a very important antimicrobial agent in drug discovery <sup>[12]</sup>.

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## 2. Experimental

### 2.1. Synthesis

All melting points were measured on an electric melting point apparatus were uncorrected. The infrared spectra were recorded using potassium bromide disks on a Pye Unicam SP-3-300 infrared spectrophotometer. The  $^1\text{H-NMR}$  and  $^{13}\text{C}$  NMR spectra were measured on Bruker WP AC 300 (300 MHz) in  $\text{DMSO-d}_6$  as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as  $\delta$  in ppm. Mass spectra were determined on Finnigan Incos 500 (70 e V). Elemental analyses were carried out at the Micro analytical Centre, Faculty of Science, Cairo University, Egypt. The micro analytical data were measured in Central Lab of Cairo University, Egypt; and the Ministry of Defense Chemical Laboratories, Egypt. All the chemical reactions were monitored by TLC. Solvents and reagents were obtained from commercial sources and without purification. Column chromatography was carried out on silica gel (300e400mm) the eluent according to suitable compound.

#### 2.1.1. 2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (1)

This compound obtained via Gewald reaction of malononitrile with Cyclopentanone and elemental sulfur according to literature [13].

#### 2.1.2. 2-((3-Amino-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazanyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile(4)

Dissolve (1.64g, 0.01 mol) of amino thiophene derivative **1** in 20 ml of HCl solution (10%) then added 30 ml (0.01 mol) of sodium nitrite solution, stirring for one hour in ice path at temperature (0-5°C) to form the corresponding diazonium salt (2). Dissolve (0.01 mol) of 5-amino-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (3) in 10 ml NaOH (10%) the solution was cooled and kept the temperature between 0-5°C for 45 minutes, the diazonium salt (2) was added drop wise in a period of half hour, stirring well for 4 hours in ice bath, filtered off the solid obtained, crystallized with ethanol: dioxin (1:3) to obtain dark brown crystals, Yield: 3.08 g (88%); m.p: above 300°C. IR spectrum (KBr)  $\text{cm}^{-1}$  showed three absorption bands at: 3424  $\text{cm}^{-1}$  broad for  $\text{NH}_2$ , 2221  $\text{cm}^{-1}$  for CN, 1640  $\text{cm}^{-1}$  amide C=O.  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ): the chemical shift  $\delta$  (ppm) 2.12 (qui, 2H,  $\text{CH}_2$ ), 2.66 (t, 2H,  $\text{CH}_2$ ), 3.05 (t, 2H,  $\text{CH}_2$ ), 4.26 (s, 1H, CH-N=N), 6.38 (s, 2H,  $\text{NH}_2$ ), 7.28 (t, 1H, aromatic H), 7.65 (t, J=8.3Hz, 2H, aromatic H), 7.99

(d, J=8.3Hz, 2H, aromatic H).  $^{13}\text{C-NMR}$ (DMSO) 25.89,27.91,34.85 (3C of cyclopentano nucleus), 67.02 (CH-N=N), 99.03 (C $\equiv$ N), 115.44, 124.43, 128.03, 129.51 (4C of thiophene ring); 134.00, 135.50, 139.42, 142.11 (4C of benzene ring); 154.90 (C=N), 169.93 (C=O). MS showed the parent peak at m/z (% abundance) 350 (15) [ $\text{M}^+$ ]. Anal. for  $\text{C}_{17}\text{H}_{14}\text{N}_6\text{OS}$  (350.09). Calculated C, 58.27; H, 4.03; N, 23.98; Found: C, 58.53; H, 4.24; N, 23.74.

### 2.2. General procedure for Synthesis of diazo dyes 6-10

Dissolve (3.5g,0.01 mol) of 2-((3-amino-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazanyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (**4**) in 20 ml of HCl solution (10%) then added (0.01 mol) of sodium nitrite, stirring for one hour in ice path to form the corresponding diazonium salt **5**. Dissolve (0.01 mol) of salicylic acid, resorcinol, 4,5-dihydroxynaphthalene-2,7-disulfonic acid di salt, 2-naphthol 10 ml NaOH (10%) and/or (0.01 mol) of N, N Dimethyl aniline in 10 ml HCl (10%) the solution was cooled and kept the temperature between 0-5°C for 45 minutes, the diazonium salt (5) was added drop wise in a period of half hour, stirring well for 4 hours in ice bath, filtered off the solid obtained, washed by water three time (50 mL) to gives the corresponding diazo dyes **6-10**.

#### 2.2.1. 5-((4-((3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)diazanyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)diazanyl)-2-hydroxybenzoic acid(6)

Crystallize with ethanol: dioxin (1:3) to obtain brown crystals, Yield: 3.59 g (72%); m.p. above 300°C. IR spectrum (KBr)  $\text{cm}^{-1}$  showed absorption bands at : broad band 3400-2500  $\text{cm}^{-1}$  include sharp band at 3235  $\text{cm}^{-1}$  for free OH of phenol, 2210  $\text{cm}^{-1}$  for CN, 1658  $\text{cm}^{-1}$  for C=O acid, 1612  $\text{cm}^{-1}$  for C=O amide.  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ): the chemical shift  $\delta$  (ppm) 2.17 (qui, 2H,  $\text{CH}_2$ ), 2.72 (t, 2H,  $\text{CH}_2$ ), 3.06 (t, 2H,  $\text{CH}_2$ ), 4.28 (s, 1H, CH), 6.88 (d, J= 8.3Hz, 1H, aromatic H), 7.28 (t, 1H, aromatic H), 7.48 (d, J= 8.3Hz, 1H, aromatic H), 7.59 (t, 2H, aromatic H), 7.89 (s, 1H, aromatic H), 7.99 (d, J= 8.2Hz, 2H, aromatic H), 9.83 (s, 1H, OH of phenol), 11.78 (s, 1H, OH of acid).  $^{13}\text{C-NMR}$ (DMSO) 23.05, 25.68, 32.32 (3C of cyclopentano nucleus); 66.92 ((CH-N=N)); 101.32 (C $\equiv$ N); 122.51, 126.98, 127.89, 128.92; 113.25, 115.05, 118.14 (4C of thiophene ring) 132.05, 133.41, 137.87, 141.05 (aromatic C of benzene ring); 156.32 (C=N);

167.91 (C-OH phenolic); 171.03 (C=O of amide); 172.16 (C=O of acid). MS showed the parent peak at  $m/z$  (% abundance) 499 (18)  $[M^+]$ . *Anal.* for  $C_{24}H_{17}N_7O_4S$  (499.11). Calculated: C, 57.71; H, 3.43; N, 19.63; Found: C, 57.93; H, 3.64; N, 19.49.

2.2.2. 2-((3-((2,4-dihydroxyphenyl)diazanyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazanyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile(7)

Crystallize from ethanol: dioxin (1:3) to obtain red crystals, Yield: 3.7 g (78%); m.p. above 300°C. IR spectrum (KBr)  $cm^{-1}$  showed three absorption bands at: 3122  $cm^{-1}$  for free OH of phenol, 2222  $cm^{-1}$  of CN, 1651  $cm^{-1}$  (C=O amide).  $^1H$ -NMR (DMSO- $d_6$ ): the chemical shift  $\delta$  (ppm) 2.168 (qui, 2H,  $CH_2$ ), 2.723 (t, 2H,  $CH_2$ ), 3.05 (t, 2H,  $CH_2$ ), 4.28 (s, 1H, CH), 6.503 (s, 1H, aromatic H), 6.60 (s, 1H, aromatic H), 6.68 (d,  $J=7.9$ Hz, 1H, aromatic H), 7.14 (d,  $J=7.9$ Hz, 1H, aromatic H), 7.40 (t, 1H, aromatic H), 7.58 (t, 2H, aromatic H), 7.97 (d,  $J=8.1$ Hz, 2H, aromatic H), 9.99 (s, 1H, OH of phenol), 10.03 (s, 1H, OH of phenol).  $^{13}C$  NMR (DMSO) 23.21, 25.72, 32.05 (3 C of cyclopentano nucleus); 66.02 (CH-N=N); 93.34 (C $\equiv$ N); 101.15, 105.01, 113.76, 118.54, 122.81, 135.56, 137.45, 141.41 (aromatic C of benzene ring); 128.56, 130.15, 132.35, 133.27 (4C of thiophene ring), 156.03 (C=N); 160.96 (C-OH of phenol); 167.64 (C-OH of phenol); 172.02 (C=O of amide). *Anal.* for  $C_{23}H_{17}N_7O_3S$  (471.11). Calculated; C, 58.59; H, 3.63; N, 20.80; Found: C, 58.72; H, 3.87; N, 20.69.

2.2.3. 1-((4-((3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophen-2-yl)diazanyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)diazanyl)-4,5-dihydroxynaphthalene-2,7-disulfonicacid(8)

Crystallize from ethanol: dioxin (1:3) to obtain light brown crystals, Yield: 4.42 g (65%) m.p. above 300°C. IR spectrum (KBr)  $cm^{-1}$  showed three absorption bands at: 3448.48  $cm^{-1}$  for OH, 2183.94  $cm^{-1}$  (CN), 1638.59  $cm^{-1}$  (C=O amide).  $^1H$ -NMR (DMSO- $d_6$ ): the chemical shift  $\delta$  (ppm) 2.16 (qui, 2H,  $CH_2$  b), 2.75 (t, 2H,  $CH_2$  C), 3.05 (t, 2H,  $CH_2$  a), 4.28 (s, 1H,  $CH_2$  d), 7.39 (t, 1H, aromatic H), 7.58 (t, 2H, aromatic H), 7.63 (d,  $J=7.9$ Hz, 2H, aromatic H), 7.89 (s, 1H, aromatic H), 8.19 (s, 1H, aromatic H), 8.21 (s, 1H, aromatic H), 8.58 (s, 1H, 2OH), 9.693 (s, 1H,  $SO_3H$ ).  $^{13}C$ -NMR (DMSO) 23.14, 25.62, 32.34 (3C of cyclopentano nucleus); 66.01 (CH); 101.02 (C $\equiv$ N); 125.37, 128.61, 130.08, 132.15 (4C of thiophene ring),

133.17, 135.49, 137.35, 141.43, 143.14, 146.82 (aromatic C of benzene ring); 156.01 (C=N); 167.57 (C-OH phenolic); 172.13 (C=O of amide). *Anal.* Calculated for  $C_{27}H_{19}N_7O_9S_3$  (681.04): C, 47.57; H, 2.81; N, 14.38; Found: C, 47.83; H, 3.03; N, 14.12.

2.2.4. 2-((3-((2-hydroxynaphthalen-1-yl)diazanyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazanyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile(9)

Crystallize from ethanol: dioxin (1:3) to obtain red crystals, Yield: 3.7 g (74%) m.p. above 300°C. IR spectrum (KBr)  $cm^{-1}$  showed bands at: 3139  $cm^{-1}$  broad for (OH), 2216  $cm^{-1}$  (CN), 1613  $cm^{-1}$  (C=O amide).  $^1H$ -NMR (DMSO- $d_6$ ): the chemical shift  $\delta$  (ppm) 2.16 (qui, 2H,  $CH_2$ ), 2.72 (t, 2H,  $CH_2$ ), 3.05 (t, 2H,  $CH_2$ ), 4.28 (s, 1H, CH), 6.49 (d,  $J=7.9$ Hz, 1H, aromatic H), 6.82-7.16 (m, 4H, aromatic H), 7.61 (t, 2H, aromatic H), 7.88 (d,  $J=8.1$ Hz, 2H, aromatic H), 8.15 (d,  $J=8.1$ Hz, 2H, aromatic H), 10.15 (s, 1H, OH).  $^{13}C$ -NMR (DMSO) 23.25, 25.78, 32.01 (3C of cyclopentano nucleus); 66.25 (CH); 101.31 (C $\equiv$ N); 113.41, 116.30, 118.24, 120.51 (4C of thiophene ring), 125.43, 128.56, 130.26, 132.32, 133.28, 135.14, 137.15, 141.10, 143.42, 146.98 (aromatic C of benzene ring); 156.36 (CH=N); 167.14 (C=N); 172.20 (C=O of amide). *Anal.* Calculated for  $C_{27}H_{19}N_7O_2S$  (505.13): C, 64.15; H, 3.79; N, 19.39; Found: C, 64.38; H, 3.94; N, 19.57.

2.2.5. 2-((3-((4-(dimethylamino)phenyl)diazanyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazanyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (10)

Crystallize from ethanol: dioxin (1:3) to obtain red crystals, Yield: 2.55 g (53%) m.p. above 300°C, IR spectrum (KBr)  $cm^{-1}$  showed bands at: 2222  $cm^{-1}$  (CN), 1659  $cm^{-1}$  (C=O amide).  $^1H$ -NMR (DMSO- $d_6$ ): the chemical shift  $\delta$  (ppm) 2.16 (qui, 2H,  $CH_2$ ), 2.725 (t, 2H,  $CH_2$ ), 2.953 (t, 2H,  $CH_2$ ), 2.99 (s, 6H,  $N(CH_3)_2$ ), 4.28 (s, 1H, CH), 6.49 (d,  $J=8.1$ Hz, 2H, aromatic H), 6.79 (t, 2H, aromatic H), 7.08-7.18 (m, 3H, aromatic H), 8.21 (d,  $J=8.1$ Hz, 2H, aromatic H).  $^{13}C$ -NMR (DMSO) 23.13, 25.79, 32.21 (3C of cyclopentano nucleus); 44.12 ( $N(CH_3)_2$ ); 66.59 (CH); 101.25 (C $\equiv$ N); 113.02, 115.23, 126.88, 127.80 (4C of thiophene ring), 128.71, 132.22, 133.56, 136.36, 137.69, 139.54, 141.14 (aromatic C of benzene ring); 156.33 (CH=N); 167.79 (C=N); 172.09 (C=O of amide). MS showed the parent peak at  $m/z$  (% abundance) 482 (24)  $[M^+]$ . *Anal.* Calculated for  $C_{25}H_{22}N_8OS$  (482.16): C, 62.22;

H, 4.60; N, 23.22; Found: C, 62.46; H, 4.87; N, 23.58.

### 2.3. Materials and methods

Woven wool fabric (weight (255g/m<sup>2</sup> scoured and bleached in advance) supplied by Golden Tex Co., Tenth of Ramadan-Egypt, sodium carbonate and acetic acid for adjusting the pH were purchased from Sigma Aldrich.

#### 2.3.1. Wool dyeing procedure

Before dyeing, the samples were washed thoroughly with a solution of 5 g L<sup>-1</sup> non-ionic detergent (Hostapal CV, Clariant), at 50°C for 30 min. Then, the fabrics were rinsed with water and then air dried. Dyeing procedure was carried out using the synthesized dyes as shown in scheme 2; the selected dyeing conditions were then applied for all dyes. Dyes were applied to wool component (2g samples) using 3% dye shade with a liquor ratio of 50:1, the pH of the dye bath was adjusted to 4.5-5 by diluted acetic acid and samples were introduced into the dye bath at 40°C then the temperature was raised to 90°C, and the dyeing continued at the mentioned temperature for further 60 min as shown in figure (1). After which time, the dye bath temperature was reduced to 50°C, and then the samples were removed from the dye bath, rinsed in cold and then washed with a solution of 3g/l nonionic detergent at 50°C for 30mins, then the samples were rinsed and air dried.

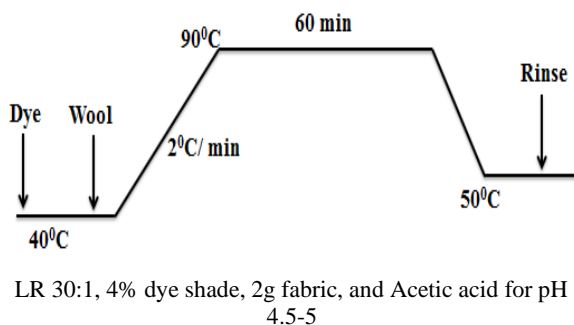


Figure 1: Dyeing profile method for the used dyes

#### 2.3.2. Color measurement

The colorimetric data of the dyed fabrics was measured using spectrophotometer with pulsed xenon lamps as light source (Ultra Scan Pro, Hunter Lab, USA) 10° observer with D65 illuminant, d/2 viewing geometry and measurement area of 2mm. All measurements were occurred at 425 nm wavelength. The corresponding color strength value (K/S) was assessed by applying the KubelkaMunk<sup>[14]</sup> (Eq.(1))

The colorimetric measurement of the dyed samples was measured using a Hunter Lab ultra Scan® PRO spectrophotometer. The corresponding colour strength value (K/S) was assessed by applying the KubelkaMunk equation as follows

$$K/S = (1-R)^2/2R - (1-R_0)^2/2R_0 \dots\dots\dots \text{Eq. 1}$$

Where: R = Decimal fraction of the reflectance of the dyed fabric

R<sub>0</sub> = Decimal fraction of the reflectance of the undyed fabric

K = Absorption coefficient.

S = Scattering coefficient [15]

#### 2.3.3. CIE Lab Difference

Color measurements of dyed fabrics were tested according to the CIE (L\*, a\*, b\*) system to evaluate the color coordinates. Where, the L\* value refers to lightness/darkness from 100 to 0 representing white to black, a\* values applied from green (negative) to red (positive) while the b\* values run from negative (blue) to positive (yellow). The dyed fabric was folded twice to prevent the penetration of incident light. Measurements were taken at three different positions for each dyed fabric, and their average was the final K/S value<sup>[16]</sup>.

#### 2.3.4. Fastness testing

The dyed samples were tested, after washing-off using 2g/l non-ionic detergent (Hostapal CV) at 60°C for 30 min, according to ISO standard methods. The specific tests were: ISO 105-X12 (1987), colour fastness to rubbing; ISO 105-C02 (1989)<sup>[17]</sup>, colour fastness to washing; and ISO 105-E04 (1989), colour fastness to perspiration. While the light fastness was tested using samples 10x10 cm of dyed fabrics mounted on a white chart paper and irradiated using a XENOTEST 1200 apparatus at a relative air humidity of 65% and 50°C with duration 4 hours.

#### 2.3.5. Antimicrobial activity assessment

The microorganism used in this assay was *Staphylococcus aureus* (ATCC 25923) selected according to the standards. An inoculum of *S. aureus* was prepared in 20.0 ± 0.1 mL of NB (Nutrient broth medium) and incubated for 24 h at 37 ± 1 °C. After incubation, the bacterial concentration was adjusted to 1 × 10<sup>7</sup> cells/ml cells/mL via absorbance readings, and based on a corresponding calibration curve. 1.0 ± 0.1 ml from the adjusted NB medium was added to 15 ml of NA (nutrient agar medium)

warmed at 45–46°C. The inoculated NB medium was poured in a sterile Petri dish. After solidification of agar, the textiles samples (2 x 2 cm each) were placed on the agar surface and incubated for 24 h at 37 ± 2°C. The evaluation of the antimicrobial activity was based on the measure of the halo formed around the edges of the samples [18].

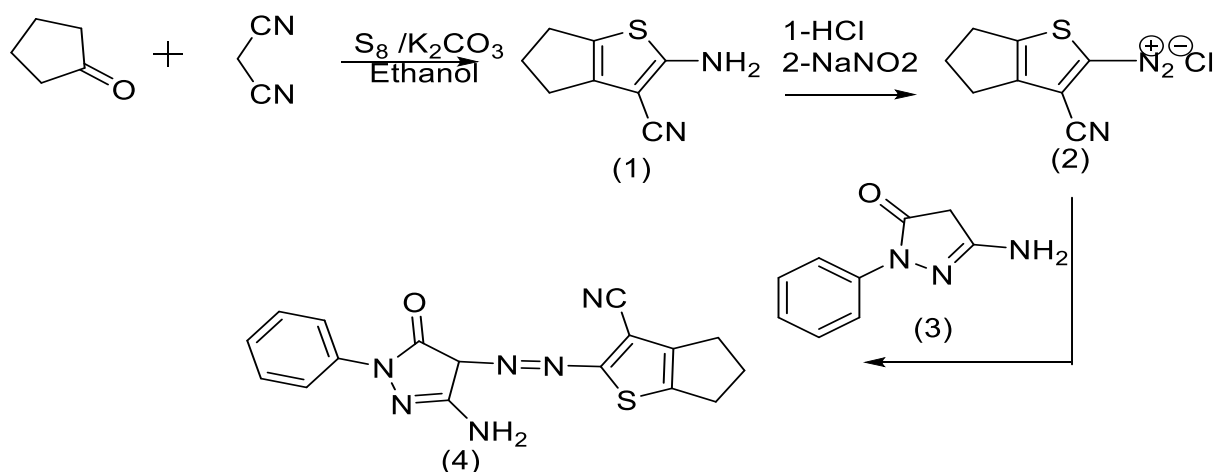
### 3. Results and Discussion

#### 3.1. Chemistry

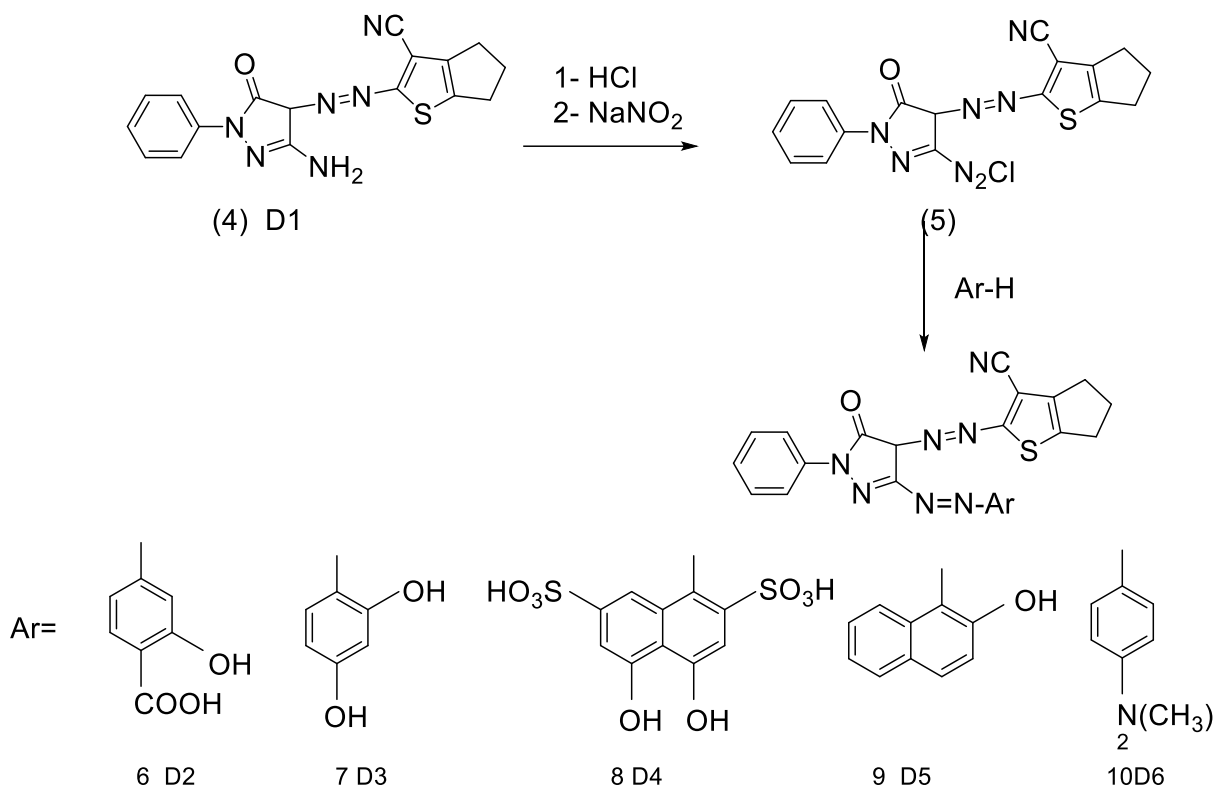
2-Amino-5,6-dihydro-4H-cyclopenta[b] thiophene-3-carbonitrile (1) was prepared according to Gewald reaction of malononitrile with cyclopentanone and elemental sulfur, the final product's structure was confirmed by IR and melting point 150-152°C. Usually, it is preferred in azo dyes synthesis molecules with small size and low molecular weight. The target mono azo dye **4** was obtained by dissolve of amino thiophene **1** in dilute HCl then added NaNO<sub>2</sub> solution to give the corresponding diazonium salt **2**, which subsequent coupling with 5-amino-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (3) to form 2-((3-amino-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (4) scheme (1). Compound **4** was characterized and confirmed by IR, exhibiting bands at 2221 and 1640 cm<sup>-1</sup> corresponding to (CN) and (C=O), respectively. The <sup>1</sup>H-NMR which display a two singlet signals at 4.26 and 6.38 ppm attributable to (CH-N=N) and free amino

groups, in addition to signals of cyclopentano and phenyl rings. We found in <sup>13</sup>C-NMR showed signals at 67.02, 99.03, 154.9 and 169.93 corresponding to (CH-N=N), (CN), (C=N) and (C=O) groups, respectively.

The mono azo dye **4** contain free primary amino group on the pyrazolone nucleus, which able to formation of the corresponding stable diazonium salt **5** at 0-5°C on dissolve in HCl then added NaNO<sub>2</sub> solution. The diazonium salt **5** is used in synthesis of target diazo dyes **6** to **10** by coupling with different activated aromatic phenols and/or amine such as salicylic acid, resorcinol, 4,5-dihydroxynaphthalene-2,7-disulfonic acid, 2-naphthol and N,N-dimethyl aniline as in scheme(2). The structures of diazo dyes **6** to **10** were confirmed by their spectral data. The IR spectra of **6** and **8** showed the absorption bands for carboxylic and sulfonic acids in addition to the absorption of -OH phenolic group. The obtained data from the <sup>1</sup>HNMR spectra was further confirmation evidence, where disappearance the singlet signal at 6.38 ppm (NH<sub>2</sub>) and showed two singlet signals at 9.83 (OH) and 11.78 (COOH) for compound **6** and 9.99, 10.03 for 2(OH) of compound **7**. 2-((3-((4-(Dimethylamino)phenyl)diazenyl)-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazenyl)-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carbonitrile (**10**) was confirmed by using <sup>1</sup>HNMR spectrum, which display at 2.99 ppm of (CH<sub>3</sub>)<sub>2</sub>N. Moreover, <sup>13</sup>CNMR and mass spectra were consistent with assigned structures of dyes **6** to **10**.



Scheme1: Formation of monoazo dye



Scheme 2: Formation of diazo dye.

### 3.2. Color measurements and fastness properties

The color data of various dyed fabrics, the reflectance values and K/S values (at the appropriate value of wavelength of maximum absorption for each dye) of the colored samples were evaluated using Hunter Lab ultra-Scan® PRO spectrophotometer (Ultra Scan Pro, Hunter Lab, USA).

Table 1 shows the average results of the color strength of the dyed wool fabrics with the synthesized dyes, the values of the color strength determine the shade depth, i.e. the deeper the shade, the higher the color strength. The color strength of the dyed wool fabric samples reached color strength up to 23.02 for dye **8**, 13.94 for dye **6**, 9.55 for dye **7**, and 7.09 for dye **9** respectively. The obtained high K/S value in case of dye **8** could be attributed to the presence of sulfonic groups which would ionic bonding with the protonized amino groups (NH<sub>3</sub><sup>+</sup>) of the wool fibers, also the presence of hydroxyl groups will form hydrogen bonds as well with the wool fibers which will directly support the fastness properties of the dyed fabrics, same explanation could be applied to dye **6** as it contains carboxylic and hydroxyl groups which will also form ionic

and hydrogen bonds respectively with the wool structure. The K/S values of dye **7** and **9** were comparatively lower than those of dye **8** and **6** as dye **7** and **9** contains only hydroxyl groups which will only hydrogen bonds with the wool structure, while the obtained K/S values in case of dye **4** and dye **10** were 1.58 and 1.64 respectively, which could be explained due to the absence of any strong bonding groups could hold with or build on the wool structure leading to poor dye uptake and weak fastness properties.<sup>[19,20]</sup>

Table 1. The color strength (K/S) values and colorimetric data of the dyed wool fabrics

| Sam-ple   | Wave-length | K/S   | L*    | a*    | b*    |
|-----------|-------------|-------|-------|-------|-------|
| <b>4</b>  | 360         | 1.58  | 75.20 | 0.49  | 11.05 |
| <b>6</b>  | 375         | 13.94 | 51.32 | 11.40 | 19.11 |
| <b>7</b>  | 370         | 9.55  | 59.09 | 7.27  | 22.83 |
| <b>8</b>  | 385         | 23.02 | 26.25 | 8.28  | 3.82  |
| <b>9</b>  | 370         | 7.09  | 41.02 | 11.08 | 12.76 |
| <b>10</b> | 360         | 1.64  | 74.18 | 0.66  | 12.33 |

The CIE L\*a\*b\* values of the dyed wool fabrics using the synthesized 4,6,7,8,9 and 10 dyes were in agreement with the obtained K/S values, as the depth of the shade is inversely related to the lightness (L\*), i.e. the lower the depth of shade the higher the lightness value. The L\* values of the dyes fabrics using dyes 4 and 10 were relatively high, indicating the low affinity of the mentioned dyes to the wool fabrics resulting in the low depth of shade, which preventing migration of the dyes from the dye bath to the fiber resulting low depth of shade. On the other hands, the L\* values for the dyed wool fabrics with dyes 8, 6, 7, and 9 were decreasing as the depth of shade were increased respectively as seen in table 1.

### 3.3. Fastness Properties

Table 2 shows a range of good to excellent fastness properties to rubbing, washing, acid and alkali perspiration, in the range of 4 and 5. The dyed wool fabrics

with the synthesized dyes shows very good to excellent results as dyes **8, 6, 7, and 9** shows very good to excellent light fastness which could ascribed to the strong ionic and hydrogen bonding between the wool structure and the synthesized dyes which possess hydroxyl and/or sulfonic and/or carboxylic groups leading to more dye uptake and fixation enhancing the fastness properties as mentioned before. On the other hand, the fastness properties of the dyed wool samples with dyes **4 and 10** exhibited relatively lower fastness compared to the rest of the dyes, which could be attributed to absence of strong bonding groups in the dye structure leading to less dye's uptakes and fixation. [21]

As a result of the obtained data from the CIE L\*, a\*, and b\* coordinates, after dyeing of wool fabrics at 3% owf shade using the prepared dyes, indicating an average of very good leveling properties for all the prepared dyes.

Table 2. Fastness properties of the dyed wool fabrics

| Dye       | Washing |       |      | Rubbing |     | perspiration |       |      |          |       |      | Light |     |
|-----------|---------|-------|------|---------|-----|--------------|-------|------|----------|-------|------|-------|-----|
|           | St.*    | St.** | Alt. | Dry     | Wet | Acidic       |       |      | Alkaline |       |      |       |     |
|           |         |       |      |         |     | St.*         | St.** | Alt. | St.*     | St.** | Alt. |       |     |
| <b>4</b>  | 4-5     | 4-5   | 4-5  | 4       | 4   | 4-5          | 4-5   | 4-5  | 4-5      | 4-5   | 4-5  | 4-5   | 4   |
| <b>6</b>  | 5       | 5     | 5    | 4-5     | 4-5 | 5            | 5     | 5    | 5        | 5     | 5    | 5     | 5-6 |
| <b>7</b>  | 5       | 5     | 5    | 4-5     | 4-5 | 4-5          | 4-5   | 4-5  | 4-5      | 4-5   | 4-5  | 5     | 5   |
| <b>8</b>  | 5       | 5     | 5    | 4-5     | 4-5 | 5            | 5     | 5    | 5        | 5     | 5    | 5     | 5-6 |
| <b>9</b>  | 5       | 5     | 5    | 4-5     | 4-5 | 4-5          | 4-5   | 4-5  | 4-5      | 4-5   | 4-5  | 5     | 4-5 |
| <b>10</b> | 4-5     | 4-5   | 4-5  | 4       | 4   | 4-5          | 4-5   | 4-5  | 4-5      | 4-5   | 4-5  | 4-5   | 4   |

St.\* = Staining on cotton; St.\*\* = Staining on wool; Alt. = alteration in color.

### 3.4. Antimicrobial activity assessment

The microorganisms (bacteria and fungi) used in this assay were: Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis), Candida albicans (C. albicans) and Aspergillus flavus (A. flavus) selected according to the standards. An inoculum of each bacterium and fungus was prepared in 20.0 ± 0.1 mL of NB (Nutrient broth medium) and incubated for 24 h at 37 ± 1 °C. After incubation, the bacterial concentration was adjusted to 1 × 10<sup>7</sup> cells/ml via absorbance readings, and based on a corresponding calibration curve. 1.0 ± 0.1 ml from the adjusted NB medium was added to 15 ml of NA (nutrient agar medium) warmed at 45–46°C. The inoculated NB medium was poured in a sterile Petri dish. After solidification of agar, the textiles samples (2 x 2 cm each) impregnated with synthesized dyes were placed on the agar surface and incubated for 24 h at 37 ± 2°C for bacteria, and 5 days at 28 ± 2°C for fungi. The evaluation of the antimicrobial activity was based

on the measure of the halo formed around the edges of the samples [22].

The prepared compound was tested as an antimicrobial agent against a panel of two Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), and two Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis). Each of the compounds was dissolved in DMSO and solution of the concentration 1 mg /ml were prepared separately paper discs of Whatman filter paper were prepared with standard size (5cm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solution were placed aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g + peptone 5g) seeded with Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus flavus. The petri dishes were incubated and the inhibition zones were recorded as describe above. Each treatment was replicated three times. The antibacterial activity of

a common standard antibiotic ampicillin and antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as below:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diametre)}}{\text{Zone of inhibition by standard (diametre)}} \times 100$$

In this study, JIS L 1902–Halo method was used to check the antimicrobial activity of the prepared compounds. A Halo zone was formed around compounds (6, 8, 9 and 10) indicating the presence of antimicrobial activity. The obtained results of the current study

exhibit a wide range of microbial activity resistance. It explains that the antimicrobial activity only depends on the substituent. The data listed in table 3 and illustrated in Figure (1) showing that compounds 6 & 8 could be considered as good antimicrobial candidates. The presence of –COOH and –SO<sub>3</sub>H groups in compounds (6 & 8) could explain the higher antimicrobial activity of these two compounds as it is well known that the carboxylic and the sulphonic groups possess an excellent metal-binding capacity [17].

**Table 3: Antimicrobial activity of the dyed wool fabrics**

| Compound      | <i>E. coli</i>                   |                  | <i>P. aeruginosa</i>             |                  | <i>S. aureus</i>                 |                  | <i>B. subtilis</i>               |                  | <i>C. Albicans</i>               |                  | <i>A. flavus</i>                 |                  |
|---------------|----------------------------------|------------------|----------------------------------|------------------|----------------------------------|------------------|----------------------------------|------------------|----------------------------------|------------------|----------------------------------|------------------|
|               | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index | Diameter of inhibition zone (mm) | % Activity index |
| 4             | NA                               | ----             | NA                               | ----             | NA                               | ----             | 2                                | 8.7              | NA                               | ----             | NA                               | ----             |
| 6             | 7                                | 28.0             | 9                                | 39.1             | 10                               | 41.7             | 12                               | 52.2             | 7                                | 25.9             | 8                                | 32.0             |
| 7             | NA                               | ----             | 3                                | 13.0             | 3                                | 12.5             | 4                                | 17.4             | NA                               | ----             | 4                                | 16.0             |
| 8             | 9                                | 36.0             | 13                               | 56.5             | 11                               | 45.8             | 15                               | 65.2             | 8                                | 29.6             | 10                               | 40.0             |
| 9             | 5                                | 20.0             | 8                                | 34.8             | 7                                | 29.2             | 10                               | 43.5             | 4                                | 14.8             | 6                                | 24.0             |
| 10            | 2                                | 8.0              | 6                                | 26.1             | 5                                | 20.8             | 7                                | 30.4             | 2                                | 7.4              | 5                                | 20.0             |
| Ampicillin    | 25                               | 100              | 23                               | 100              | 24                               | 100              | 23                               | 100              | NA                               | ----             | NA                               | ----             |
| Colitrimazole | NA                               | ----             | NA                               | ----             | NA                               | ----             | NA                               | ----             | 27                               | 100              | 25                               | 100              |

NA: not available

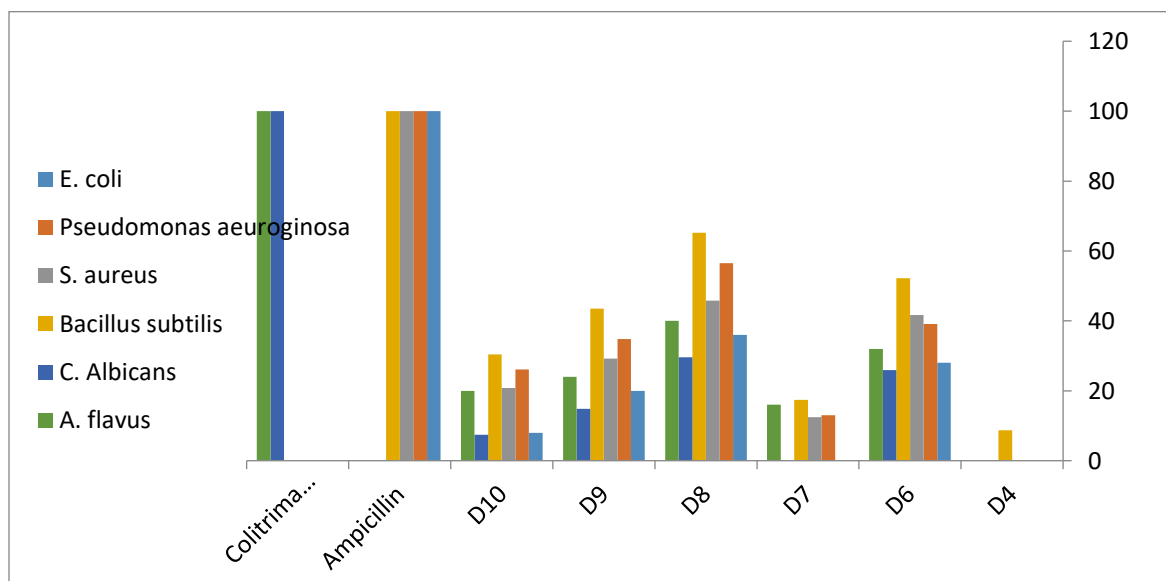


Fig. 2 Compounds tested with JIS L 1902–Halo method



#### 4. Conclusion

In this study a group of six novel azo dyes were successfully synthesized and fully characterized, the prepared dyes were applied as a potential acid dyes for dyeing of wool fabrics. The dyeing performance in terms of K/S values and CIE lab coordinates showed significant higher values in case of dye **6**, **7**, **8** and **9** than those of dye **4** and dye **10**. Accordingly, the fastness properties of the dyed samples were ranging good to excellent for the mentioned dyes. Antimicrobial activity of the dyed samples in case of dyes (**6**, **8**, **9** and **10**) showed good to very good microbial resistance which could support such dyes as good candidates for protective textile application.

#### 5. Conflicts of interest

“The authors declare that they have no conflict of interest”

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