

In vitro Biological Activity of Some Synthesized Monoazo Heterocycles Based On Thiophene and Thiazolyl-Thiophene Analogues

M. E. Khalifa, A. A. Gobouri

Abstract—Potential synthesis of a series of 3-amino-4-arylazothiophene derivatives from reaction of 2-cyano-2-phenylthiocarbamoyl acetamide and the appropriate α -halogenated reagents, followed by coupling with different aryl diazonium salts (Japp-Klingemann reaction), and another series of 5-arylaazo-thiazol-2-ylcarbonyl-thiophene derivatives from base-catalyzed intramolecular condensation of 5-arylaazo-2-(*N*-chloroacetyl)amino-thiazole with selected β -keto compounds (Thorpe-Ziegler reaction) was performed. The biological activity of the two series was studied *in vitro*. Their versatility for pharmaceutical purposes was reported, where they displayed remarkable activities against selected pathogenic microorganisms; *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative bacteria), and *Aspergillus flavus*, *Candida albicans* (fungi) with various degrees related to their chemical structures.

Keywords—2-Aminothiazole, antimicrobial, monoazo compounds, thiophene, pathogenic microorganisms.

I. INTRODUCTION

A wide variety of thiophenes was prepared directly by the Gewald synthesis or after subsequent dramatization to produce novel derivatives constituting the active part of several biologically active compounds [1]-[8]. 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 (RSV) infections, besides their antioxidant activity [9]-[11]. On the other hand, 2-Aminothiazoles building block is of widespread use in chemistry, where they are known as biologically active compounds with a broad range of activity, and as intermediates in the synthesis of antibiotics such as the well known sulfa drugs [12]. A large number of 2-aminothiazoles have been substituted with different groups for pharmaceutical purposes [13]-[17].

In continuation of our previous studies on the synthesis of a variety of several new sulfur and/or nitrogen heterocyclic monoazo compounds [18]-[25], therefore we focused on the facile synthesis of 3-amino-4-arylaazo-thiophene and thiazolyl-

thiophene moieties as potential biological active compounds against selected gram positive bacteria, gram negative bacteria and Fungi.

II. RESULTS AND DISCUSSION

A. Synthesis

Synthesis of poly functionally substituted arylazo-thiophenes (5a-c-7a-c) [24] was passed through progressive steps (Fig. 1). Initially, we synthesized 2-phenylthiocarbamoyl derivative (1) through the base catalyzed addition of phenyl isothiocyanate (10mmol) to the highly reactive cyanoacetamide (10mmol) in presence of 30ml dimethyl formamide (DMF) containing finely divided solid potassium hydroxide (10mmol). The mixture of 2-cyano-2-phenylthiocarbamoyl acetamide (1) (5mmol) and the appropriate α -halogenated reagents- e.g. chloroacetonitrile, chloroacetone and/or phenacyl chloride (5mmol)- was stirred overnight in 25ml DMF containing anhydrous K_2CO_3 (10 mmol). It was suggested that the reaction had started through nucleophilic attack of the thiolate group which underwent nucleophilic substitution and intramolecular cycloaddition to yield the corresponding poly functionally 3-amino-2-substituted-thiophene-4-carboxamide derivatives (2-4). Then, coupling of 3-amino-2-substituted-thiophene-4-carboxamide derivatives (2-4) with the appropriate aryl diazonium salts in ethanol containing potassium hydroxide (20%) underwent cleavage of amide group (Japp-Klingemann reaction) [26] afforded the corresponding 3-amino-4-arylaazo-thiophenes (5a-c-7a-c) (Fig. 1). The IR spectra displayed bands at 2190-2195 cm^{-1} assignable to the cyano group (5a-c), 1622-1636 cm^{-1} for carbonyl functions (6a-c), 1609-1618 cm^{-1} for carbonyl functions (7a-c) and bands at 3384, 3312, 3248 cm^{-1} for the NH and NH_2 functions. The 1H NMR spectra of the synthesized compounds revealed signals secured the compounds' structure as reported in Table II.

On the other hand, coupling of 2-aminothiazole 8 with a variety of aromatic diazonium salts in ethanol and sodium acetate, yielded the corresponding 2-amino-5-arylaazothiazoles (9a-c). The 2-Amino-5-arylaazothiazoles (9a-c) were reacted with chloroacetyl chloride in dimethyl formamide (DMF) containing some drops of triethyl amine (TEA) to afford the corresponding 5-arylaazo-2-(*N*-chloroacetyl)amino-thiazole derivatives (10a-c). Addition reaction of ethyl cyanoacetate, cyanoacetamide, and ethyl acetoacetate with phenyl isothiocyanate in DMF and potassium hydroxide afforded the

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corresponding thiocarbamoyl derivatives (11a, 11b, and 11c) [27], which underwent condensation reaction with 5-arylaazo-2-(*N*-chloroacetyl)amino-thiazoles (10a-c) in ethanol and sodium ethoxide to furnish the corresponding ethyl 4-amino-5-(5-arylaazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-

3-carboxylates 12, 4-amino-5-(5-arylaazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxamides 13 and ethyl 4-methyl-5-(5-arylaazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxylates 14, respectively (Fig. 2) [25].

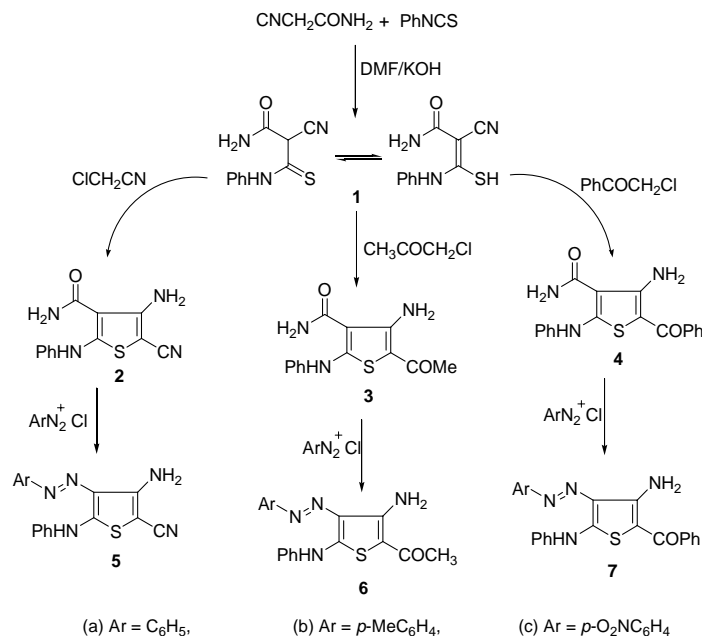


Fig. 1 Synthesis of corresponding 3-amino-4-arylaazo-thiophenes (5-7a-c)

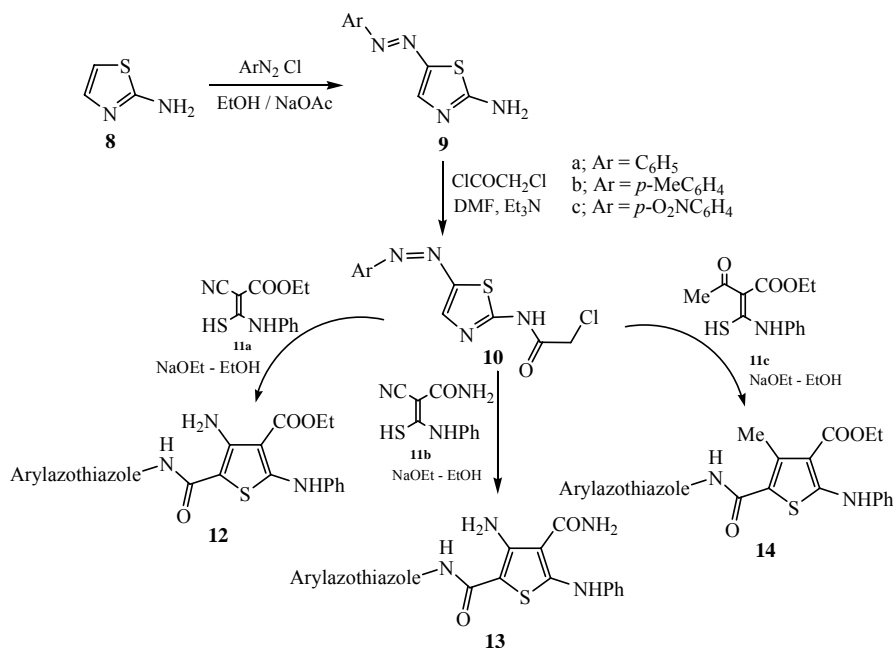


Fig. 2 Synthesis of substituted 5-arylaazo-thiazol-2-ylcarbamoyl-2-phenylamino-thiophene compounds (12a-c-14a-c)

The IR spectrum of e.g. 12b displayed bands at 1644, 1662 cm⁻¹ assignable to the carbonyl functions and bands at 3384, 3312, 3248cm⁻¹ for the NH and NH₂ functions. The ¹H NMR spectrum of the same compound revealed a triplet signal at δ 1.30ppm due to methyl protons (OCH₂CH₃), a singlet signal at

δ 2.40ppm for methyl protons (Ar-CH₃), a quartet signal at δ 4.25ppm for methylene protons (OCH₂CH₃), a multiplet signal in the range δ 7.00-7.80ppm for the aromatic protons and C-4 thiazole proton, a singlet signal at δ 9.30ppm corresponding to NH₂ protons and two singlet signals at δ 10.85 and 12.70ppm

for two NH protons. The structures of the functionalized 4-arylazo-thiophenes (12a-c-14a-c) were secured on the basis of their spectroscopic data and elemental analysis (Table II).

B. Screening of Antimicrobial Activities for the Synthesized Compounds (5a-c-7a-c) and (12a-c-14a-c)

The antimicrobial activities of the synthesized compounds (5a-c-7a-c) and (12a-c-14a-c) were screened *in vitro* against selected pathogenic microorganisms; Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) [24], [25] and fungi (*Aspergillus flavus* and *Candida albicans*) [25] referring to standard species Tetracycline (antibacterial agent) and Amphotericin (antifungal agent), using the Agar Dilution Method [28] and the inhibition zones' diameters were given in Table I. It was shown that the synthesized compounds displayed variable degrees of antimicrobial activities against

the different strains tested as indicated by Figs. 3-5. The functionalized thiophene compounds (5a-c-7a-c) and thiazolyl-thiophene compounds (12a-c-14a-c) showed higher activity against microorganisms especially against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*), while presence of electordonating group in thiophene moiety (e.g. 6-7b) inhibited the activity against selected bacteria. On the other hand, thiophenes (5-7a) showed noticeable activity against *Aspergillus flavus* fungal strain. Whereas, the presence of electro-donating, electro-withdrawing groups (5c, 6b-c and 7b-c), and thiazolyl moiety (12a-c-14a-c) as well, inhibited the activities against all of the tested fungi used in this study. On contrary the remarkable activity of 5b against *Aspergillus flavus* fungal strain, may be due to presence of the nitrile group.

TABLE I
THE INHIBITION ZONE (MM) VALUES OF THE SYNTHESIZED COMPOUNDS (5a-c-7 a-c) and (12a-c-14a-c)

Compounds	Inhibition zone diameter (mm / mg Sample)					<i>Aspergillus flavus</i> (Fungus)	<i>Candida albicans</i> (Fungus)
	<i>Bacillus subtilis</i> G+	<i>Staphylococcus aureus</i> G+	<i>Escherichia coli</i> G-	<i>Pseudomonas aeruginosa</i> (G)			
Tetracycline acterial standard)	30	28	30	31	-	-	
Amphotericin Fungal standard)	-	-	-	-	16	20	
5a	8.20	10.4	7.3	12.5	8.2	0.0	
5b	1.70	4.30	7.2	12.3	9.1	0.0	
5c	12.5	11.5	8.0	11.0	0.0	1.4	
6a	12.5	10.7	7.2	11.1	5.9	2.1	
6b	0.70	0.60	0.0	0.0	0.0	0.0	
6c	10.5	9.20	8.4	4.2	0.0	0.0	
7a	17.6	11.7	6.8	10.1	5.1	2.4	
7b	0.90	0.70	0.0	0.0	0.0	0.0	
7c	11.5	10.3	8.1	2.1	0.0	0.0	
12a	12	13	12	13	0.0	0.0	
12b	12	13	14	12	0.0	0.0	
12c	14	13	10	12	0.0	0.0	
13a	14	14	15	14	0.0	0.0	
13b	10	14	12	10	0.0	0.0	
13c	11	10	10	10	0.0	0.0	
14a	9	10	12	9	0.0	0.0	
14b	13	17	15	14	0.0	0.0	
14c	13	15	15	14	0.0	0.0	

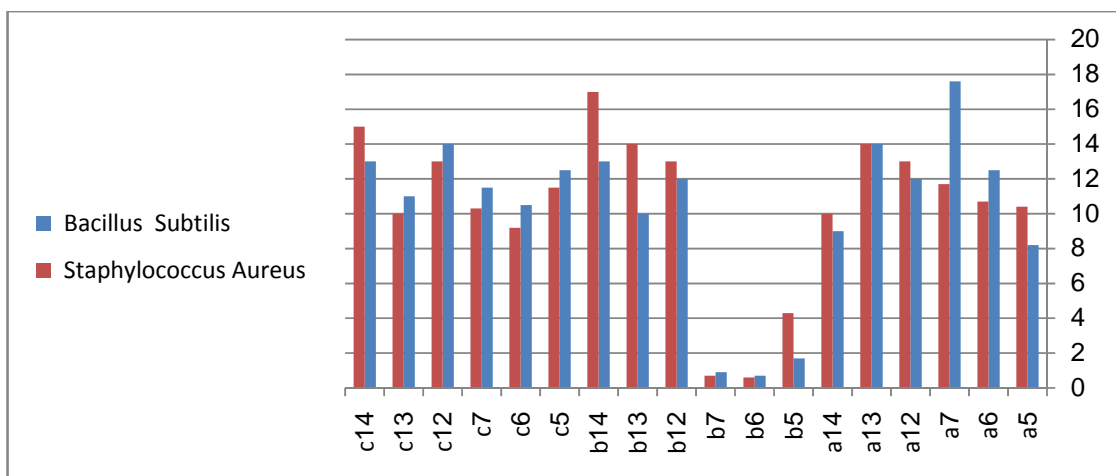


Fig. 3 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against G+ bacteria

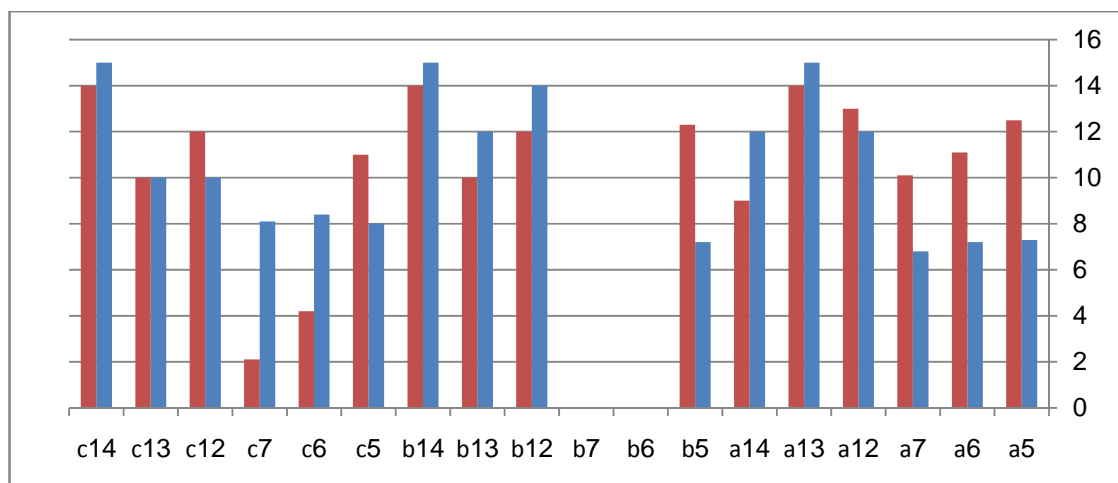


Fig. 4 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against G- bacteria

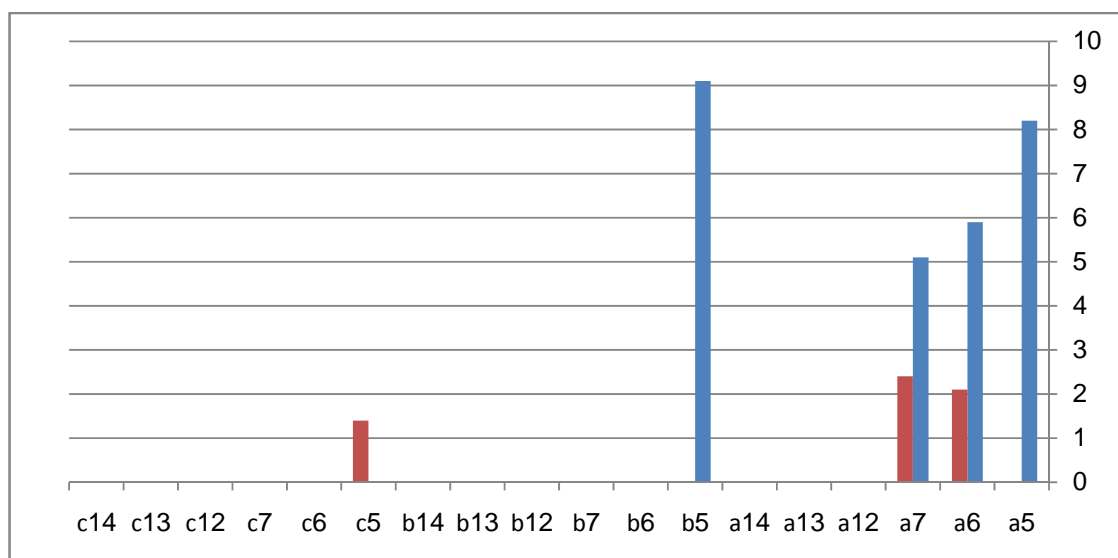


Fig. 5 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against selected fungi

III. EXPERIMENTAL

A. Materials and Instrumentation

The reagents were analytical grade or chemically pure. Elemental analyses (C, H, N) were conducted using the Perkin-Elmer 2400 Analyzer, series II (Perkin Elmer Co., Shelton, UK), their results were found to be in good agreement ($\pm 0.3\%$) with the calculated values. All of the corrected melting points were determined using a Stuart SMP 20 melting point apparatus (Bibby Scientific Limited, Staffordshire, UK). The infrared spectra were recorded on a Perkin Elmer Alpha platinum-ATR spectrometer, and the ^1H NMR spectra were measured on a Bruker WP 300 (Bruker, MA, USA) in CF_3COOD using TMS as an internal standard. All of the microanalyses and spectral analyses were performed at the Micro Analytical Centres of Taif (IR, CHN) and King Abdel-Aziz University (^1H NMR analysis), Saudi Arabia. The biological tests were performed by the "Biotechnology Unit", Faculty of Agriculture, Cairo University, Egypt.

B. Synthesis and Spectroscopic Characterisation

1. Synthesis of 2-Cyano-2-Phenylthiocarbonyl Acetamide (1)

To a cold suspension of finely divided KOH (0.56g, 10.0 mmol) in DMF (30ml), the cyanoacetamide (0.84g, 10.0 mmol) was added, followed by phenyl isothiocyanate (1.20ml, 10.0mmol). The reaction mixture was stirred at room temperature overnight, poured into ice-cold water and then neutralized with dilute HCl. The resultant solid product was collected by filtration, washed with water, dried, and recrystallized from ethanol to afford compound **1**.

Brown solid; yield: 85%; mp 160-4°C (EtOH); IR: $\bar{\nu} = 1642$ (C=O), 2190 (CN), 3421, 3330 (NH_2) cm^{-1} . Anal. Calcd for $\text{C}_{10}\text{H}_9\text{N}_3\text{OS}$: C, 54.78; H, 4.14; N, 19.16; S, 14.62%. Found: C, 54.71; H, 4.11; N, 19.14; S, 14.60%.

2. General Procedure for Synthesis of 3-Amino-2-Substituted-Thiophene-4-Carboxamide Compounds (2-4)

Into a solution of 2-cyano-2-phenylthiocarbonyl acetamide (5mmol) in 25ml dimethyl formamide (DMF) were added solid potassium carbonate (2 equiv) and the appropriate α -halogenated reagent e.g. chloroacetonitrile, chloroacetone and/or phenacyl chloride (1 equiv), stirred for 12 hours at room temperature. The reaction mixture was then poured into cold H₂O and neutralized with dilute HCl. The solid product was filtered, washed with water, dried and recrystallized from ethanol or ethanol:DMF (2:1) mixture to afford the corresponding thiophene compounds 2-4.

2.1. 3-Amino-2-Cyano-5-Phenylamino-Thiophene-4-Carboxylic Acid Amide (2)

Brown solid; yield 74%; mp 186°C (EtOH); IR: $\bar{\nu}$ = 1635 (C=O), 2189 (C≡N), 3420, 3328, 3233 (NH and NH₂) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ = 7.05-7.45 (m, 5H, Ar-H), 8.65 (s, 2H, NH₂), 11.20 (s, 2H, NH₂), 12.75 (s, 1H, NH). Anal. Calcd for C₁₂H₁₀N₄OS: C, 55.80; H, 3.90; N, 21.69; S, 12.41%. Found: C, 55.78; H, 3.88; N, 21.66; S, 12.38%.

2.2. 2-Acetyl-3-Amino-5-Phenylamino-Thiophene-4-Carboxylic Acid Amide (3)

Pale brown solid; yield 84%; mp 136 °C (EtOH); IR: $\bar{\nu}$ = 1628 (broad, C=O), 3408, 3311, 3227 (NH and NH₂) cm⁻¹; ¹H NMR (CDCl₃): δ = 2.20 (s, 3H, COCH₃), 7.15-7.70 (m, 5H, Ar-H), 8.55 (s, 2H, NH₂), 11.35 (s, 1H, NH), 14.10 (s, 2H, NH₂). Anal. Calcd for C₁₃H₁₃N₃O₂S: C, 56.71; H, 4.76; N, 15.26; S, 11.65%. Found: C, 56.68; H, 4.73; N, 15.22; S, 11.61%.

2.3. 3-Amino-2-Benzoyl-5-Phenylamino-Thiophene-4-Carboxylic Acid Amide (4)

Brown solid; yield 74%; mp 220 °C (EtOH); IR: $\bar{\nu}$ = 1622 (broad, C=O), 3398, 3305, 3187 (NH and NH₂) cm⁻¹; ¹H NMR (CDCl₃): δ = 7.10-7.75 (m, 10H, Ar-H), 8.40 (s, 2H, NH₂), 11.20 (s, 1H, NH), 14.25 (s, 2H, NH₂). Anal. Calcd for C₁₈H₁₅N₃O₂S: C, 64.08; H, 4.48; N, 12.45; S, 9.50%. Found: C, 64.04; H, 4.45; N, 12.42; S, 9.48%.

3. General Procedure for Synthesis of 3-Amino-4-Arylazo-2-Substituted-Thiophene Compounds (5a-c-7a-c)

A suspension of 3-amino-2-substituted-thiophene-4-carboxamide derivatives 2-4 (5mmol) in 25ml EtOH was added 20% aq KOH and was kept in refrigerator overnight. A freshly cooled solution of aryl diazonium chloride, which prepared by adding cold sodium nitrite solution (5mmol) to cold suspension of different aromatic amines (5mmol) in 3ml concentrated HCl, was then added dropwise with stirring to the cold suspension of thiophene derivatives 2-4. The reaction mixture was allowed to stir at (0-5°C) for additional 2 hours and kept overnight in refrigerator. The solid arylazo products (5-7a-c) were then filtered, dried and recrystallized from ethanol or ethanol-DMF mixture. The characterization data for compounds (5-7a-c) was shown in Table II.

4. General Procedure for Synthesis of 2-Amino-5-Arylazothiazoles (9a-c)

A solution of sodium nitrite (0.70g, 10mmol) in 10ml water was gradually added to a well cooled solution of the aromatic amine (10mmol) in conc. HCl (3.0ml). The diazonium salt solution was added with continuous stirring to an ice cooled solution of the 2-aminothiazole 8 (1g, 10mmol) in ethanol (50ml) and sodium acetate (3.8g). The reaction mixture was allowed to stand for 2 h and then filtered. The obtained 2-amino-5-arylazothiazoles (9a-c) were dried and recrystallized from the appropriate solvent.

5. Synthesis of 2-[N-(Chloroacetyl)Amino]-5-Arylazo-Thiazoles (10a-c)

To a solution of 2-amino-5-arylazothiazoles (9a-c) (10mmol) in 25ml DMF containing 0.5ml triethyl amine, chloroacetyl chloride (15mmol, 1.2ml) was added dropwise with stirring at room temperature. Stirring was continued for 2 hours and the reaction mixture was poured to ice cooled water to afford the corresponding 2-N-chloroacetyl amino derivatives (10a-c). The precipitate which formed was collected by filtration, dried and recrystallized from the appropriate solvent.

5.1. 2-[N-(Chloroacetyl)Amino]-5-Phenylazo-Thiazole (10a)

Greenish yellow solid (EtOH); yield 78%; mp 220-222°C; IR: $\bar{\nu}$ = 1686 (C=O), 3234 (NH) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ = 4.10 (s, 2H, CH₂), 7.10-7.50 (m, 5H, Ar-H), 7.70 (s, 1H, C-4 thiazole-H), 11.55 (s, 1H, NH). Anal. Calcd. for C₁₁H₉ClN₄OS (Mol. Wt.: 280.73): C, 47.06; H, 3.23; N, 19.96%. Found: C, 47.18; H, 3.26; N, 19.91%.

5.2. 2-[N-(Chloroacetyl)Amino]-5-(p-Tolyl)Azo-Thiazole (10b)

Yellowish brown solid (EtOH); yield 82%; mp 235-237 °C; IR: $\bar{\nu}$ = 1695 (C=O), 3204 (NH) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ = 2.35 (s, 3H, CH₃), 4.10 (s, 2H, CH₂), 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 C₁₂H₁₁ClN₄OS (Mol. Wt.: 294.76): C, 48.90; H, 3.76; N, 19.01%. Found: C, 48.79; H, 3.70; N, 19.08%.

5.3. 2-[N-(Chloroacetyl)Amino]-5-(p-Nitrophenyl)Azo-Thiazole (10c)

Dark brown solid (EtOH-DMF); yield 85%; mp 186-187 °C; IR: $\bar{\nu}$ = 1705 (C=O), 3276 (NH) cm⁻¹; ¹H NMR (300 MHz, DMSO): δ 4.20 (s, 2H, CH₂), 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 C₁₁H₈ClN₅O₃S (Mol. Wt.: 325.73): C, 40.56; H, 2.48; N, 21.50%. Found: C, 40.61; H, 2.46; N, 21.46%.

6. General Procedure for Synthesis of Thiocarbonyl Derivatives (11a, 11b And 11c)

The thiocarbonyl (11a, 11b and 11c) derivatives were prepared according to the previous literature [27].

TABLE II
CHARACTERIZATION DATA FOR COMPOUNDS (5a-c-7a-c) AND (12a-c-14a-c)

Compd. No. (Ar =)	Mol. Formula (M.Wt.)	Color	Yield %	M.p. °C	IR ν (cm ⁻¹)	¹ H NMR (Solvent) δ (ppm)	Elemental Analysis		
							C % Calc.	H (% Found)	N (% Found)
5a (C ₆ H ₆)	C ₁₇ H ₁₃ N ₅ S	Brown	68	226 (EtOH)	2192 (C≡N), 3401, 3334, 3208 (NH and NH ₂)	(DMSO-d ₆) 7.20-7.75 (m, 10H, Ar-H), 11.20 (s, 2H, NH ₂), 12.35 (s, 1H, NH)	63.93 (63.90)	4.10 (4.08)	21.93 (21.90)
5b (p-Me.C ₆ H ₄)	C ₁₈ H ₁₅ N ₅ S	Dark brown	57	165 (EtOH)	2195 (C≡N), 3392, 3283, 3210 (NH and NH ₂)	(DMSO-d ₆) 2.40 (s, 3H, CH ₃), 7.10-7.60 (m, 9H, Ar-H), 10.85 (s, 2H, NH ₂), 12.80 (s, 1H, NH).	64.84 (64.83)	4.53 (4.52)	21.01 (20.9)
5c (p-NO ₂ .C ₆ H ₄)	C ₁₇ H ₁₂ N ₆ O ₂ S	Violet	82	260 (EtOH)	2190 (C≡N), 3393, 3315, 3216 (NH and NH ₂)	(DMSO-d ₆) 7.25-7.75 (m, 9H, Ar-H), 11.85 (s, 2H, NH ₂), 13.30 (s, 1H, NH).	56 (56.01)	3.32 (3.28)	23.06 (23.02)
6a (C ₆ H ₆)	C ₁₈ H ₁₆ N ₄ OS	Greenish brown	74	155 (EtOH)	1622 (C=O), 3376, 3278, 3198 (NH and NH ₂)	(CDCl ₃) 2.20 (s, 3H, COCH ₃), 7.20-7.55 (m, 10H, Ar-H), 11.40 (s, 1H, NH), 13.75 (s, 2H, NH ₂)	64.26 (64.23)	4.79 (4.75)	16.65 (16.61)
6b (p-Me.C ₆ H ₄)	C ₁₉ H ₁₈ N ₄ OS	Dark brown	86	147 (EtOH)	1636 (C=O), 3399, 3288, 3211 (NH and NH ₂)	(CDCl ₃) 2.20 (s, 3H, CH ₃), 2.40 (s, 3H, CH ₃), 7.15-7.65 (m, 9H, Ar-H), 11.15 (s, 1H, NH), 13.90 (s, 2H, NH ₂).	65.12 (65.08)	5.18 (5.14)	15.99 (15.96)
6c (p-NO ₂ .C ₆ H ₄)	C ₁₈ H ₁₅ N ₅ O ₃ S	Green	90	239 (EtOH- DMF)	1635 (C=O), 3422, 3328, 3221 (NH and NH ₂)	(DMSO-d ₆) 2.20 (s, 3H, COCH ₃), 7.25-7.75 (m, 9H, Ar-H), 12.55 (s, 1H, NH), 14.45 (s, 2H, NH ₂)	56.68 (56.63)	3.96 (3.94)	18.36 (18.31)
7a (C ₆ H ₆)	C ₂₃ H ₁₈ N ₄ OS	Red	78	181 (EtOH)	1609 (C=O), 3412, 3326, 3174 (NH and NH ₂)	(DMSO-d ₆) 7.15-7.75 (m, 15H, Ar-H), 10.85 (s, 1H, NH), 13.25 (s, 2H, NH ₂)	69.32 (69.30)	4.55 (4.51)	14.06 (14.01)
7b (p-Me.C ₆ H ₄)	C ₂₄ H ₂₀ N ₄ OS	Red	84	174 (EtOH)	1611 (C=O), 3421, 3308, 3185 (NH and NH ₂)	(DMSO-d ₆) 2.35 (s, 3H, CH ₃), 7.10-7.80 (m, 14H, Ar-H), 11.30 (s, 1H, NH), 13.55 (s, 2H, NH ₂)	69.88 (69.85)	4.89 (4.85)	13.58 (13.56)
7c (p-NO ₂ .C ₆ H ₄)	C ₂₃ H ₁₇ N ₅ O ₃ S	Violet	82	286 (EtOH- DMF)	1618 (C=O), 3435, 3343, 3227 (NH and NH ₂)	(DMSO-d ₆) 7.10-7.90 (m, 14H, Ar-H), 11.85 (s, 1H, NH), 14.15 (s, 2H, NH ₂)	62.29 (62.25)	3.86 (3.82)	15.79 (15.76)
12a (C ₆ H ₆)	C ₂₃ H ₂₀ N ₆ O ₃ S ₂ (492.57)	Brown	74	187 (EtOH)	1646, 1658 (C=O), 3417, 3352, 3218 (NH and NH ₂)	(CDCl ₃) 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)	56.08 (56.26)	4.09 (4.16)	17.06 (17.02)
12b (p-Me.C ₆ H ₄)	C ₂₄ H ₂₂ N ₆ O ₃ S ₂ (506.6)	Yellowish brown	62	181 (EtOH)	1644, 1662 (C=O), 3384, 3312, 3248 (NH and NH ₂)	(CDCl ₃) 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).	56.90 (56.77)	4.38 (4.32)	16.59 (16.67)
12c (p-NO ₂ .C ₆ H ₄)	C ₂₃ H ₁₉ N ₇ O ₅ S ₂ (537.57)	Brown	78	173 (EtOH- DMF)	1650, 1664 (C=O), 3422, 3367, 3274 (NH and NH ₂)	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)	51.39 (51.46)	3.56 (3.52)	18.24 (18.32)
13a (C ₆ H ₆)	C ₂₁ H ₁₇ N ₇ O ₂ S ₂ (463.54)	Yellowish brown	72	232 (EtOH)	1645 (C=O), 3411, 3346, 3262 (NH and NH ₂)	(CDCl ₃ /DMSO) 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)	54.41 (54.20)	3.70 (3.62)	21.15 (21.22)
13b (p-Me.C ₆ H ₄)	C ₂₂ H ₁₉ N ₇ O ₂ S ₂ (477.56)	Brown	64	196 (EtOH)	1648 (C=O), 3384, 3342, 3262 (NH and NH ₂)	(CDCl ₃ /DMSO) 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)	55.33 (55.12)	4.01; (4.07)	20.53 (20.62)
13c (p-NO ₂ .C ₆ H ₄)	C ₂₁ H ₁₆ N ₈ O ₄ S ₂ (508.53)	Dark brown	62	173 (EtOH)	1651 (C=O), 3412, 3440, 3267 (NH and NH ₂)	(DMSO) 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)	49.60 (49.82)	3.17 (3.10)	22.03 (22.09)
14a (C ₆ H ₆)	C ₂₄ H ₂₁ N ₅ O ₃ S ₂ (491.59)	Brown	77	193 (EtOH)	1641, 1661 (C=O), 3311,	(CDCl ₃) 1.30 (t, 3H, CH ₃), 2.70 (s, 3H, CH ₃),	58.64 (58.48)	4.31 (4.26)	14.25 (14.28)

14b	C ₂₅ H ₂₃ N ₃ O ₃ S ₂ (<i>p</i> -Me.C ₆ H ₄) (505.61)	Brown	83	175 (<i>EtOH</i>)	1640, 1664 (C=O), 3288, 3214 (NH)	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) (CDCl ₃)	59.39 (59.46)	4.59 (4.62)	13.85 (13.78)
14c	C ₂₄ H ₂₀ N ₆ O ₅ S ₂ (<i>p</i> -NO ₂ .C ₆ H ₄) (536.58)	Brown	81	161 (<i>EtOH</i>)	1644, 1665 (C=O), 3308, 3216, (NH)	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) (CDCl ₃ /DMSO)	53.72 (53.87)	3.76 (3.78)	15.66 (15.57)

7. General Procedure for The Synthesis of 2-(Phenylamino)-5-(5-Arylazothiazol-2-yl)-Thiophene Compounds (12a-C-14a-C)

Mixtures of thiocarbamoyl derivatives (11a-c) (5 mmol) was heated with sodium ethoxide solution (5 mmol, 0.1 g sodium metal in 10 ml absolute ethanol), then the chloroacetyl derivatives (10a-c) (5 mmol) were added. The mixtures were refluxed for 15 minutes. The resultant solid products were collected by filtration, dried and recrystallized from the appropriate solvent. The characterization data for compounds (12a-c- 14a-c) was shown in Table II.

C. Antimicrobial Test Method

The Agar Dilution method was used to evaluate the *in vitro* antimicrobial activity of the synthesized compounds (5a-c-7c and 12a-c-14a-c), where stationary – phase cultures of bacteria were prepared at 37°C and used to inoculate fresh 5.0ml culture to an optical density at 600nm (OD₆₀₀) of 0.05 the 5.0 ml cultures were then incubated at 37°C until an OD₆₀₀ of 0.10 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6 x 10⁵ CFU/ml. Serial dilutions from the treatments (0–320µg/ml) were prepared and mixed with 5.0ml of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37°C. The colony forming units (CFU) 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 mm. The relationships between the biological activity and the chemical structure of the synthesized compounds were discussed.

IV. CONCLUSION

We synthesized a set of functionalized 3-amino-4-arylaazo-2-substituted-thiophene compounds (5a-c-7a-c), by the reaction of 2-cyano-2-phenylthiocarbamoyl acetamide with various α -halogenated reagents, followed by azo-coupling with different diazotized aromatic, and another set of 5-(5-arylaazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophenes (12a-c-14a-c) by cyclocondensation of 5-arylaazo-2-(*N*-chloroacetyl)amino-thiazoles with various thiocarbamoyl derivatives. In general, all the synthesized compounds displayed positive antimicrobial activities (>10 mm inhibition zone in most cases) against the different pathogenic strains

tested Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus flavus*) with various degrees according to their chemical structure and attached functional groups, whereas the thiazolyl-thiophene compounds showed no activities against fungal streams used in this study (*Aspergillus flavus* and *Candida albicans*).

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