Disperse Dyes Based on 5-Arylazo-thiazol-2-ylcarbamoyl-thiophenes: Synthesis, Antimicrobial Activity and Their Application on Polyester

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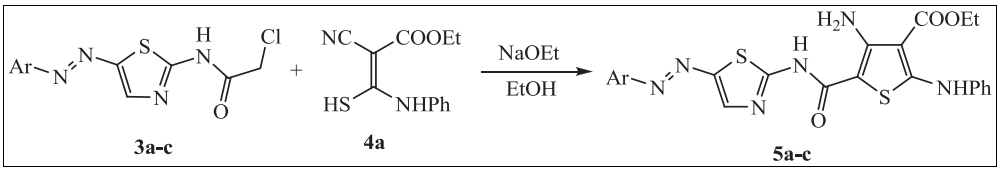


3a-c 4a 5a-c

A series of novel 5-arylazo-thiazol-2-ylcarbamoyl-thiophene derivatives was synthesized, and their chemical structures were secured by elemental and spectroscopic analyses. Their versatility for pharmaceutical purposes and textile dyeing as disperse dyes were reported. The synthesized dyes were applied to polyester fabrics by using high temperature dyeing method at 130°C. The dyed polyester fabrics displayed very good washing and perspiration fastness and moderate light fastness. Finally, the synthesized compounds showed biological activities against *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli*, and *Pseudomonas aeruginosa* (Gram-negative bacteria), while no effect had been reported against fungi. The minimum inhibitory concentration of the most active compound was evaluated.

INTRODUCTION

2-Aminothiazoles are mainly known as biologically active compounds with a broad range of activities and as intermediates in the synthesis of antibiotics such as the well known sulfa drugs [1]. A large number of 2-aminothiazoles have been substituted with different groups for pharmaceutical purposes [2–5] and are also used in the syntheses of various types of dyes [6–8].

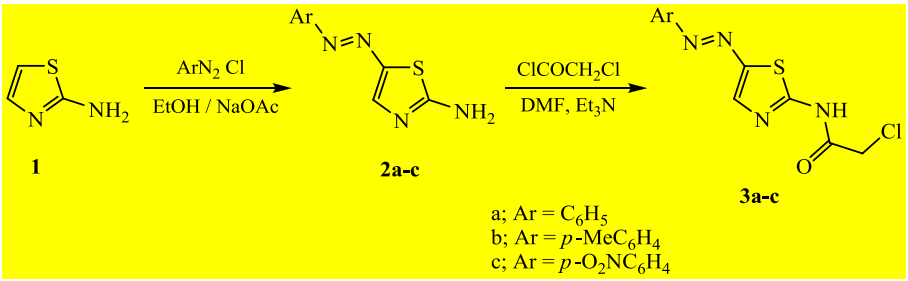
On the other hand, thiophene nucleus and its derivatives were proven to constitute the active part of several biologically active compounds, where the activity of thiophenes is to treat allergy, asthma, rhinitis, dermatitis, β-cell lymphomas, tumors, and diseases associated with bacterial, rhinovirus, or respiratory syncytial virus infections, besides their antioxidant activity [9–14].

In continuation of our previous studies on the synthesis of a variety of several new sulfur and/or nitrogen heterocyclic azo-disperse dyes from the readily obtainable cheapest starting materials for dyeing polyester fabrics, which provide strong shades that range from yellow, orange, red, and brown colors [15–19]. The polyester fabric has several advantages over traditional fabrics such as cotton. It does not absorb moisture but does absorb oil; this quality makes polyester the perfect fabric for the application of water-resistant, soil-resistant, and fire-resistant finishes and stains resistance besides its non-allergenic property, and disperse dyes are considered to be very popular and important class of dyes for dyeing polyester fabrics because of their ease of synthesis, structural variability, higher chromophoric strength, brilliancy, wide range of hues, and excellent fastness properties, in addition to their environmental and economic importance [20–22]. Therefore, we focused on the synthesis of novel thiazolyl-thiophene moiety as potential monoazo disperse dyes for dyeing polyester fabrics. In addition, the biological activities of the synthesized dyes against selected Gram-positive bacteria, Gram-negative bacteria, and fungi were also evaluated.

RESULTS AND DISCUSSION

Synthesis and reactions. Coupling of 2-aminothiazole **1** with a variety of aromatic diazonium salts in ethanol and sodium acetate, yielded the corresponding 2-amino-5-arylazothiazoles **2a–c**. The structure of the synthesized compounds was confirmed by elemental and spectrochemical analyses. The infrared spectrum of **2c** exhibited bands at 1640 (C═N) and 3384, 3263 cm^{-1} (NH_2). The ^1H NMR spectrum of **2c** showed the signal of aromatic protons as two doublet signals at δ = 7.50 and 8.25 ppm, a singlet signal at δ 7.80 for the C-4 thiazole ring proton, and a singlet signal at δ = 8.55 due to NH_2 protons. 2-Amino-5-arylazothiazoles **2a-c** were reacted with chloroacetyl chloride in DMF containing some drops of triethyl amine to afford the corresponding 5-arylazo-2-(*N*-chloroacetyl)amino-thiazole derivatives **3a–c** (Scheme 1). The chemical structures of **3a–c** were secured by elemental and spectral analyses. The IR spectrum of, for example, **3b** revealed intense bands at 1695 and 3204 cm^{-1} assignable to the (C═O) and (NH) functions, respectively. The ^1H NMR spectrum of **3b** revealed two singlet signals

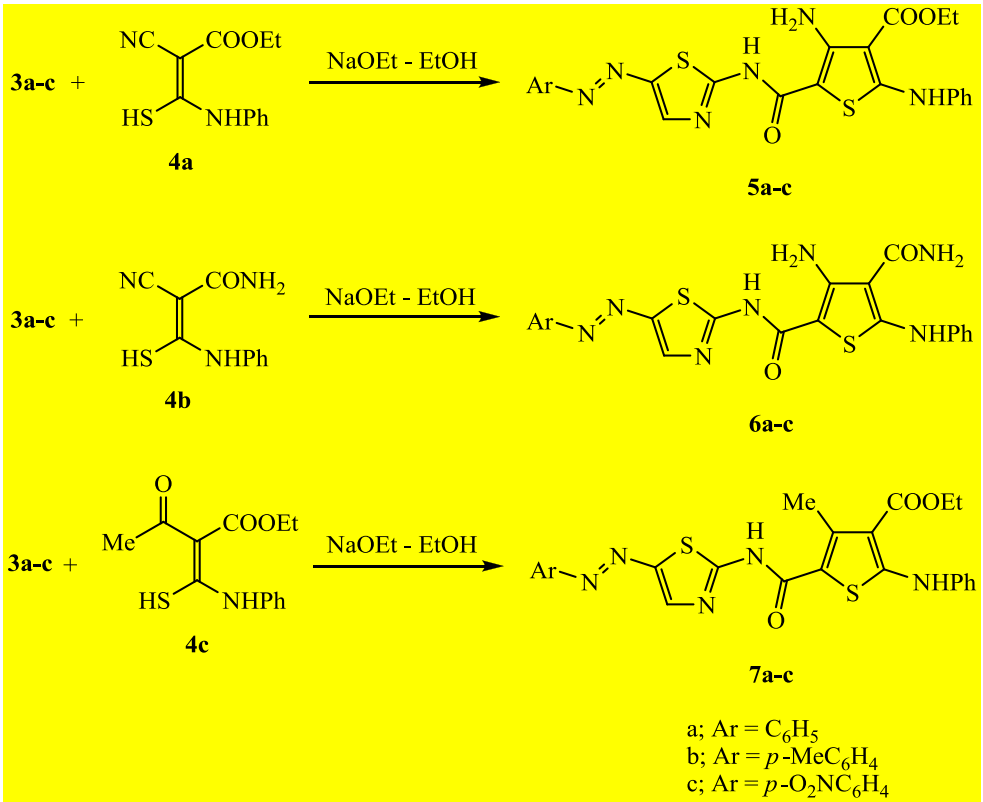
Scheme 1. Synthesis of 5-Arylazo-2-(*N*-chloroacetyl)amino-thiazoles **3a–c**.



at δ 2.35 and 4.10 ppm due to the CH_3 and CH_2 protons, two doublet signals at δ 7.25 and 7.50 ppm for aromatic protons, a singlet signal at δ 7.70 for the C-4 thiazole proton, and a singlet signal at δ 11.10 due to NH proton.

Addition reaction of ethyl cyanoacetate, cyanoacetamide, and ethyl acetoacetate with phenyl isothiocyanate in DMF and potassium hydroxide afforded the corresponding thiocarbamoyl derivatives **4a**, **4b**, and **4c** [23,24], which underwent condensation reaction with 5-arylazo-2-(*N*-chloroacetyl)amino-thiazoles **3a–c** in ethanol and sodium ethoxide to furnish the corresponding ethyl 4-amino-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxylates **5a–c**, 4-amino-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxamides **6a–c**, and ethyl 4-methyl-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxylates **7a–c**, respectively (Scheme 2). The IR spectrum of, for example, **5b** displayed bands at 1644 and 1662 cm^{-1} assignable to the carbonyl functions and bands at 3384, 3312, and 3248 cm^{-1} for the NH and NH_2 functions. The ^1H NMR spectrum of the same compound revealed a triplet signal at δ 1.30 ppm due to methyl protons ($OCH_2\underline{CH_3}$), a singlet signal at δ 2.40 ppm for methyl protons (Ar-$\underline{CH_3}$), a quartet signal at δ 4.25 ppm for methylene protons (($O\underline{CH_2}CH_3$), a multiplet signal in the range δ 7.00–7.80 ppm for the aromatic protons and C-4 thiazole proton, a singlet signal at δ 9.30 ppm corresponding to NH_2 protons and two singlet signals at δ 10.85 and 12.70 ppm for two NH protons.

Scheme 2. Synthesis of 5-(5-Arylazothiazol-2-yl)-2-phenylamino-thiophenes **5a–c**, **6a–c**, and **7a–c**.



Dyeing and fastness properties. For dyeing polyester fabrics, in practical terms, only disperse dyes are suitable. Through their hydrophobic properties, these dyes are capable of penetrating into the similarly hydrophobic polyester fiber. The synthesized disperse dyes under investigation were applied to polyester fabrics at 2% color depth (weight of dye to fabric w.o.f.) using high-temperature dyeing technique (pH 5.5, liquor ratio 20:1, temperature 130°C for 60 min), where range of visual color hues varied from yellow to brown were obtained. The dyes on polyester fabrics were evaluated in terms of their fastness properties and given in Table 1 using standard method, where fastness to perspiration, sublimation, and light were assessed in accordance with AATCC-15 (1985), rubbing fastness test was assessed in accordance with AATCC-88 (1988), and wash fastness test was assessed in accordance with IS: 765–1979 [25]. The dyes obtained gave excellent leveling, uniformity of coloration, and exhaustion of dye liquor. Furthermore, excellent behavior in fastness to washing and perspiration was shown. Most of the dyes have good rubbing fastness and good sublimation fastness. The light fastness of the synthesized dyes on polyester is significantly affected by the nature of substituent in the diazo component. The light fastness of each of the dyes was measured by employing the standard method for determination of color fastness of textiles. Several reports [26] suggested that fading of azo dyes is mainly a consequence of decomposition of the –N=N– moiety by oxidation, reduction or photolysis. The rates of these processes should be sensitive to the chemical structure of the dye, the type of substrate, and treatment conditions. Because the dyed substrate employed in this study is polyester fabrics (i.e., non-proteinic), the fading process likely occurs by oxidation [27]. The ease of oxidation of azo linkages should be a function of electron density. Therefore, electron donating substituent on this moiety should increase the fading rate. This proposal is in agreement with the observed results (Table 1), which demonstrate that the presence of a methyl group in synthesized dyes causes decrease of light fastness to 3–4.

Color assessment. The colors of synthesized dyes on polyester fabrics were expressed in terms of CIELAB values (Table 2), and the following CIELAB coordinates were measured by the reflectance spectrometer, where (L*) values represent color lightness, chroma (C*) values represent color purity, hue angle (h) values, which varies from 0 to 360°, to specify the color related to the principle colors, (a*) values represent the degree of redness (positive) and greenness (negative), and (b*)

Table 1

Fastness properties of the synthesized dyes 5–7 on 100% polyester fabrics.

			Perspiration		Rubbing		Sublimation fastness		
Dye	Visual color	Washing	Acid	Alkali	Dry	Wet	Staining at 180ºC	Staining at 210ºC	Light (40 h)
5a	Brown	5	5	5	5	4–5	5	4	4–5
5b	Yellowish brown	5	5	5	4	4	4	3–4	4
5c	Pale brown	5	5	5	4	4	4	4	5–6
6a	Pale brown	5	5	5	4–5	4–5	4–5	4–5	4
6b	Yellowish brown	5	5	5	4	4	5	4–5	3–4
6c	Pale brown	5	5	5	4–5	4–5	4–5	4–5	5
7a	brown	5	5	5	4	4	4	4	4–5
7b	Reddish brown	5	5	5	4–5	4–5	5	4–5	3–4
7c	brown	5	5	5	4	4	5	4–5	6

Table 2

Optical measurements of dyes 5–7.

Dye	K/S	L*	a*	b*	C*	h	ΔL	ΔC	ΔH	ΔE
5a	12.26	68.95	10.29	36.28	37.71	74.17	—	—	—	—
5b	7.90	61.08	5.33	29.33	29.81	79.70	−7.87	−7.90	5.53	7.68
5c	8.56	61.11	7.28	30.92	31.76	76.75	−7.84	−5.95	2.04	6.85
6a	8.08	56.19	7.89	24.99	26.21	72.48	—	—	—	—
6b	9.48	59.29	6.49	23.47	24.35	74.54	3.1	−1.86	2.06	3.03
6c	6.12	54.77	10.48	29.24	31.06	70.28	−1.42	4.85	−2.20	3.10
7a	9.46	61.90	8.89	35.09	36.20	75.79	—	—	—	—
7b	13.31	62.18	4.93	21.81	22.36	77.27	0.28	−13.84	1.48	6.32
7c	15.03	63.65	6.21	22.68	23.51	74.69	1.75	−12.69	−1.10	5.95

values represent the degree of yellowness (positive) and blueness (negative). Also, color strength (K/S) values were calculated at λ_{max} and directly correlated with the dye concentration on the dyed substrate according to the Kubelka–Munk equation: $K/S = (1-R)^2/2R$, where K = absorbance coefficient, S = scattering coefficient, and R = reflectance ratio. The difference in color strength depends on the substitutes present and/or the position of the substitutes on the structure of the synthesized dyes. A chromophore-containing compound is called a "chromogen": its color depends on the nature, number, and position of the "auxochromes". Auxochromes may shift the absorption towards higher wavelengths and thus the color of the dye will deepen. Such groups are called "bathochromes" (i.e., having electron-donating substitutes such as OH, NH_2 and CH_3); these are said to have a bathochromic effect. A group having the opposite, hyposochromic, effect is called a "hypochrome" (i.e., having electron-attracting substitutes such as CHO, COR, and NO_2). As shown in Table 2, the application of the dyes **5–7** on polyester fabrics showed that such dyes had good affinity to polyester fabrics through the satisfactory color yields and accepted values of K/S and the color hue represented degrees of redness to yellowness color hue as indicated from the positive values of a* and b* on the red–green axis. The dyes **6** found to be lighter than the other derivatives **5** and **7** according to the color lightness values L*. These results were in line with the previously reported by Műller on the effect of substituent in the dye structure and hue [28].

Screening of antimicrobial activities for the synthesized dyes. The antimicrobial activities of the synthesized dyes **5a–c**, **6a–c** and **7a–c** were screened against selected species of test strains, as per the ASTM specifications, from Gram-positive bacteria and fungi (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungi (*Aspergillus flavus* and *Candida albicans*) using the agar dilution method [29], and their inhibition zones' diameter were given in Table 3. In general, the synthesized dyes displayed uniform antimicrobial activities and approximately half effect of the standard antibacterial agent (*Tetracycline*) against the different strains tested (>10 mm inhibition zone). On the other hand, they showed no activities against all of the tested fungi used in this study. The enhanced antibacterial activity is clearly linked to the introduction of ester group in the thiophene moiety (e.g., **7b**) and/or nitro group at the side chain of the backbone of the molecule (e.g., **7c**) as electron withdrawing groups, while presence of electron donating groups such as amino, amide, and methyl groups (e.g., compounds **5**, **6a–c**, and **7a**) deactivate the thiophene moiety against tested bacteria.

MIC_{90} determination. Minimum inhibitory concentration, which inhibits 90% of the bacterial count compared with control microorganism, was evaluated, where compound **7b** was selected, for example, because of its highly active behavior among other samples against both types of bacteria (*S. aureus* and *E. coli*). It was found out that the minimum inhibitory concentration (MIC_{90}) of **7b** against *S. aureus* (G^+) was 164 μg/mL, comparing with *Tetracycline* as standard (104 μg/mL) indicating its acceptable efficiency.

CONCLUSION

A series of novel monoazo disperse dyes were synthesized based on 5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenyl-amino-thiophenes by cyclocondensation of 5-arylazo-2-(N-chloroacetyl)amino-thiazoles with various thiocabamoyl

Table 3

The inhibition zone (mm) values of the synthesized compounds **5a–c**, **6a–c**, and **7a–c**.

		Inhibition zone diameter (mm/mg sample)					
	Sample	*Bacillus subtilis* (G^+)	*Staphylococcus aureus* (G^+)	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Aspergillus flavus* (Fungus)	*Candida albicans* (Fungus)
Standard	Tetracycline *Antibacterial Agent*	30	28	30	31	0.0	0.0
	Amphotericin B *Antifungal agent*	—	—	—	—	16	20
5a		12	13	12	13	0.0	0.0
5b		12	13	14	12	0.0	0.0
5c		14	13	10	12	0.0	0.0
6a		14	14	15	14	0.0	0.0
6b		10	14	12	10	0.0	0.0
6c		11	10	10	10	0.0	0.0
7a		9	10	12	9	0.0	0.0
7b		13	17	15	14	0.0	0.0
7c		13	15	15	14	0.0	0.0

derivatives. All of the synthesized dyes were then applied for dyeing 100% polyester fabrics by using high temperature dyeing method at 130°C. The dyed polyester fabrics, which display yellowish brown to brown hues, exhibited very good fastness to washing, perspiration, and sublimation fastness properties with little variation in the good to excellent rubbing and moderate light fastness depending on the amount of dye fixed. Finally, all the synthesized dyes exhibited antimicrobial efficiency against Gram-positive bacteria (*B. subtilis* and *S. aureus*), Gram-negative bacteria (*E. coli* and *P. aeruginosa*), with various degrees according to the chemical structure of the synthesized dyes and attached functional groups, while showed no activities against fungi used in this study (*A. flavus* and *C. albicans*).

EXPERIMENTAL

All melting points were determined using Stuart SMP 20 melting point apparatus (Bibby Scientific Limited, Staffordshire, UK). Infrared spectra were recorded on a Perkin Elmer Alpha platinium-ATR spectrometer (Perkin Elmer Co., Shelton, UK), and ^{1}H NMR spectra were measured on a Bruker WP 300 (Bruker Co., MA, USA), in $CDCl_3$ and/or DMSO as solvent, using TMS as an internal standard. All the microanalysis and spectral analysis were performed by Micro analytical centers of Taif (IR, CHNS) and King Abdel-Aziz universities (^{1}H NMR analysis), Kingdom of Saudi Arabia. Microanalysis of the elements: carbon, hydrogen, and nitrogen was performed using Perkin-Elmer 2400 Analyzer, series II (Perkin Elmer Co., Shelton, UK). The results were in satisfactory agreement with the calculated values. The dyeing process was carried out using Galvanin-Marino VI dyeing machine (Galvanin Co., Saint Marino, Italy), the colorimetric measurements for the dyed polyester fabrics were carried out using a reflectance GretagMacbeth CE 7000a spectrophotometer (GretagMacbeth Co., Windsor, USA). Fastness to washing was carried out using the automatic launder Rota dyer (Texcare Co., Delhi, India), sponsored by the British Standard Institute – Society of Dyers and Colourists, fastness to perspiration was assessed according to the test sponsored by the (BSS), fastness to rubbing was carried out according to the standard method of testing (BSS) using Crock meter FD-17 type (Electric Hungarian Co., Budapest, Hungary), fastness to sublimation was carried out using the scorch tester M247 A (Atlas Electric Devices Co., Chicago, IL, USA) and fastness to light was carried out using the "Weather-o-meter" (Atlas Electric Devices Co., Chicago, IL USA), AATCC standard test method. Dyeing processes, color assessments, and color fastness properties were performed by Chemical laboratory, Dakahlia for Spinning and Weaving Company, Mansoura, Egypt. Antimicrobial tests were performed by the Biological Analysis Centre, Cairo University, Egypt.

General procedure for synthesis of 2-Amino-5-arylazothiazoles (2a–c). A solution of sodium nitrite (0.70 g, 10 m*M*) in 10 mL water was gradually added to a well-cooled solution of the aromatic amine (10 m*M*) in conc. HCl (3.0 mL). The diazonium salt solution was added with continuous stirring to an ice cooled solution of the 2-aminothiazole **1** (1 g, 10 m*M*) in ethanol (50 mL) and sodium acetate (3.8 g). The reaction mixture was allowed to stand for 2 h and then filtered. The obtained 2-amino-5-arylazothiazoles were dried and recrystallized from the appropriate solvent.

2-Amino-5-phenylazo-thiazole (2a). Reddish brown solid (EtOH); yield 84%; mp 270–1ºC; Lit. mp 270–271ºC [30].

2-Amino-5-(p-tolyl)azo-thiazole (2b). Brown solid (EtOH); yield 73%; mp 207–209ºC; Lit. mp 205ºC [31].

2-Amino-5-(p-nitrophenyl)azo-thiazole (2c). Brown solid (EtOH); yield 80%; mp 167–169ºC; IR: $\bar{\nu}$ = 1640 (C═N), 3384, 3263 (NH_2) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 7.50 (d, 2H, Ar-H), 7.80 (s, 1H, C-4 thiazole-H), 8.25 (d, 2H, Ar-H), 8.55 (s, 2H, NH_2). *Anal*. Calcd for $C_9H_7N_5O_2S$ (MW: 249.25): C, 43.37; H, 2.83; N, 28.10%. Found: C, 43.28; H, 2.86; N, 28.14%.

Synthesis of 2-[*N*-(Chloroacetyl)amino]-5-arylazo-thiazoles (3a–c). To a solution of 2-amino-5-arylazothiazoles **2**a–c (10 m*M*) in 25 mL DMF containing 0.5 mL triethyl amine, chloroacetyl chloride (1.2 mL, 15 m*M*) was added dropwise with stirring at room temperature. Stirring was continued for 2 h, and the reaction mixture was poured to ice cooled water. The precipitate that formed was collected by filtration, dried, and recrystallized from the appropriate solvent.

2-[N-(Chloroacetyl)amino]-5-phenylazo-thiazole (3a). Greenish yellow solid (EtOH); yield 78%; mp 220–222ºC; IR: $\bar{\nu}$ = 1686 (C═O), 3234 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.10 (s, 2H, CH_2), 7.10–7.50 (m, 5H, Ar-H), 7.70 (s, 1H, C-4 thiazole-H), 11.55 (s, 1H, NH). *Anal*. Calcd for $C_{11}H_9ClN_4OS$ (MW: 280.73): C, 47.06; H, 3.23; N, 19.96%. Found: C, 47.18; H, 3.26; N, 19.91%.

2-[N-(Chloroacetyl)amino]-5-(p-tolyl)azo-thiazole (3b). Yellowish brown solid (EtOH); yield 82%; mp 235–237ºC; IR: $\bar{\nu}$ = 1695 (C═O), 3204 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.35 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 7.25 (d, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 7.70 (s, 1H, C-4 thiazole-H), 11.10 (s, 1H, NH). *Anal*. Calcd for $C_{12}H_{11}ClN_4OS$ (MW: 294.76): C, 48.90; H, 3.76; N, 19.01%. Found: C, 48.79; H, 3.70; N, 19.08%.

2-[N-(Chloroacetyl)amino]-5-(p-nitrophenyl)azo-thiazole (3c). Dark brown solid (EtOH-DMF); yield 85%; mp 186–187ºC; IR: $\bar{\nu}$ = 1705 (C═O), 3276 (NH) cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ 4.20 (s, 2H, CH_2), 7.50 (d, 2H, Ar-H), 7.80 (s, 1H, C-4 thiazole-H), 7.50 (d, 2H, Ar-H), 12.35 (s, 1H, NH). *Anal*. Calcd for $C_{11}H_8ClN_5O_3S$ (MW: 325.73): C, 40.56; H, 2.48; N, 21.50%. Found: C, 40.61; H, 2.46; N, 21.46%.

General procedure for the synthesis of thiocarbamoyl derivatives (4a–c). The thiocarbamoyl derivatives were prepared according to the previous literature [23,24].

General procedure for the synthesis of 2-(Phenylamino)-5-(5-arylazothiazol-2-yl)-thiophenes 5-(7a–c). A mixture of thiocarbamoyl derivatives **4a–c** (5 m*M*) was heated with sodium ethoxide solution (5 m*M*, 0.1 g sodium metal in 10 mL absolute ethanol), then the chloroacetyl derivative **3a–c** (5 m*M*) was added. The mixture was refluxed for 15 min. The resultant solid product was collected by filtration, dried, and recrystallized from the appropriate solvent.

4-Amino-3-carbethoxy-2-(phenylamino)-5-(5-phenylazothiazol-2-yl)-thiophene (5a). Brown solid (EtOH); yield 74%; mp 186–187ºC; IR: $\bar{\nu}$ = 1646, 1658 (C═O), 3417, 3352, 3218 (NH and NH_2) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, CH_3), 4.25 (q, 2H, CH_2), 7.00–7.70 (m, 11H, Ar-H and C-4 thiazole-H), 8.65 (s, 2H, NH_2), 10.45 (s, 1H, NH), 12.20 (s, 1H, NH). *Anal*. Calcd for $C_{23}H_{20}N_6O_3S_2$ (MW: 492.57): C, 56.08; H, 4.09; N, 17.06%. Found: C, 56.26; H, 4.16; N, 17.02%.

4-Amino-3-carbethoxy-2-(phenylamino)-5-[5-(p-tolyl)azothiazol-2-yl]-thiophene (5b). Yellowish brown solid (EtOH); yield 62%; mp 180–181ºC; IR: $\bar{\nu}$ = 1644, 1662 (C═O), 3384, 3312, 3248 (NH and NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.25 (q, 2H,CH_2), 7.00–7.80 (m, 10H, Ar-H and C-4 thiazole-H), 9.30 (s, 2H, NH_2), 10.85 (s, 1H, NH), 12.70 (s, 1H, NH). *Anal*. Calcd for $C_{24}H_{22}N_6O_3S_2$ (MW: 506.6): C, 56.90; H, 4.38; N, 16.59%. Found: C, 56.77; H, 4.32; N, 16.67%.

4-Amino-3-carbethoxy-2-(phenylamino)-5-[5-(p-nitrophenyl) azothiazol-2-yl]-thiophene (5c). Brown solid (EtOH-DMF); yield 78%; mp 172–173ºC; IR: $\bar{\nu}$ = 1650, 1664 (C═O), 3422, 3367, 3274 (NH and NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/DMSO): δ = 1.30 (t, 3H, CH_3), 4.30 (q, 2H, CH_2), 7.10–7.70 (m, 8H, Ar-H and C-4 thiazole-H), 8.05 (d, 2H, Ar-H), 9.80 (s, 2H, NH_2), 11.75 (s, 1H, NH), 12.70 (s, 1H, NH). *Anal*. Calcd for $C_{23}H_{19}N_7O_5S_2$ (MW: 537.57): C, 51.39; H, 3.56; N, 18.24%. Found: C, 51.46; H, 3.52; N, 18.32%.

4-Amino-2-(phenylamino)-5-(5-phenylazothiazol-2-yl)-thiophene-3-carboxamide (6a). Yellowish brown solid (EtOH); yield 72%; mp 230–232ºC; IR: $\bar{\nu}$ = 1645 (C═O), 3411, 3346, 3262 (NH and NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/DMSO): δ = 7.10-7.80 (m, 11H, Ar-H and C-4 thiazole-H), 9.25 (s, 2H, NH_2), 10.10 (s, 2H, NH_2), 11.80 (s, 1H, NH), 13.05 (s, 1H, NH). Anal. Calcd for $C_{21}H_{17}N_7O_2S_2$ (MW: 463.54): C, 54.41; H, 3.70; N, 21.15%. Found: C, 54.20; H, 3.62; N, 21.22%.

4-Amino-2-(phenylamino)-5-[5-(p-tolyl)azothiazol-2-yl]-thiophene-3-carboxamide (6b). Brown solid (EtOH); yield 64%; mp 195–196ºC; IR: $\bar{\nu}$ = 1648 (C═O), 3384, 3342, 3262 (NH and NH_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/DMSO): δ = 2.40 (s, 3H, CH_3), 7.10–7.85 (m, 10H, Ar-H and C-4 thiazole-H), 8.90 (s, 2H, NH_2), 9.90 (s, 2H, NH_2), 11.70 (s, 1H, NH), 12.85 (s, 1H, NH). *Anal*. Calcd for $C_{22}H_{19}N_7O_2S_2$ (MW: 477.56): C, 55.33; H, 4.01; N, 20.53%. Found: C, 55.12; H, 4.07; N, 20.62%.

4-Amino-2-(phenylamino)-5-[5-(p-nitrophenyl)azothiazol-2-yl]-thiophene-3-carboxamide (6c). Dark brown solid (EtOH); yield 62%; mp 171–173ºC; IR: $\bar{\nu}$ = 1651 (C═O), 3412, 3440, 3267 (NH and NH_2) cm^{-1}; ^1H NMR (300 MHz, DMSO): δ = 7.20–7.80 (m, 8H, Ar-H and C-4 thiazole-H), 8.20 (d,2H, Ar-H), 9.15 (s, 2H, NH_2), 10.15 (s, 2H, NH_2), 11.65 (s, 1H, NH), 12.80 (s, 1H, NH). *Anal*. Calcd for $C_{21}H_{16}N_8O_4S_2$ (MW: 508.53): C, 49.60; H, 3.17; N, 22.03%. Found: C, 49.82; H, 3.10; N, 22.09%.

3-Carbethoxy-4-methyl-2-(phenylamino)-5-(5-phenylazothiazol-2-yl)-thiophene (7a). Brown solid (EtOH); yield 77%; mp 192–193ºC; IR: $\bar{\nu}$ = 1641, 1661 (C═O), 3311, 3256 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.30 (q, 2H, CH_2), 6.90–7.70 (m, 11H, Ar-H and C-4 thiazole-H), 10.15 (s, 1H, NH), 11.80 (s, 1H, NH). *Anal*. Calcd for $C_{24}H_{21}N_5O_3S_2$ (MW: 491.59): C, 58.64; H, 4.31; N, 14.25%. Found: C, 58.48; H, 4.26; N, 14.28%.

3-Carbethoxy-4-methyl-2-(phenylamino)-5-[5-(p-tolyl)azothiazol-2-yl]-thiophene (7b). Brown solid (EtOH); yield 83%; mp 174–175ºC; IR: $\bar{\nu}$ = 1640, 1664 (C═O), 3288, 3214 (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.30 (q, 2H, CH_2), 6.90–7.80 (m, 10H, Ar-H and C-4 thiazole-H), 10.25 (s, 1H, NH), 11.70 (s, 1H, NH). *Anal*. Calcd for $C_{25}H_{23}N_5O_3S_2$ (MW: 505.61): C, 59.39; H, 4.59; N, 13.85%. Found: C, 59.46; H, 4.62; N, 13.78%.

3-Carbethoxy-4-methyl-2-(phenylamino)-5-[5-(p-nitrophenyl) azothiazol-2-yl]-thiophene (7c). Brown solid (EtOH); yield 81%; mp 160–161ºC; IR: $\bar{\nu}$ = 1644, 1665 (C═O), 3308, 3216, (NH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/DMSO): δ = 1.30 (t, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.30 (q, 2H, CH_2), 6.90–7.70 (m, 8H, Ar-H and C-4 thiazole-H), 8.20 (d, 2H, Ar-H), 10.20 (s, 1H, NH), 11. (s, 1H, NH). *Anal*. Calcd for $C_{24}H_{20}N_6O_5S_2$ (MW: 536.58): C, 53.72; H, 3.76; N, 15.66%. Found: C, 53.87; H, 3.78; N, 15.57%.

Dye bath preparation and dyeing procedure. Dispersion of the dye was produced by dissolving the appropriate amount of dye (0.1 g dye/5 g fiber, 2% shade) in 1 mL acetone and then added drop wise with stirring to a solution of Setamol WS (0.5–1.5), an anionic dispersing agent of BASF (sodium salt of a condensation product of naphthalene sulfonic acid and formaldehyde). The dyestuff dispersion was then added to the dye bath at 60ºC through a fine mesh sieve or filter cloth. The dye bath was prepared with liquor ratio 20:1 using sealed stainless steel dye pots of 250 mL capacity in "Galvanin-Marino VI-Italy" dyeing machine. Additional dispersing agent (0.5–1.0 g/L Setamol WS of BASF) was added and the pH of the bath adjusted to 5.5 using glacial acetic acid. The polyester fabric was immersed and dyeing carried out by raising the dye bath temperature from 20 to 130ºC at a rate of 3ºC/min and holding at this temperature for 60 min before rapidly cooling to 50ºC at 9.9ºC/min. The dyed fabric was then rinsed with cold water, reduction-cleared using mixture of 2 mL/L caustic soda solution 32.5% (71ºTw), 2 g/L sodium hydrosulphite and 0.5 g/L Hostapal CV conc., non-ionic wetting agent of Clariant, at 75–85ºC for 15–30 min and soaped with 2% non-ionic detergent and ammonia (pH 8.5) at 50ºC for 30 min to improve washing fastness then drying in a pre-drier without contact up to a residual moisture of approximately 30% followed by final drying which be carried out on a hot flue.

Color fastness tests. The color fastness properties of the dyed fabrics against washing, perspiration, rubbing, sublimation, and light were evaluated using standard methods [25]. Results were listed in Table 1, where the staining of adjacent cotton fabrics was assessed using the gray scale: 1-poor, 2-fair, 3-moderate, 4-good, and 5-excellent, other than light fastness, which is scaled from 1–8 on the gray scale.

Color assessment. The colorimetric measurements of the dyed fabric (Table 3) were assessed in terms of tristimulus colorimetry values. The values of the chromaticity coordinates, luminance factor, and the positions of colors in the CIELAB color solid were reported.

Antimicrobial test method. The agar dilution method was used to evaluate the antimicrobial activity of the synthesized dyes **5a–c**, **6a–c**, and **7a–c**. Stationary-phase cultures of bacteria were prepared at 37°C and used to inoculate fresh 5.0 mL culture to an OD_{600} of 0.05. The 5.0 mL cultures were then incubated at 37°C until an OD_{600} of 0.10 was achieved, from which standardized bacterial suspensions were prepared to a final cell density of 6×10^5 colony forming unit/mL. Serial dilutions from the treatments (0–320 µg/mL) were prepared and mixed with 5.0 mL of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37°C. The colony forming units were counted for each dilution (NCCLS: M7 – A4, 1997). The antimicrobial activity was evaluated by measuring the average of inhibition zone diameter against the test microorganisms and the values were expressed in millimeter. The relationships between the biological activity and the chemical structure of the synthesized compounds and their dyed fabrics were discussed.

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