Biological Impact of Certain Substituted Monoazo Thiazole-thiophene and Their Seleno Like Moieties: Part I

Mohamed E Khalifa[1,2*] and Adil A Gobouri[1]

***Abstract*—** Thiophene and thiazole along with their seleno like nuclei are still of interest in organic chemistry due to their medicinal and valuable applications. In continuation of our interest in synthesis of S/N heterocyclic azo compounds, the synthesis of 3-amino-*N*-(4-aryl-5-arylazo-2-thiazolyl)-thieno[2,3-*b*]pyridine-2-carboxamide) along with their seleno like derivatives of pyridine, pyridazine and quinolone, were accomplished. All the synthesized compounds were *in vitro* screening of their antioxidant activity, antitumor activity against Ehrlich ascites carcinoma cell EACC cell line and antimicrobial activity against various pathogenic microorganisms, such as Gram-positive bacteria *(Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli* and *Salmonella typhimurium*) and fungi strains of *Aspergillus flavus* and *Candida albicans.*The structural–activity relationship was studied based on the obtained data.

***Keywords*—**2- Aminothiazole, thiophene, Antioxidant, Antitumor agents, pathogenic microorganisms, Selenium

I. Introduction

THE heterocyclic based mono azo compounds are not only important due to their excellent properties as dyes for polyester textiles but they also have been utilised in non-textile applications, such as photodynamic therapy, lasers, reprographic technology, functional dye applications and non-linear optical systems [1], [2]. The attraction interest in the field of thiazolyl azo compounds has grown, and these compounds have been extensively investigated to produce medicinal properties (i.e., antitumor activity, cytotoxic, antimicrobial, anti-inflammatory, mitodepressive, hypotensive, anti-HIV, hypoallergenic, tuberculosis, and agriculture pesticide action) [3]. Organo-selenium compounds have a wide range of unique properties, which include antimicrobial, antitumor and anticancer activities as well, where many medicinal preparations have been produced based on organic derivatives of selenium [4], [5]. In addition, organo-selenium compounds are capable of sensitizing processes in living organisms, and the selenium atom is also a primary constituent of four proteins, where its deficiency in human and animal organisms might be related to various chronic diseases, especially necrosis of the liver [6]. In a continuation of our previous researches on the synthesis and important applications of S/N-Se heterocyclic compounds [7]-[11], we introduce an interesting biological study of their extensive activities as antitumor, antioxidant and antimicrobial compounds.

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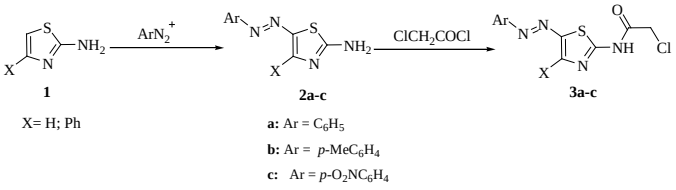
II. Results and Discussion

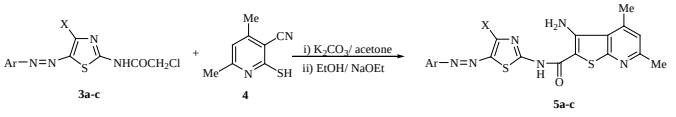
A. Chemistry

A set of 4-substituted-2-(*N*-chloroacetyl)-5-arylazothiazole derivatives **3a-c**, was prepared by azo coupling of 4-substituted-2-aminothiazole **1** with various diazotized aromatic amines to yield the 4-substituted-2-amino-5-arylazothiazole derivatives **2a-c**, followed by active condensation with chloroacetyl chloride as shown in scheme 1 [10], [11].



Scheme 1 Synthesis of 5-arylazo-2-chloro-*N*-(thiazol-2-yl)acetamide derivatives **3a-c.**

The 2-(*N*-chloroacetyl)-5-arylazo-4-substituted-thiazole derivatives **3a-c** were reacted with 4,6-dimethyl-2-mercaptonicotinonitrile **4** by refluxing in acetone containing sodium carbonate, followed by cyclization upon heating in a solution of ethanol containing sodium ethoxide to afford the corresponding thieno-[2,3-*b*]pyridine derivatives **5a-c** (Scheme 2) [9].



Scheme 2 Synthesis of thieno-[2,3-*b*]pyridine derivatives **5a-c**

The chemical structures of **5a-c** were established on the basis of their elemental analyses and spectral data and in

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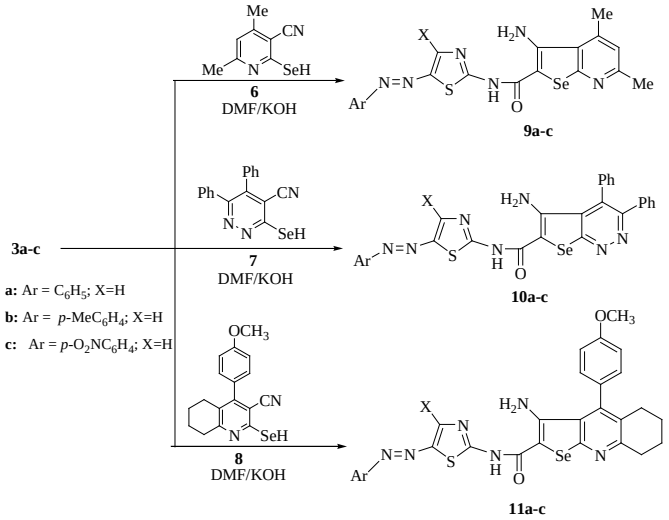
agreement with the literature. Where the IR spectrum of **5a** for example, clearly indicated the lack of cyano absorption band and revealed the characteristics of NH_2 absorption bands at 3177, 3280 cm^{-1} in addition to the carbonyl absorption band at 1632 cm^{-1}. The strong decrease in the carbonyl absorption frequencies is attributed to the highly chelated intramolecular H-bond structure. The ^1H NMR spectrum of **5b** for example, confirmed the lack of the singlet signal that characterized the methylene protons and showed three singlet signals corresponding to the three methyl protons at δ 2.4, 2.8 and 3.0, in addition to the singlet signal at δ 7.3 for the pyridine C_5-H proton and multiplet signal at δ 7.4-7.9 for the aromatic protons.

A one pot synthesis reaction of 4-substituted-5-arylazo-2-chloro-*N*-(thiazol-2-yl)acetamide derivatives **3a-c** with selenol derivatives of pyridine **6**, pyridazine **7** and quinoline **8** in dimethyl formamide (DMF) containing 10 % aqueous potassium hydroxide, followed by Thorpe-Ziegler cyclization by treatment with an excess of 10% aqueous potassium hydroxide in (DMF), yield the corresponding 3-amino-4,6-dimethyl-*N*-(5-arylazothiazol-2-yl)selenopheno[2,3-b]pyridine-2-carboxamide derivatives **9a-c**, 3-amino-4,6-diphenyl-*N*-(5-arylazothiazol-2-yl)selenopheno[2,3-b]pyridazine-2-carboxamide derivatives **10a-c** and 3-amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-*N*-(5-arylazothiazol-2-yl)selenopheno[2,3-b]quinoline-2-carboxamide derivatives **11a-c** (Scheme 3) [11].



Scheme 3 Synthesis of 3-amino-*N*-(5-arylazothiazol-2-yl)selenopheno[2,3-b]pyridine, pyridazine and/or quinoline-2-carboxamide derivatives "**9a-c-11a-c**"

The chemical structures of selenol derivatives "**9a-c-11a-c**" were established based on their elemental analysis and spectral data and in agreement with the literature. The IR spectra of 3-amino-*N*-(5-arylazothiazol-2-yl)selenopheno[2,3-*b*]pyridine, pyridazine and/or quinoline-2-carboxamide derivatives "**9a-c-11a-c**" in common, clearly indicated the absence of the cyano group band and the appearance of characteristics absorption bands at 3177 and 3280 cm^{-1}corresponding to the NH_2 group as well as at 1652 cm^{-1} corresponding to the carbonyl group. The strong decrease in the carbonyl absorption frequencies was due to the highly chelated intramolecular H-bonded structure. For example, the ^1H NMR spectrum of **9b** confirmed the absence of the characteristic signal of the methylene group. In addition, the results exhibited three singlet peaks corresponding to the three methyl protons at δ 2.44, 2.83, 3.09, a singlet peak at δ 7.33 corresponding to the pyridine C5-H proton and multiplet peaks at δ 7.47-7.95 corresponding to the aromatic protons. In addition, the peaks corresponding to the NH groups were absent due to the use of CF_3COOD as a solvent.

B. In vitro antioxidant potential of synthesized compounds

Antioxidant efficiency was performed using the DPPH free radical scavenging method (H-donor method) and hydroxyl radical scavenging assay, which based upon different mechanisms to provide complementary insight into the antioxidant activity of the synthesized compounds [12]. The results are indicated and listed in tables 1 and 2.

Table 1 Antioxidant activity percentage of synthesized compounds "**5a-c** and **9a-c -11a-c**" at different concentrations using the DPPH method

Compound #	% Antioxidant activity		
	100 µM	200 µM	300 µM
5a	18^{gh} ±0.54	22^{g} ±0.95	34^{i} ±0.54
5b	19^{f} ±0.74	34^{f} ±0.42	33^{g} ±0.23
5c	31^{d} ±0.75	45^{d} ±0.60	66^{d} ±0.91
9a	21^{gh} ±0.56	39^{g} ±1.25	43^{i} ±0.56
9b	25^{f} ±0.79	46^{f} ±0.46	57^{g} ±0.30
9c	33^{d} ±0.78	58^{d} ±0.62	72^{d} ±0.98
10a	28^{e} ±0.30	52^{e} ±1.32	74^{c} ±0.70
10b	37^{c} ±0.36	61^{c} ±0.72	70^{e} ±0.98
10c	49^{b} ±0.46	70^{b} ±1.59	78^{b} ±0.72
11a	17^{i} ±0.61	28^{i} ±0.61	34^{j} ±0.79
11b	20^{h} ±0.44	34^{h} ±0.89	46^{h} ±0.50
11c	22^{g} ±0.82	47^{t} ±1.15	61^{t} ±0.72
Ascorbic acid (standard)	53^{a} ±0.50	78^{a} ±0.52	95^{a} ±0.53
LSD	1.002	1.678	1.208

Values are expressed as means ± SD (n = 3).
Values with different superscript letters within the same column are significantly different (P < 0.05).

Table 2 Hydroxyl radical scavenging activity of synthesized compounds "**5a-c** and **10a-c -12a-c**" at different concentrations

Compound #	% OH radical scavenging activity		
	100 µM	200 µM	300 µM
5a	13^{h} ±0.67	22^{g} ±0.94	30^{g} ±0.72
5b	16^{f} ±0.35	29^{f} ±0.95	33^{e} ±1.32
5c	20^{d} ±0.64	39^{d} ±0.97	55^{cd} ±0.60
9a	15^{h} ±0.69	27^{g} ±0.95	35^{g} ±0.78
9b	19^{f} ±0.35	38^{f} ±0.95	49^{e} ±1.32
9c	28^{d} ±0.75	49^{d} ±1.15	62^{cd} ±0.61
10a	22^{e} ±0.46	42^{e} ±0.69	63^{c} ±0.75
10b	32^{c} ±0.96	54^{c} ±0.56	61^{d} ±1.06
10c	43^{b} ±0.46	63^{b} ±1.23	65^{b} ±0.95
11a	13^{i} ±0.36	23^{h} ±0.35	26^{h} ±0.26
11b	16^{gh} ±0.75	27^{g} ±0.53	35^{g} ±0.36
11c	17^{g} ±0.45	38^{f} ±1.42	41^{f} ±0.92
Ascorbic acid (standard)	49^{a} 2.10	73^{a} 0.89	84^{a} ±0.30
LSD	1.508	1.584	1.369

Values are expressed as means ± SD (n = 3).
Values with different superscript letters within the same column are significantly different (P < 0.05).

As shown in tables 1 and 2, the antioxidant and hydroxyl radical scavenging activities were increased when doubling the concentration of the tested compounds, and most of the synthesized compounds exhibited very good antioxidant activity (i.e., pyridazine > pyridine > quinoline analogues) relative to ascorbic acid, which was used as a standard due to its higher antioxidant activity. The high antioxidant and scavenging activities of the tested compounds may be due to the resonance phenomena of double bonds and lone pair electrons on nitrogen. This structure may lead to radical formation in more than one site, especially on the benzene ring attached to the nitro group, which is a highly electron withdrawing group that enables the benzene ring to convert to a radical form and forms a new covalent bond with another radical (e.g.,**10c** and **11a-c**). In addition, protection against peroxides, peroxynitrite, glutathione, peroxidase-like activity and metal–binding capacity due to organoselenium analogues leads to antioxidant activity. This conclusion is also supported by previously reported results [13].

C. Acute toxicity and antitumor activity

The median lethal dose (LD50) of the selected compounds (**9a** and **10a-c**) based on their in vitro antioxidant potential results, was measured in mice. The results indicated that the selected compounds were non–toxic at doses up to 500 mg kg^{-1}B.wt (bodyweight of tested mice).

The tested compounds were evaluated *in vitro* for their cytotoxic activity against the Ehrlich Ascites Carcinoma Cell (EACC) line, and the viability percentages were determined and listed in table 3.

Table 3 Viability percentages of carcinoma cells treated with synthesized compounds"**5a-c** and **9a-c–11a-c**" at different concentrations

Compound #	% Viability of carcinoma cells		
	100 µM	200 µM	300 µM
5a	22^{bc} ±0.72	12^{c} ±0.51	11^{c} ±0.74
5b	20^{d} ±0.95	14^{d} ±0.90	7^{f} ±0.15
5c	21^{e} ±0.63	16^{e} ±0.94	6^{g} ±0.24
9a	34^{bc} ±0.62	24^{c} ±0.53	17^{c} ±0.78
9b	29^{d} ±0.95	19^{d} ±0.92	8^{f} ±0.17
9c	25^{e} ±0.66	17^{e} ±0.98	5^{g} ±0.26
10a	35^{ab} ±0.95	24^{c} ±0.52	16^{d} ±0.62
10b	28^{d} ±0.85	18^{e} ±1.32	4^{h} ±0.36
10c	23^{f} ±0.79	13^{f} ±0.44	0^{i} ±0.00
11a	36^{a} ±0.46	30^{a} ±0.82	25^{a} ±0.26
11b	33^{c} ±0.26	28^{b} ±0.46	18^{b} ±0.44
11c	28^{d} ±1.04	16^{e} ±0.53	12^{e} ±0.40
Control	100	100	100
LSD	1.325	1.336	0.734

Values are expressed as means ± SD (n = 3).
Values with different superscript letters within the same column are significantly different (P < 0.05).

As shown in table 3, the tested compounds exhibited broad-spectrum antitumor activity in the following order: pyridazine > pyridine > quinoline analogues. The thieno-pyridine derivatives **5a-c** in common, showed less activity than seleno derivatives. Compounds **9b,c** and **10b,c** exhibited higher antitumor activity compared to the other tested compounds, and compound **10c** was the most active member in this study. The viability percentage of the treated EACC decreased from 100 % in the control sample to 0 %, 4 %, 5 % and 8 % at a concentration of 300 µM for compounds **10c, 10b, 9c** and **11b**

respectively. However, compounds **9a, 10a, 11b** and **11c**, exhibited moderate cytotoxicity and a decrease in the viability percentage from 18 % to 12 % at a concentration of 300 µM of each compound. The presence of the 4-phenyl function with a nitro group (e.g.,**10c** and **9c**) and a methyl group (e.g.,**11b** and **10b**) increased the antitumor activity due to their high antioxidant and free radical scavenging activities, as previously discussed and supported by previously reported results [14].

D. Antimicrobial performance

An antimicrobial screening of synthesized compounds "**5a-c** and **9a-c–11a-c**" against selected Gram-positive, Gram-negative bacteria and fungi compared to tetracycline, which is a standard antibacterial agent, and Amphotericin B, which is a standard antifungal agent, was performed, and the results are listed in table 4. All of the tested compounds exhibited high broad-spectrum antimicrobial activities against both gram-positive and gram-negative bacteria with inhibition percentages in the range of 50 % to 78 %. These compounds exhibited moderate activities against the studied fungi with inhibition percentages in the range of 24 % to 40 %. The presence of a strong electron-withdrawing group (e.g., compound **10c**) resulted in higher activity against the microorganisms under study.

Table 4 Inhibition zone diameter (mm) of synthesized compounds "**5a-c and 9a-c– 11a-c**"

Compounds #		Inhibition zone diameter (mm /µ g Sample)					
		Bs	Sa	Ec	St	Af	Ca
		(G^+)		(G^-)		Fungi	
Control (DMSO)		0	0	0	0	0	0
Standard	Tetracycline	22^{a} ±0.46	24^{a} ±0.53	20^{a} ±0.26	19^{a} ±0.30	-	-
Standard	Amphotericin B	-	-	-	--	27^{a} ±0.56	25^{a} ±0.66
5a		10^{f} ±0.35	12^{e} ±0.50	8^{d} 0.41	11^{f} 0.09	6^{cd} ±0.66	8^{d} ±0.53
5b		12^{e} ±0.53	11^{de} ±0.40	10^{d} 0.42	11^{d} 0.35	7^{bc} ±0.31	9^{c} ±0.21
5c		13^{c} ±0.41	16^{bc} ±0.61	10^{c} 0.44	11^{c} 0.51	9^{b} ±0.44	10^{b} ±0.22
9a		11^{f} ±0.36	14^{e} ±0.52	10^{d} 0.44	11^{f} 0.10	8^{cd} ±0.70	8^{d} ±0.46
9b		14^{e} ±0.56	15^{de} ±0.44	11^{d} 0.40	13^{d} 0.35	9^{bc} ±0.36	9^{c} ±0.26
9c		16^{c} ±0.44	17^{bc} ±0.60	12^{c} 0.44	14^{c} 0.53	10^{b} ±0.44	10^{b} ±0.26
10a		14^{e} ±0.26	15^{de} ±0.85	10^{d} 0.46	11^{f} 0.10	8^{cd} ±0.36	7^{e} ±0.17
10b		14^{e} ±0.36	16^{cd} ±0.17	10^{d} 0.20	12^{e} 0.56	7^{d} ±0.10	6^{f} ±0.20
10c		17^{b} ±0.17	18^{b} ±0.36	13^{b} 0.50	15^{b} 0.46	10^{b} ±0.87	8^{d} ±0.10
11a		15^{d} ±0.26	16^{cd} ±0.60	11^{d} 0.00	13^{d} 0.20	9^{bc} ±0.1	7^{e} ±0.3
11b		16^{c} ±0.30	15^{de} ±0.20	11^{d} 0.56	12^{e} 0.20	7^{d} ±0.78	6^{f} ±0.10
11c		17^{b} ±0.56	18^{b} ±0.17	12^{c} 0.50	14^{c} 0.20	9^{bc} ±0.10	6^{f} ±0.30
LSD		0.668	0.838	0.969	0.577	0.877	0.552

Values are expressed as means ± SD (n = 3).
Values with different superscript letters within the same column are significantly different (P < 0.05).

Bs: Bacillus subtilis; Sa: Staphylococcus aureus; Ec: Escherichia coli; St: Salmonella typhimurium; Af: Aspergillus Flavus; Ca: Candida albicans G+: Gram-positive bacteria; G-: Gram-negative bacteria.

All of the tested compounds exhibited high broad-spectrum antimicrobial activities against both gram-positive and gram-negative bacteria with inhibition percentages in the range of 50 % to 78 %. These compounds exhibited moderate activities against the studied fungi with inhibition percentages in the range of 24 % to 40 %. The presence of a strong electron-withdrawing group (e.g., compound **11c**) resulted in higher activity against the microorganisms under study.

III. EXPERIMENTAL

A. Materials and instrumentation

The reagents were analytical grade or chemically pure. Elemental analyses (C, H, N, S) 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 and ^{13}C NMR analysis), Kingdom of Saudi Arabia. The biological tests were performed by the "Biotechnology Unit", Faculty of Agriculture, Cairo University, Egypt. The Ehrlich Ascites Carcinoma Cell (EACC) line was supplied by "The National Cancer Institute", Egypt. Biological statistical analysis was carried out according to Fisher, indicating the standard deviation ‘‘SD’’ and the standard error ‘‘SE’’ [15]. LSD (Least significant difference) test was used to compare the significant differences between means of treatment. The statistical package for social science S.P.S.S. (1999) program version was used for all analysis [16].

B. Synthesis and characterisation

*General method for the synthesis of 5-aryl azo-2-aminothiazol derivatives **2a-c***

Coupling of molar ratio of 2-aminothiazole **1** with different diazotised aromatic amines (aniline, *p*-toluidine, *p*-nitroaniline) in ethanol/sodium acetate at -5 °C was performed to obtain the corresponding derivatives of 5-arylazo-2-aminothiazol **2a-c**. The precipitate which formed was collected by filtration, dried and recrystallized from proper solvent. The IR and ^{1}H NMR spectra for the obtained compounds were recorded to characterise the structure of these compounds, and were found to be in agreement with the literature [10],[11], [17].

2-Amino-5-phenylazo-thiazole **(2a).** X=H: Reddish brown solid (EtOH), yield 84%, m.p. 270-271 ºC, Lit. m.p. 270-271 ºC; X= Ph: Red solid (EtOH), yield 83%, m.p. 278-279 ºC, Lit. m.p. 280 ºC.

2-Amino-5-(*p*-tolyl)azo-thiazole **(2b).** X=H: Brown solid (EtOH), yield 73%, m.p. 207-209 ºC, Lit. m.p. 205 ºC; X= Ph: Brown solid (EtOH), yield 94%, m.p. 204 ºC, Lit. m.p. 203-205 ºC.

2-Amino-5-(*p*-nitrophenyl)azo-thiazole **(2c).** X=H: Brown solid (EtOH), yield 80%; m.p. 167-169 ºC, Lit. m.p. 167-169 ºC; X= Ph: Brown solid (DMF), yield 76%, m.p. 261-262 ºC, Lit. m.p. 261-262 ºC.

*General method for the synthesis of 2-[N-(chloroacetyl)amino]-5-arylazo-thiazoles **3a-c***

To a solution of 2-amino-5-arylazothiazoles **2a-c** (10 mmol) in DMF (25 ml) containing 0.5 ml triethyl amine, chloroacetyl chloride (1.2 ml, 15 mmol) was added dropwise with stirring at room temperature. Stirring was continued for 2 hours and the reaction mixture was poured to ice cooled water. The precipitate which formed was collected by filtration, dried and recrystallized from the appropriate solvent. The structure of the synthesized compounds were characterised using IR and ^{1}H NMR spectra, and were found to be in agreement with previously reported [9], [10].

2-[*N*-(chloroacetyl)amino]-5-phenylazo-thiazole **(3a).** X= H: Greenish yellow solid (EtOH); yield 78%; m.p.: 220-222 ºC; Lit. m.p.: 220-222 ºC; X= Ph: Orange solid (EtOH); yield 79%; m.p.: 227-228 ºC; Lit. m.p.: 229 ºC.

2-[*N*-(chloroacetyl)amino]-5-(*p*-tolyl)azo-thiazole **(3b).** X= H: Yellowish brown solid (EtOH); yield 82%; m.p.: 235-237 ºC; Lit. m.p.: 235-237 ºC; X= Ph: Orange solid (EtOH); yield 77%; m.p.: 221 ºC; Lit. m.p.: 222 ºC.

2-[*N*-(chloroacetyl)amino]-5-(*p*-nitrophenyl)azo-thiazole (**3c**). X= H: Dark brown solid (EtOH-DMF), yield 85%, m.p. 186-187 ºC; Lit. m.p. 186-187 ºC; X= Ph: Brown solid (DMF), yield 82%, m.p. 215-216 ºC, Lit. m.p. 216 ºC.

*Synthesis of 3-amino-N-(4-phenyl-5-arylazo-2-thiazolyl)-thieno[2,3-b]pyridine-2-carboxamide dyes **5a-c***

A mixture of 2-(*N*-chloroacetyl)-5-arylazo-thiazole derivatives **3a-c** (0.01 mol), 4,6-dimethyl-2-mercaptonicotinonitrile **4** (0.01 mol), and anhydrous potassium carbonate (0.01 mol) in acetone (30 ml) was refluxed for 4 hours. The nicotinonitrile derivatives formed were added to a solution of sodium ethoxide (from 0.005 mol sodium metal) in absolute ethanol (30 ml). The solution was refluxed for 2 hours, left to cool, and diluted with cooled water (50 ml). The solid obtained was filtered and recrystallized from ethanol [11].

5a; Red solid, yield 43%, mp >300 °C, Lit. m.p. >265 °C.

5b; Red solid, yield 50%, mp 298 °C, Lit. m.p. >265 °C.

8d; Greenish brown solid, yield 56%; mp >300 °C; Lit. m.p. >265 °C.

*Synthesis of hydroseleno-(pyridine, pyridazine and quinoline)carbonitrile derivatives **6-8***

2-hydroseleno-4,6-dimethylpyridine-3-carbonitrile **6**, 3-hydroseleno-5,6-diphenylpyridazine-4-carbonitrile **7** and 5,6,7,8-tetrahydro-2-hydroseleno-4-(4'-methoxyphenyl)quinoline-3-carbonitrile **8** were synthesized according to previously reported methods and their characterized data (m.p., IR and ^{1}H NMR) were in agreement with literature [11].

2-Hydroseleno-4,6-dimethylpyridine-3-carbonitrile **(6).** Brown solid (EtOH), yield 79%, m.p. 214-215 °C, Lit. 214-216 °C.

3-Hydroseleno-5,6-diphenylpyridazine-4-carbonitrile **(7**). Brown solid (EtOH), yield 90%, m.p. 219 °C, Lit. 218-220 °C.

5,6,7,8-Tetrahydro-2-hydroseleno-4-(4'-methoxyphenyl)quinoline-3-carbonitrile **(8).** Dark brown solid (EtOH), yield 67%, m.p. 171 °C, Lit.170-172 °C.

*General procedure for the synthesis of 3-amino substituted N-(5-arylazothiazol-2-yl)selenopheno-pyridine, pyridazine and/or quinoline-2-carboxamide Derivatives **9a-c- 11a-c***

The mixtures of 2-(*N*-chloroacetyl)-5-arylazothiazole derivatives **3a-c** (10 mmol) with 2-hydroseleno-4,6-dimethylpyridine-3-carbonitrile **4** (2.12 g, 10 mmol), 3-hydroseleno-5,6-diphenylpyridazine-4-carbonitrile **5** (3.36 g, 10 mmol) and/or 5,6,7,8-tetrahydro-2-hydroseleno-4-(4'-methoxyphenyl)quinoline-3-carbonitrile **6** (3.43 g, 10 mmol) dissolved in DMF (30 ml) containing aqueous KOH (10 %, 5 ml), were stirred for 2 hrs. Treatment of reaction mixture with an excess amount of aqueous 10 % KOH (5 ml), and stirring for an additional 2 hrs., affording the corresponding derivatives "**9a-c-11a-c**". The precipitates formed were filtered and recrystallised from ethanol. The characterization for the products was in agreement with the literature [11].

3-Amino-4,6-dimethyl-*N*-(5-phenylazothiazol-2-yl)selenopheno[2,3-*b*]pyridine-2-acetamide **(9a).** Orange solid, yield 39 %, m.p. >300 °C, Lit. m.p. >300 °C.

3-Amino-4,6-dimethyl-*N*-[5-(4-tolylazo)thiazol-2-yl]selenopheno[2,3-*b*]pyridine-2-actamid **(9b).** Yellowish brown solid, yield 42 %, m.p. 156-157 °C, Lit. m.p. 156-157 °C.

3-Amino-4,6-dimethyl-*N*-[5-(4-nitrophenylazo)thiazol-2-yl]selenopheno[2,3-b]pyridine-2-actamide **(9c).** Dark brown solid, yield 62 %, m.p. 176-177 °C, Lit. m.p. 176 °C.

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3-Amino-4,6-diphenyl-*N*-(5-phenylazothiazol-2-yl)selenopheno[2,3-*b*]pyridazine-2-acetamide **(10a).** Brown solid, yield 41 %, m.p. 167-168 °C, Lit. m.p. 168-169 °C.

3-Amino-4,6-diphenyl-*N*-[5-(4-tolylazo)thiazol-2-yl]selenopheno[2,3-*b*]pyridazine-2-acetamide **(10b).** Reddish brown solid, yield 57 %, m.p. 232 °C, Lit. m.p. 230-233 °C.

3-Amino-4,6-diphenyl-*N*-[5-(4-nitrophenylazo)thiazol-2-yl]selenopheno[2,3-*b*]pyridazine-2-acetamide **(10c).** Dark brown solid, yield 62 %, m.p. 180-181 °C, Lit. m.p. 180 °C.

3-Amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-*N*-(5-phenylazothiazol-2-yl)selenopheno[2,3-*b*]quinoline-2-acetamide **(12a).** Brown solid, yield 43 %, m.p. 142-143 °C, Lit. m.p. 142-144 °C.

3-Amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-*N*-[5-(4-tolylazo)thiazol-2-yl]selenopheno[2,3-*b*]quinoline-2-acetamide **(12b).** Reddish brown solid, yield 50 %, m.p. 155 °C, Lit. m.p. 154-155 °C.

3-Amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-*N*-[5-(4-nitrophenylazo)thiazol-2-yl]selenopheno[2,3-*b*]quinoline-2-acetamide **(12c).** Dark reddish brown solid, yield 58 %, m.p. 169-170 °C, Lit. m.p. 168-170 °C.

C. Screening of biological activities of the synthesized dyes

In vitro antioxidant potential of synthesized compounds

DPPH radical scavenging activity

The scavenging effect of the 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical was measured by the method reported by Chou et al. and is expressed in terms of the inhibition percentage (I %) [19]. Different concentrations of the synthesized compounds (100, 200 and 300µM) with 0.1 ml of a 1 mM DPPH-methanol solution were incubated at room temperature for 30 min. The absorbance (A) of each solution was measured at 517 nm against a blank containing ascorbic acid using equation 1.

$$I\% = \frac{Acontrol - Asample}{Acontrol} * 100 \qquad (1)$$

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was measured according to Nagai et al., [12] where mixtures of various concentrations of the synthesized compounds (100, 200, 300 µM) with 0.45 ml of a sodium phosphate buffer solution (0.2M), 0.15 ml of a 2-deoxyribose solution (10 mM), 0.15 ml of an $FeSO_4$-EDTA solution (10 mM) and 0.15 ml of H_2O_2 (10 mM) were prepared and filled to the final volume (1.5 ml) with distilled water. The solutions were incubated at 37 °C for 4 hours, and the reaction was terminated by adding 0.75 ml of a trichloroacetic acid solution (70 % w/v) and 0.75 ml of a thiobarbituric acid solution (50 mM). The absorbance (A) was measured at 520 nm, and the inhibition of deoxyribose degradation as a percentage (I %) was calculated according to equation 1.

In vitro acute toxicity and antitumor activity

Acute toxicity (LD50)

The median lethal dosage (LD_{50}) values of selected compounds (**9c, 10a, 10b** and **10c**) based on *in vitro* antioxidant potential results were determined in mice. LD_{50} represents the individual dose required to kill 50 % of a population of tested animals (e.g., rats, fish, mice, cockroaches). A group of five female adult albino mice (25-30g) was injected intraperitoneally (I.P) with graded doses of 100-1000 mg Kg^{-1} of body weight for each selected compound suspended in DMSO. The percentage of mortality was determined 72 hours after injection. The LD_{50} calculation was processed using a graphical method [18].

In vitro antitumor activity

Female Swiss albino mice (25-30g) were housed at a constant temperature (24±2 ^oC) with alternating 12 hours of light and dark cycles and were fed standard laboratory food (Milad Co) along with ad libitum water. The care and handling of the animals were performed according to the guidelines of "The World Health Organization, Geneva, Switzerland". A strain of Ehrlich Ascites Carcinoma Cells (EACC) was supplied by "The National Cancer Institute", Egypt. The tumour cell line was maintained in female Swiss albino mice through serial intraperitoneal inoculation at 7 or 8 day intervals in the form of ascites.

In vitro cytotoxicity

The EACC cells were obtained by needle aspiration of the ascitic from preinoculated mice under aseptic conditions according to the method reported by Uma Dev et al. [19]. The tumour cell suspension (2×10^6 cells per ml) was prepared in RPMI-1640 media, 10 % Foetal bovine serum and L-glutamine. The tested compounds with different concentrations (i.e., 100, 200, 300 µM) in DMSO were incubated overnight with 2 ml of suspended tumour cells under 5 % CO_2 at 37 ^oC. The trypan blue exclusion method reported by Bennett et al. was used to calculate the viability percentage of tumour cells using equation 2 [20].

$$I\% = \frac{No.of\ variables}{Total\ no.\ of\ cells} * 100 \qquad (2)$$

The antioxidant and hydroxyl radical scavenging activities increased when the concentration of the tested compounds was doubled, and most of the synthesized compounds exhibited very good antioxidant activity (i.e., pyridazine > pyridine > quinoline analogues) relative to ascorbic acid, which was used as a standard. The high antioxidant and scavenging activities of the tested compounds may be due to the resonance phenomena of double bonds and lone pair electrons on nitrogen. This structure may lead to radical formation in more than one site, especially on the benzene ring attached to the nitro group, which is a highly electron withdrawing group that enables the benzene ring to convert to a radical form and forms a new covalent bond with another radical (e.g.,**9c** and **10a-c**). In addition, protection against peroxides, peroxynitrite, glutathione, peroxidase-like activity and metal–binding capacity due to organoselenium analogues leads to antioxidant

activity. This conclusion is also supported by previously reported results [13].

Antibacterial and antifungal activities

The antibacterial activity of novel synthesized compounds **"5a-c** and **9a-c – 11a-c"** (100 µg/ml in DMSO) was determined *in vitro* using the disc diffusion method [21], against a variety of pathogenic microorganisms, such as Gram-positive bacteria *(*i.e., *Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria *(*i.e., *Escherichia coli* and *Salmonella typhimurium*), in nutrient agar media by measuring the zone of inhibition in mm. The antifungal screening of the synthesized compounds was also carried out *in vitro* using the same method against two fungi strains of *Aspergillus flavus* and *Candida albicans.* In addition, *Tetracycline* and *Amphotericin B* served as standard antibacterial and antifungal agents, respectively, and both served as positive controls for antimicrobial activity.

IV. Conclusion

A series of monoazo compounds based on 4-substituted-3-amino-*N*-(4-aryl-5-arylazo-2-thiazolyl)-thieno[2,3-b]pyridine-2-carboxamide) along with their seleno like derivatives of pyridine, pyridazine and quinolone was synthesized and *in vitro* tested for their biological activity. They exhibited variable antioxidant activity due to the protection against peroxide and peroxynitrite radicals, as well as antitumor activity. In addition, these compounds are emerging as promising downstream candidates for cancer therapy due to their ability to modulate multiple physiological functions implicated in cancer development due to their antioxidant and anticancer chemo preventive or apoptotic activities while being nontoxic. These compounds also have the potential for use as antibacterial agents against different pathogenic bacteria and fungi.

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