International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:4, 2014

# *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-arylazo-thiazol-2-ylcarbamoyl-thiophene derivatives from base-catalyzed intramolecular condensation of 5-arylazo-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-arylazo-thiophene and thiazolyl-thiophene moieties as potential biological active compounds against selected gram positive bacteria, gram negative bacteria and Fungi.

M. E. Khalifa (corresponding author) is with the Faculty of Science, Department of Chemistry, Taif University, Taif 21974, Saudi Arabia , and with the Higher Institute of Engineering and Technology, Department of Chemical Engineering, New Damietta, Egypt (phone: +966569846966, e-mail: mohamedezzat200@hotmail.com).

A. A. Gobouri is with the Faculty of Science, Department of Chemistry, Taif University, Taif 21974, Saudi Arabia (phone:+966530059592, e-mail: agobouri00@yahoo.com).

## 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 nuceophillic 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-arylazo-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-arylazothiazoles (9a-c). The 2-Amino-5-arylazothiazoles (9a-c) were reacted with chloroacetyl chloride in dimethyl formamide (DMF) containing some drops of triethyl amine (TEA) to afford the corresponding 5-arylazo-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

corresponding thiocarbamoyl derivatives (11a, 11b, and 11c) [27], which underwent condensation reaction with 5-arylazo-2-(*N*-chloroacetyl)amino-thiazoles (10a-c) in ethanol and sodium ethoxide to furnish the corresponding ethyl 4-amino-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxylates 12, 4-amino-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino- thiophene-3-carboxamides 13 and ethyl 4-methyl-5-(5-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophene-3-carboxylates 14, respectively (Fig. 2) [25].

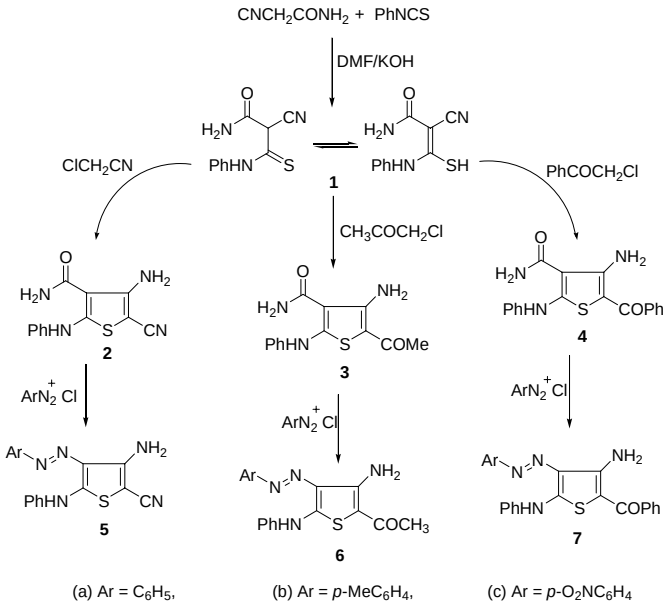


Fig. 1 Synthesis of corresponding 3-amino-4-arylazo-thiophenes (5-7a-c)

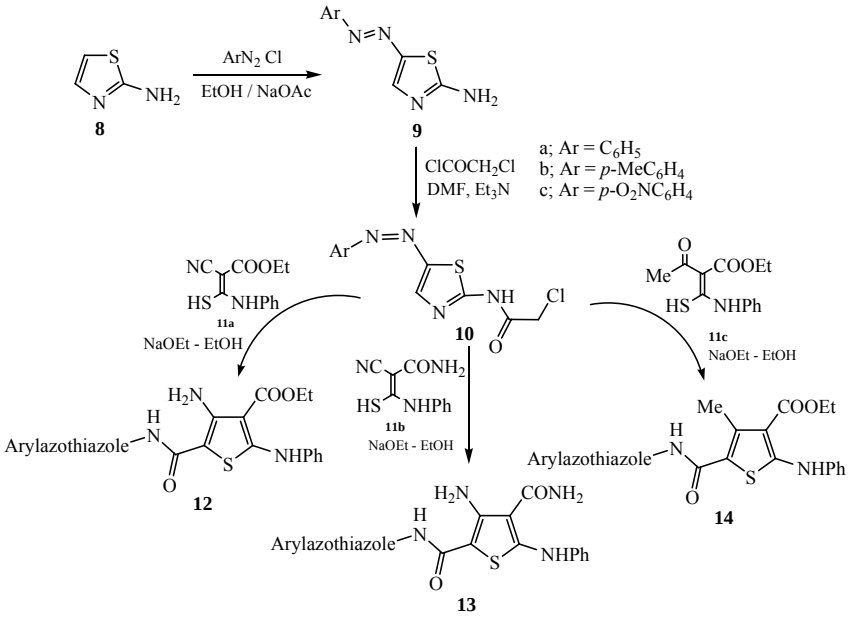


Fig. 2 Synthesis of substituted 5-arylazo-thiazol-2-ylcarbamoyl-2-phenylamino-thiophene compounds (12a-c-14a-c)

The IR spectrum of e.g. 12b displayed bands at 1644, 1662 $cm^{-1}$ assignable to the carbonyl functions and bands at 3384, 3312, 3248$cm^{-1}$ for the NH and $NH_2$ functions. The $^1H$ NMR spectrum of the same compound revealed a triplet signal at δ 1.30ppm due to methyl protons ($OCH_2\underline{CH_3}$), a singlet signal at δ 2.40ppm for methyl protons (Ar-$\underline{CH_3}$), a quartet signal at δ 4.25ppm for methylene protons ($O\underline{CH_2}CH_3$), 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 $NH_2$ 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) | | | | | |
|---|---|---|---|---|---|---|
| | ***Bacillus subtilis*** **G+** | ***Staphylococcus aureus*** **G+** | ***Escherichia coli*** **G-** | ***Pseudomonas aeruginosa*** **($G^-$)** | ***Aspergillus flavus*** **(Fungus)** | ***Candida albicans*** **(Fungus)** |
| **Tetracycline (Bacterial 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 |

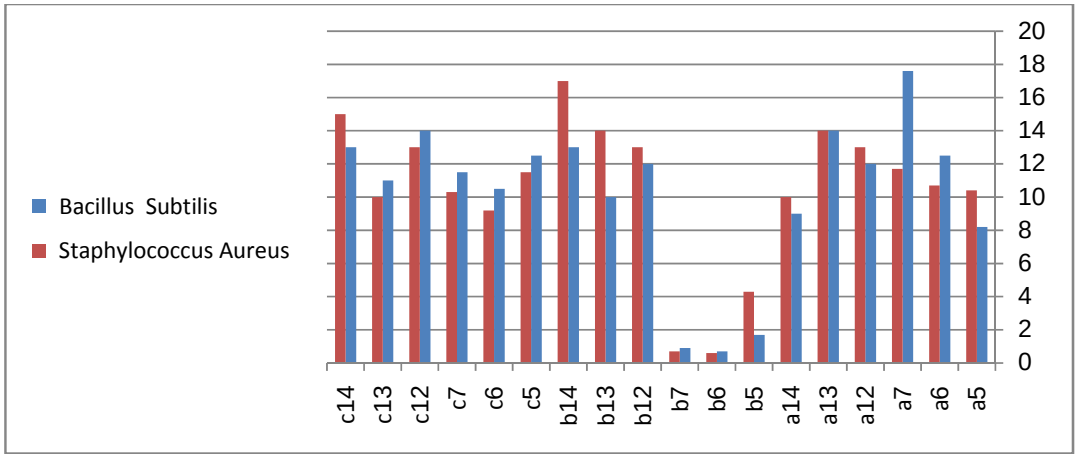


Fig. 3 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against G+ bacteria

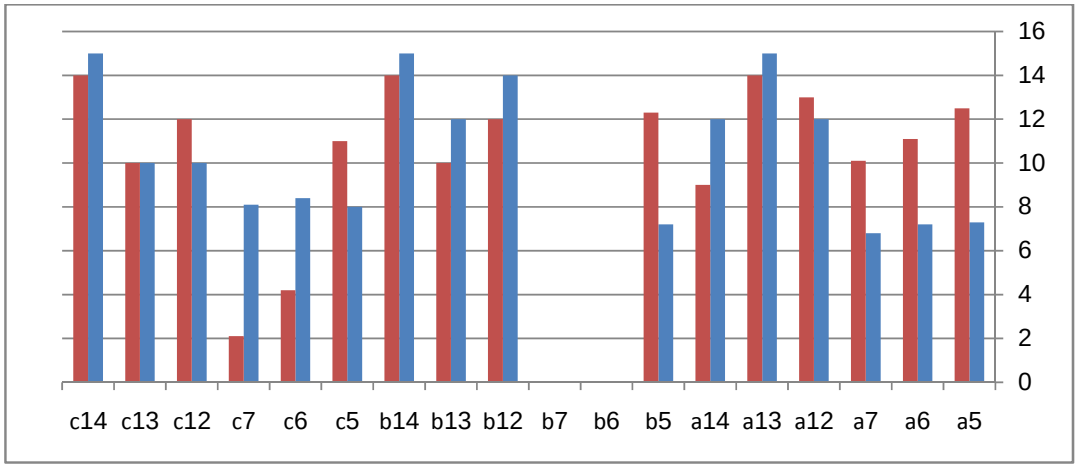


Fig. 4 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against G- bacteria

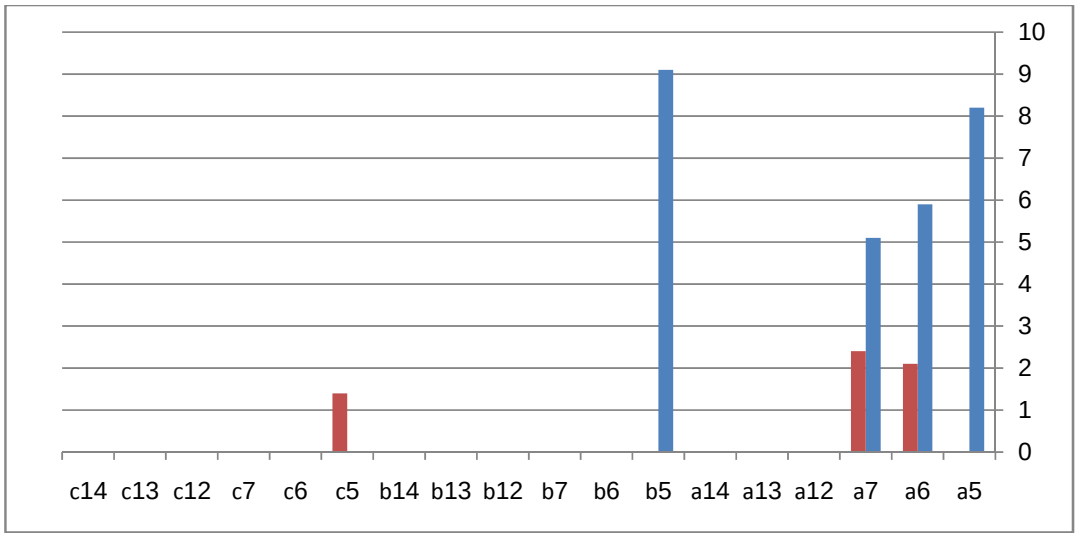


Fig. 5 Antimicrobial behavior of dyes 5a-c-7a-c and 12a-c-14a-c against selected fungi

## 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 (±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 $^{1}$H 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 ($^{1}$H 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-Phenylthiocarbamoyl 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 160-4ºC (EtOH); IR: $\overline{\nu}$ = 1642 (C=O), 2190 (CN), 3421, 3330 ($NH_2$) $cm^{-1}$. Anal. Calcd for $C_{10}H_9N_3OS$: 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-phenylthiocarbamoyl acetamide 1(5mmol) in 25ml dimethyl formamide (*DMF*) were added solid potassium carbonate (2 equiv) and the appropriate $\alpha$-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 $H_2O$ 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 186ºC (EtOH); IR: $\overline{V}$ = 1635 (C=O), 2189 (C≡N), 3420, 3328, 3233 (NH and $NH_2$) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 7.05-7.45 (m, 5H, Ar-H), 8.65 (s, 2H, $NH_2$), 11.20 (s, 2H, $NH_2$), 12.75 (s, 1H, NH). Anal. Cacld for $C_{12}H_{10}N_4OS$: 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 ºC (*Et*OH); IR: $\overline{V}$ = 1628 (broad, C=O), 3408, 3311, 3227 (NH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 2.20 (s, 3H, $COCH_3$), 7.15-7.70 (m, 5H, Ar-H), 8.55 (s, 2H, $NH_2$), 11.35 (s, 1H, NH), 14.10 (s, 2H, $NH_2$) ). Anal. Cacld for $C_{13}H_{13}N_3O_2S$: 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 ºC (*Et*OH); IR: $\overline{V}$ = 1622 (broad, C=O), 3398, 3305, 3187 (NH and $NH_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ = 7.10-7.75 (m, 10H, Ar-H), 8.40 (s, 2H, $NH_2$), 11.20 (s, 1H, NH), 14.25 (s, 2H, $NH_2$). Anal. Cacld for $C_{18}H_{15}N_3O_2S$: 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 (0-5ºC) 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 220-222ºC; IR: $\overline{V}$ = 1686 (C=O), 3234 (NH) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 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 $C_{11}H_9ClN_4OS$ (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 ºC; IR: $\overline{V}$ = 1695 (C=O), 3204 (NH) $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ = 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 $C_{12}H_{11}ClN_4OS$ (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 ºC; IR: $\overline{V}$ = 1705 (C=O), 3276 (NH) $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO): $\delta$ 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 $C_{11}H_8ClN_5O_3S$ (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 Thiocarbamoyl Derivatives (11a, 11b And 11c )

The thiocarbamoyl (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 % Calc. (% Found) C | H | N |
|---|---|---|---|---|---|---|---|---|---|
| 5a ($C_6H_6$) | $C_{17}H_{13}N_5S$ | Brown | 68 | 226 (*Et*OH) | 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.$C_6H_4$) | $C_{18}H_{15}N_5S$ | Dark brown | 57 | 165 (*Et*OH) | 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*-$NO_2$.$C_6H_4$) | $C_{17}H_{12}N_6O_2S$ | Violet | 82 | 260 (*Et*OH) | 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 ($C_6H_6$) | $C_{18}H_{16}N_4OS$ | Greenish brown | 74 | 155 (*Et*OH) | 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.$C_6H_4$) | $C_{19}H_{18}N_4OS$ | Dark brown | 86 | 147 (*Et*OH) | 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*-$NO_2$.$C_6H_4$) | $C_{18}H_{15}N_5O_3S$ | Green | 90 | 239 (*Et*OH-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 ($C_6H_6$) | $C_{23}H_{18}N_4OS$ | Red | 78 | 181 (*Et*OH) | 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.$C_6H_4$) | $C_{24}H_{20}N_4OS$ | Red | 84 | 174 (*Et*OH) | 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*-$NO_2$.$C_6H_4$) | $C_{23}H_{17}N_5O_3S$ | Violet | 82 | 286 (*Et*OH-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 ($C_6H_6$) | $C_{23}H_{20}N_6O_3S_2$ (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.$C_6H_4$) | $C_{24}H_{22}N_6O_3S_2$ (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*-$NO_2$.$C_6H_4$) | $C_{23}H_{19}N_7O_5S_2$ (537.57) | Brown | 78 | 173 *(EtOH-DMF)* | 1650, 1664 (C=O), 3422, 3367, 3274 (NH and $NH_2$) | $CDCl_3$/DMSO): $\delta$= 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 ($C_6H_6$) | $C_{21}H_{17}N_7O_2S_2$ (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.$C_6H_4$) | $C_{22}H_{19}N_7O_2S_2$ (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*-$NO_2$.$C_6H_4$) | $C_{21}H_{16}N_8O_4S_2$ (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 ($C_6H_6$) | $C_{24}H_{21}N_5O_3S_2$ (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) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 3256 (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) | | | |
| 14b ($p$-Me.$C_6H_4$) | $C_{25}H_{23}N_5O_3S_2$ (505.61) | Brown | 83 | 175 *(EtOH)* | 1640, 1664 (C=O), 3288, 3214 (NH) | ($CDCl_3$) 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) | 59.39 (59.46) | 4.59 (4.62) | 13.85 (13.78) |
| 14c ($p$-$NO_2$.$C_6H_4$) | $C_{24}H_{20}N_6O_5S_2$ (536.58) | Brown | 81 | 161 *(EtOH)* | 1644, 1665 (C=O), 3308, 3216, (NH) | ($CDCl_3$/DMSO) 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) | 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 ($OD_{600}$) 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 x $10^5$ 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-arylazo-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-arylazo-thiazol-2-ylcarbamoyl)-2-phenylamino-thiophenes (12a-c-14a-c) by cyclocondensation of 5-arylazo-2-(*N*-chloroacetyl)amino-thiazoles with various thiocabamoyl 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*).

## Acknowledgment

The authors want to dedicate this work with great honor to their great master, Prof. Dr. Mohamed A. Metwally (May God bless his soul) for his fabulous efforts as long as his life, serving the human beings in the field of teaching and scientific research.

## References

[1] K. Gewald, E. Schinke and H. Böttcher, "Heterocyclen aus CH-aciden Nitrilen, VIII. 2-Amino-thiophene aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel," *Chem Ber*., vol. 99, pp. 94-100.

[2] T. A. Grese, S. Cho, D. R. Finley, A. G. Godfrey, C. D. Jones, C. Lugar, M. Martin, K. .Matsumoto, L. Pennington, M. A. Winter, M. D. Adrian, H. W. Cole, D. E. Magee, D. L. Phillips, E. R. Rowley, L. L. Short, A. L. Glasebrook and H. U. Bryant, "Structure−Activity Relationships of Selective Estrogen Receptor Modulators: Modifications to the 2-Arylbenzothiophene Core of Raloxifene," *J. Med. Chem*., vol. 40, pp. 146-167.

[3] K. Takeuchi, T. J. Kohn, D. J. Sall, M. L. Denney, J. R. McCowan, G. F. Smith and D. S. Gifford-Moore, "Dibasic benzo[b]thiophene derivatives as a novel class of active site directed thrombin inhibitors: 4. SAR studies on the conformationally restricted C3-side chain of hydroxybenzo[b]thiophenes," *Bioorg. Med. Chem. Lett*., vol. 9, pp. 759-764.

[4] R. Romagnoli, P. G. Baraldi, M. D. Carrion, C. L. Cara, D. Preti, F. Fruttarolo, M. G. Pavani, M. A. Tabrizi, M. Tolomeo, S. Grimaudo; A. Di Cristina, J. Balzarini, J.A. Hadfield, A. Brancale and E. Hamel, "Synthesis and Biological Evaluation of 2- and 3-Aminobenzo[b]thiophene Derivatives as Antimitotic Agents and Inhibitors of Tubulin Polymerization," *J. Med. Chem.*, vol. 50, pp. 2273-2277.

[5] T.A. Miller, D.J. Witter, J. David and S. Belvedene, "Preparation of thiophene and benzothiophene hydroxamic acid derivatives as histone deacetylase inhibitors," PCT Inl. Appl. WO, 2005, 034880, *Chem. Abstr*. 2005, 142, 392278.

[6] S. T. Branum, R. W. Colburn, S. L. Dax, C. M. Flores, M. C. Jetter, Y. Liu, D. Ludovici, M. J. Macielag, J. M. Matthews, J. J. Mcnally, L. M. Reaney, R. K. Russell, N. Qin, K. M. Wells, S. C. Youells and M. A. Youngman, "Sulfonamides as TRPM8 modulators," PCT Int. Appl. WO, 2009, 012430, *Chem. Abstr*. 2009, 150, 168153.

[7] M. A. A. Radwan, M. A. Shehab and S. M. El-Shenawy, "Synthesis and biological evaluation of 5-substituted benzo[b]thiophene derivatives as anti-inflammatory agents," *Monatsch. Chem*., vol. 140, pp. 445-450.

[8] G. H. Merriman, P. M. Weintraub, J. S. Sabol, R. Dharanipragada, N. J. Hrib, J. G. Jurcak, A. Gross, B. Whiteley, K. Y. Musick and J. Klein, "Preparation of substituted 3-aminoindolecarboxylates and 3-aminobenzothiophenes as interleukin-4 gene expression inhibitors," PCT Int. Appl. WO, 2003, 091215, *Chem. Abstr*. 2003, 139, 364826.

[9] T. A.Grese, L. D. Pennington, J. P. Sluka, M. D. Adrian, H. W. Cole, T. R. Fuson, D. E. Magee, D. L. Phillips, E. R. Rowley, P. K. Shetler, L. L. Short, M.Venugopalan, N. N. Yang, M. Sato, A. L. Glasebrook and H. U. Bryant, "Synthesis and Pharmacology of Conformationally Restricted Raloxifene Analogues: Highly Potent Selective Estrogen Receptor Modulators," *J. Med. Chem*., vol. 41, pp. 1272-1283.

[10] T. A. Grese, S. Cho, D. R. Finley, A. G. Godfrey, C. D. Jones, C. Lugar, M. Martin, K. .Matsumoto, L. Pennington, M. A. Winter, M. D. Adrian, H. W. Cole, D. E. Magee, D. L. Phillips, E. R. Rowley, L. L. Short, A. L. Glasebrook and H. U. Bryant, "Structure−Activity Relationships of Selective Estrogen Receptor Modulators: Modifications to the 2-Arylbenzothiophene Core of Raloxifene," *J. Med. Chem.*, vol. 40, pp. 146-167.

[11] K. C. Lee, B. C. Moon, J. H. Lee, K.-H. Chung, J. A. Katzenellenbogen and D. Y. Chi, "Synthesis and binding affinities of fluoroalkylated raloxifenes," *Bioorg. Med. Chem*., vol. 11, pp. 3649-3658.

[12] U. G. Ibatullin, T. F. Petrushina, L. Y. Leitis, I. Z. Minibaev and B. O. Logvin, "Synthesis and transformations of 4-substituted 2-aminothiazoles," Chem Hetrocycl Compd, 1993, vol. 12, pp. 612-615.

[13] P. C. Hang and J. F. Honek, "Electronic structure calculations on the thiazole-containing antibiotic thiostrepton: molecular mechanics, semi-empirical and ab initio analyses," *Bioorg. Med. Chem. Lett.*, 2005, 15, 1471-1474.

[14] P. Beuchet, M. Varache-Lembège, A. Neveu, J. M. Léger, J. Vercauteren, S. Larrouture, G. Deffieux and A. Nuhrich, "New 2-sulfonamidothiazoles substituted at C-4: synthesis of polyoxygenated aryl derivatives and in vitro evaluation of antifungal activity," *Eur. J. Med. Chem.*, 1999, 34, 773-779.

[15] A. Geronikaki, P. Vicini, N. Dabarakis, A. Lagunin, V. Poroikov, J. Dearden, H. Modarresi, M. Hewitt and G. Theophilidis, "Evaluation of the local anaesthetic activity of 3-aminobenzo[d]isothiazole derivatives using the rat sciatic nerve model," *Eur. J. Med. Chem*., 2009, vol. 44, pp. 473-481.

[16] C. Papadopoulou, A. Geronikaki and D. Hadjipavlou-Litina,"Synthesis and biological evaluation of new thiazolyl/benzothiazolyl-amides, derivatives of 4-phenyl-piperazine," *II Farmaco*, 2005, vol. 60, pp. 969-973.

[17] A. Kreutzberger and H. Schimmelpfennig, "Antivirale Wirkstoffe, 18. Mitt. 2-Aminothiazole durch Spaltung der S-S-Bindung des Disulfidodicarbamidins," *Arch. Pharm*., 1981, vol. 314, pp. 385-391.

[18] M. A. Metwally, M. E. Khalifa and F. A. Amer, "New azodisperse dyes with 4-hydroxymethyl-2-pyrazolin-5-one ring for dyeing polyester fabrics Part 5," *Dyes and Pigments*, 2008, vol. 76, pp. 379- 385.

[19] M. A. Metwally, M. E. Khalifa and F. A. Amer, " New Azo Disperse Dyes with 1,3-Diphenyl-4- Hydroxymethyl- 4- Arylazo –2- Pyrazolin – 5- Ones for Dyeing Polyester Fabrics," MensAgitat, 2009, vol. 4, pp. 81-88.

[20] H. E. Gaffer, M. E. Khalifa, S. F. El-Bially and M. A. Metwally, "New Azo disperse Dyes Containing Cyclohexanone Ring For Dyeing Polyester and Nylon Fabrics," *Macdonian Journal of Chemistry and Chemical Engineering*, 2010, vol. 29, pp. 87-95.

[21] E. Abdel-Latif, M. A. Metwally, F. A. Amer and M. E. Khalifa," Syntheses of some 5-arylazo-2-(arylidenehydrazino)-thiazole disperse dyes for dyeing polyester fibers," *Pigment and Resin Technology*, 2009, vol. 38, pp. 105–110.

[22] M. A. Metwally, M. E. Khalifa, E. Abdel-Latif and F. A. Amer,"New arylhydrazonothiazolidin-5-one disperse dyes for dyeing polyester fibers," *Polish Journal of Chemical Technology*. 2010, vol. 12, pp. 1-6.

[23] M. E. Khalifa, M. A. Metwally, E. Abdel-Latif and F. A. Amer, "Synthesis of Some New 5-Arylazothiazole Derivatives as Disperse Dyes for Dyeing Polyester Fibers," *International Journal of Textile Science*. 2012, vol. 1, pp. 62-68.

[24] M. E. Khalifa, M. A. Amin and E. Abdel-latif, "Synthesis of Some Novel 3-Amino-4-arylazothiophene Disperse Dyes of Biological Activity for Dyeing Polyester Fabrics and Their Application as Medical Textiles," *International Journal of Chemistry,* vol.34, pp. 1157-1166.

[25] M. E. Khalifa, E. Abdel-Latif and A. A. Gobouri," Disperse dyes based on 5-arylazo-thiazol-2-ylcarbamoyl-thiophenes: synthesis, antimicrobial activity and their application on polyester," *J. Heterocyclic Chem.*, 2014, under press DOI 10.1002/jhet.2153

[26] F. R. Japp, F. Klingemann, "Ueber sogenannte gemischte Azoverbindungen," *Berichte der deutschen chemischen Gesellschaft*, 1887, vol. 20, pp. 3398–3401.

[27] M. A. Metwally, E. Abdel-latif and F. A. Amer, "Synthesis and reactions of some thiocarbamoyl derivatives," *Sulfur Lett,* 2003, vol. 26, pp. 119-126.

[28] National Committee for Clinical Laboratory Standards. 1997, "Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically," Approved standard, 4th ed. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.